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Emerging researchers in frontiers in pharmacology: Obstetric and pediatric pharmacology 2022

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Emerging researchers in frontiers in pharmacology: Obstetric and pediatric pharmacology 2022

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Editorial: Emerging researchers in frontiers in pharmacology: obstetric and pediatric pharmacology 2022

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risk factors, pregnancy complication, infertility, uterine fibroids, ovary diseases, reproductive diseases, exosomes, maternal-fetal pharmacology

Editorial on the Research Topic
Emerging researchers in frontiers in pharmacology: obstetric and pediatric pharmacology 2022

1 The aim and scope of this Research Topic

Globally, while students are undertaking fundamental research as part of their education in Obstetric and Pediatric Pharmacology, most of this research needs to be conveyed to the broader audience. We acknowledge that many student researchers may find peer review daunting. Therefore, at Frontiers, where peer review is considered a collaborative process, our interactive peer review is tailored to provide researchers with hands-on guidance and constructive feedback. This specific Research Topic is to encourage emerging researchers to publish their work with Frontiers in Pharmacology. In addition, our Research Topic Editors are committed to advancing emerging researchers' and students' success at publications.

This Research Topic features 12 articles, including 5 original and 7 review articles, in a multidisciplinary collaboration among obstetrics and gynecology, pharmacology, and pediatrics. These articles cover several diseases, including pregnancy complications, children's diseases, uterine fibroids, and ovarian disorders.

2 Overview of contributors

2.1 Diseases during pregnancy

Adverse pregnancy outcomes are known to have long-term health implications for the child. Fetal arrhythmias are common cardiac abnormalities associated with high mortality by reason of ventricular dysfunction and heart failure (Keenan et al., 2022). Qin et al. conducted a

network meta-analysis to understand the efficacy and safety of various therapeutic medicines for fetal tachycardias, one of the fetal arrhythmia types. The authors concluded that flecainide monotherapy and a combination of digoxin and flecainide should be the superior therapeutic strategies for fetal tachycardia.

Ectopic pregnancy (EP) has the implantation of a fertilized ovum outside the endometrial cavity (Barnhart, 2009), most often occurring in a fallopian tube. Predictive factors of damage to the Fallopian tube may guide the treatment for patients with tubal pregnancy (Cabar et al., 2006). Some potential predictors of trophoblastic invasion of the fallopian tubes have been reported in EP. Teshima et al. investigated the link between VEGF tissue expression and the depth of trophoblastic infiltration into the tubal wall in patients with ampullary pregnancy, which is not associated with the previous finding that serum VEGF was correlated with the trophoblast invasion in the tubal wall from EP patients. This study indicates that the tissue expression of VEGF at the EP implantation site may not be primarily responsible for providing the local microenvironment triggering the trophoblast invasion.

The use of prescription drugs is prevalent during pregnancy, Pregnant women can have severe health consequences for infants if misuse of prescription drugs occurs. Currently, the knowledge about maternal-fetal safety and efficacy of drug use is limited. Hudson et al. present an overview of the current understanding of maternal-fetal drug exposure, discuss biospecimen-guided sampling design and methods for measuring fetal drug concentrations throughout gestation, and propose strategies for advancing pharmacology research in the maternal-fetal population.

Some women experience health problems during and after pregnancy. Pregnant individuals with arterial hypertension have significantly high risks of maternal mortality (Vaidy and Vaidya, 2023). Brandão et al. reported that assessment by ambulatory blood pressure monitoring (ABPM) verified the presence of hypertension in pregnant women. The ethnicity, self-reported hypertension, and the presence of hypertension during pregnancy are associated with arterial hypertension measured by ABPM. Measurement of arterial hypertension by ABPM will help improve quality of life and longevity.

Antiphospholipid syndrome (APS) is an autoimmune disorder that causes an increased risk of blood clots (Garcia and Erkan, 2018). As a result, pregnant women with APS show an increased risk of miscarriage. Wu et al. conducted a bibliometric analysis to review the studies in the field of APS and revealed that the research on APS has increased steadily in the past 10 years. Clinical studies on the mechanism and treatment of APS are recognized as encouraging research hotspots to reduce APS-associated miscarriages.

Preeclampsia complicates 2%–4% of all pregnancies and accounts for about 46,000 maternal deaths and 500,000 fetal or newborn deaths yearly (Magee et al., 2022). In the review article by Veiga et al., some inflammatory markers, including leptin, total cholesterol, triglycerides, C-reactive proteins, and TNFa, were elevated in pregnant women with preeclampsia compared to pregnant control women, indicating the correlation between the inappropriate inflammatory responses and preeclampsia pathophysiology.

2.2 Children diseases

Apnea of prematurity is a developmental disorder affecting most highly preterm infants and associates with long-term morbidity, including poor neurodevelopmental outcomes (Williamson et al., 2021). Caffeine has been used for many years to treat apnea of prematurity (Chavez and Bancalari, 2022). However, the long-term use of caffeine may cause adverse effects. In the review article by Dai et al., caffeine alters the circadian rhythms in humans and animals, and the relationship between preterm infants and circadian rhythms linked to caffeine therapy could help in the clinical practice to encourage precision therapy. Further investigation into the effect of caffeine on circadian rhythms regarding safety, dose efficacy, and duration of treatment during pregnancy is needed. In addition, obstructive sleep apnea hypopnea syndrome (OSAHS) is a sleep-related breathing disorder associated with substantial morbidity. A clinical trial by Zheng et al. tested the safety and efficacy of esketamine during drug-induced sleep endoscopy (DISE) in children with OSAHS, and compared it with dexmedetomidine, a selective alpha-2 adrenergic agonist, and recommended agent for DISE. Their studies demonstrated that esketamine provided a more effective and safer depth of anesthesia for pediatric DISE with OSAHS than dexmedetomidine.

Children experience severe repercussions from poisoning due to less capability of neutralizing harmful substances. In addition, developmental exposure to adverse exposure can increase the risk of diseases in the adult stage. Althobaiti et al. performed a retrospective cohort study on 122 children exposed to various toxic substances in Makkah, Saudi Arabia, including pharmaceutic products, household products, plant envenomation, and animal envenomation. They identified the poison forms, poisoning routes, and presenting symptoms. Their studies indicate that acute poising among children is a significant health Research Topic that necessitates more attention to raise awareness of safety requirements.

2.3 Uterine and ovarian diseases

Uterine fibroids (UFs) are the most common pelvic tumors among women of reproductive age, affecting more than 75% of women. Although benign, UFs are associated with significant morbidity, including heavy menstrual bleeding, pelvic pain, and reproductive dysfunction. UFs are the leading cause of hysterectomy (Bulun, 2013; Yang et al., 2022). Women of African descent are at a higher risk of developing UFs and frequently experience much more severe symptoms (Li et al., 2023). Sub-Saharan Africa is known to have the largest population of black women. However, most UFs studies do not include people from the continent of Africa. Sefah et al. reviewed the existing literature, emphasizing that the prevalence of UFs on Africa is not well investigated. Therefore, conducting future research on African women is highly needed.

Epithelial ovarian cancer (EOC) is the most common type of ovarian cancer that affects the female reproductive system and continues as a leading cause of death from gynecological malignancies (Qu et al., 2022). Baghban et al. reviewed the studies on the role of exosomes in EOC. They revealed that research on the exosome and EOC had been expanded, and China is much more involved than other countries in research, financial support, and international cooperation. The interest of exosome-oriented research on EOC includes exosomes as prognostic and diagnostic biomarkers, the role of exosomes in proliferation, migration, and metastasis, epithelial-mesenchymal transition, and chemoresistance. In this Research Topic, another review article by Izadi et al. presented

an overview of mesenchymal stem cells (MSC)-derived exosomes as a promising approach for treating infertility in women with polycystic ovary syndrome (PCOS). MSC-derived exosomes exhibited therapeutic effects on the PCOS via modulating immunity response, exerting an anti-inflammatory effect, and suppressing apoptosis of granulosa cells. These two reviewer articles highlight and suggest the promising role of exosomes in targeting ovary diseases, including PCOS and ovarian cancer.

3 Conclusion

In conclusion, this Research Topic has provided original research and updated reviews of early-stage researchers related to basic and translational research in obstetrics and gynecology, pharmacology, and pediatrics. These studies further advance our understanding of the risk and pathogenesis of infertility, adverse pregnancy outcomes, uterine fibroids, and ovarian diseases. The evidence collected from this Research Topic is also expected to be translated into more precise and practical clinical approaches to predict and treat relevant human disorders in the future.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Barnhart, K. T. (2009). Clinical practice. Ectopic pregnancy. *N. Engl. J. Med.* 361 (4), 379–387. doi:10.1056/NEJMcp0810384

Bulun, S. E. (2013). Uterine fibroids. *N. Engl. J. Med.* 369 (14), 1344–1355. doi:10.1056/NEJMra1209993

Cabar, F. R., Pereira, P. P., Schultz, R., and Zugaib, M. (2006). Predictive factors of trophoblastic invasion into the ampullary region of the tubal wall in ectopic pregnancy. *Hum. Reprod.* 21 (9), 2426–2431. doi:10.1093/humrep/del170

Chavez, L., and Bancalari, E. (2022). Caffeine: Some of the evidence behind its use and abuse in the preterm infant. *Neonatology* 119 (4), 428–432. doi:10.1159/000525267

Garcia, D., and Erkan, D. (2018). Diagnosis and management of the antiphospholipid syndrome. *N. Engl. J. Med.* 378 (21), 1290–2021. doi:10.1056/NEJMc1808253

Keenan, E., Karmakar, C., Udhayakumar, R. K., Brownfoot, F. C., Lakhno, I., Shulgin, V., et al. (2022). Detection of fetal arrhythmias in non-invasive fetal ECG recordings using data-driven entropy profiling. *Physiol. Meas.* 43 (2), 025008. doi:10.1088/1361-6579/ac4e6d

Li, Y., McNally, R. P., Feng, Y., Kim, J. J., and Wei, J. J. (2023). Racial differences in transcriptomics and reactive oxygen species burden in myometrium and leiomyoma. *Hum. Reprod.* 38 (4), 609–620. doi:10.1093/humrep/dead020

Magee, L. A., Nicolaides, K. H., and von Dadelszen, P. (2022). Preeclampsia. *N. Engl. J. Med.* 386 (19), 1817–1832. doi:10.1056/NEJMra2109523

Qu, Q., Liu, L., Cui, Y., Chen, Y., Wang, Y., and Wang, Y. (2022). Exosomes from human omental adipose-derived mesenchymal stem cells secreted into ascites promote peritoneal metastasis of epithelial ovarian cancer. *Cells* 11 (21), 3392. doi:10.3390/cells11213392

Vaidy, A., and Vaidya, A. (2023). Pulmonary arterial hypertension in pregnancy. *Curr. Opin. Cardiol.* 38 (3), 250–256. doi:10.1097/HCO.0000000000001034

Williamson, M., Poorun, R., and Hartley, C. (2021). Apnoea of prematurity and neurodevelopmental outcomes: Current understanding and future prospects for research. *Front. Pediatr.* 9, 755677. doi:10.3389/fped.2021.755677

Yang, Q., Ciebiera, M., Bariani, M. V., Ali, M., Elkafas, H., Boyer, T. G., et al. (2022). Comprehensive review of uterine fibroids: Developmental origin, pathogenesis, and treatment. *Endocr. Rev.* 43 (4), 678–719. doi:10.1210/endrev/bnab039

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SYSTEMATIC REVIEW
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Efficacy and Safety of Various First-Line Therapeutic Strategies for Fetal Tachycardias: A Network Meta-Analysis and Systematic Review

*Jiangwei Qin[1†], Zhengrong Deng[1†], Changqing Tang[2], Yunfan Zhang[1], Ruolan Hu[1], Jiawen Li[1], Yimin Hua[1] and Yifei Li[1]**

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Background: Fetal arrhythmias are common cardiac abnormalities associated with high mortality due to ventricular dysfunction and heart failure, particularly when accompanied by hydrops. Although several types of common fetal tachycardias have been relatively identified medications, such as digoxin, flecainide, and sotalol, there is no first-line drug treatment protocol established for the treatment of various types of fetal tachycardias.

Methods: We conducted a network meta-analysis using a Bayesian hierarchical framework to obtain a model for integrating both direct and indirect evidence. All tachycardia types (Total group), supraventricular tachycardia (SVT subgroup), atrial flutter (AF subgroup), hydrops subgroup, and non-hydrops subgroup fetuses were analyzed, and five first-line regimens were ranked according to treatment outcomes: digoxin monotherapy (D), flecainide monotherapy (F), sotalol monotherapy (S), digoxin plus flecainide combination therapy (DF), and digoxin plus sotalol combination therapy (DS). Effectiveness and safety were determined according to the cardioversion rate and intrauterine death rate.

Results: The pooled data indicated that DF combination therapy was always superior to D monotherapy, regardless of the tachycardia type or the presence of hydrops: Total, 2.44 (95% CrI: 1.59, 3.52); SVT, 2.77 (95% CrI: 1.59, 4.07); AF, 67.85 (95% CrI: 14.25, 168.68); hydrops, 6.03 (95% CrI: 2.54, 10.68); and non-hydrops, 5.06 (95% CrI: 1.87, 9.88). DF and F had a similar effect on control of fetal tachycardias. No significant differences were observed when comparing S, DS with D therapies across the subgroup analyses for the SVT, hydrops, and non-hydrops groups. No significant differences in mortality risks were among the various treatment regimens for the total group. And no significant differences were found in rates of intrauterine death rates at the same cardioversion amount.

Conclusion The flecainide monotherapy and combination of digoxin and flecainide should be considered the most superior therapeutic strategies for fetal tachycardia.

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Keywords: fetal tachycardia, first-line therapy, digoxin, flecainide, network meta-analysis

INTRODUCTION

Fetal arrhythmias refer to abnormalities of either the heart rate or rhythm observed in a fetus, which can be further categorized into tachycardias and bradycardias (Carvalho, 2019). Tachycardias are potentially more life-threatening than bradycardias, with an overall mortality rate of 8%–9%, and can progress to ventricular dysfunction or fetal heart failure, leading to intrauterine death. Fetal tachycardias are often misdiagnosed or unnoticed until the observation of hydrops, which may cause serious consequences such as lifetime neurological complications and deaths (Carvalho, 2019). Fetal hydrops is associated with a 35% intrauterine mortality rate, despite treatment, compared with a 0%–4% mortality rate in non-hydrops fetuses (Simpson, 2006).

The most common types of fetal tachycardias are supraventricular tachycardia (SVT) and atrial flutter (AF). SVT accounts for 66% of all fetal tachycardia cases (Crosson and Scheel, 1996), whereas AF has been reported in 25%–30% of cases (Krapp et al., 2003). Some types of tachycardias are rarely observed, including atrial ectopic tachycardia (AET) and permanent junctional reciprocating tachycardia (PJRT). In most fetuses with tachycardia, critical cardiac malformations are also considered to be significantly associated with tachycardia onset; however, congenital heart diseases (CHDs) represent major comorbidities.

Echocardiography and fetal cardiac magnetic resonance imaging (MRI) are the predominant methods used to diagnose fetal tachycardias. The optimal and timely administration of transplacental medications is necessary to regulate arrhythmias, which can develop into fetal hydrops, cardiomegaly, atrioventricular valve regurgitation, or intrauterine death (Nii et al., 2006; Carvalho et al., 2007). Patients should receive first-line treatments as soon as arrhythmias are detected or proceed to further treatments if necessary. Transplacental anti-arrhythmic therapy for fetal tachycardia was first reported in 1980 (Kerenyi et al., 1980). Since then, a series of treatment protocols have been described for fetal tachycardia, including the use of digoxin, flecainide, sotalol, amiodarone, verapamil, and propafenone. Digoxin is the most widely used first-line medication for controlling the heart rate *in utero*. Although several types of medication have been described for use as first-line treatment, well-designed multi-group comparison studies are difficult due to the limited cases of fetal tachycardiac patients. Several individual cohorts have reported on the efficacy and safety of currently available first-line treatments; therefore, a network meta-analysis can be conducted to demonstrate the differences across the various reported therapeutic strategies. Previously, two individual meta-analyses have investigated the efficacy of first-line treatments for fetal tachycardias (Alsaied et al., 2017; Hill et al., 2017). However, these studies only made pairwise comparisons among several therapeutic strategies using a typical meta-analysis approach, which may be associated with the potential existence of type II statistical errors. Moreover, recent European Society of Cardiology (ESC) guidelines indicated the need for future follow-up studies to establish specific and effective treatment protocols with minimal risks (Brugada et al., 2020). A rich body of retrospective studies that examine this issue is available, and a network meta-analysis can be applied to evaluate the efficacy and safety of various proposed first-line treatment regimens for fetal tachycardias.

METHODS

Study Protocol

This analysis was conducted in accordance with a predetermined protocol, following the recommendations of a guideline for the reporting of systematic reviews of prognostic factor studies (Riley et al., 2019). The data collection and reporting were performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al., 2015) and the PRISMA Extension Statement for Reporting of Systemic Review Incorporating Network Meta-Analyses (Hutton et al., 2015).

Search Strategy

We searched the PubMed, Web of Science, Medline, Embase, and Cochrane Library databases to identify studies comparing and evaluating the effects of various drug regimens used to regulate fetal tachycardias from database inception to July 2021, with no limits on the study design, country of origin, tachycardia type, administration route (placental, intravenous, intramuscular or other administration routes), or whether combination treatment was applied. Additional studies for inclusion were identified from among the references of relevant reviews (Alsaied et al., 2017; Hill et al., 2017; Carvalho, 2019; Yuan, 2020; Veduta et al., 2021). The search strategy was established previously, and the terms included digoxin; flecainide; sotalol; amiodarone; tachycardia(s); fetus; supraventricular tachycardia or SVT; and atrial flutter or AF. The PubMed search strategy was described as [Anti-Arrhythmia Agents (MeSH Terms)] OR [Amiodarone (MeSH Terms)] OR [Digoxin (MeSH Terms)] OR [Sotalol (MeSH Terms)] OR [Flecainide (MeSH Terms)] AND [Tachycardia (MeSH Terms)] OR [Tachycardia, Supraventricular (MeSH Terms)] OR [Atrial Flutter (MeSH Terms)] OR [Hydrops (MeSH Terms)] AND (fetus).

Study Selection

Two authors (Jiangwei Qin and Zhengrong Deng) independently performed the literature search and performed data extraction using a standardized, pre-established form that distinguished the tachycardia types or whether hydrops existed. The full texts of the identified studies were independently assessed for inclusion by two other authors (Yifei Li and Yimin Hua), based on predetermined criteria. Each identified study was assessed for the following inclusion criteria: 1) the study population included fetuses treated with medication for sustained SVT or AF, with a

diagnosis of fetal tachycardia based on the results of echocardiography or cardiac MRI; 2) the interventions were defined as anti-arrhythmia drugs administrated to the fetuses using a transplacental approach, so comparisons were made among first-line regimens: digoxin monotherapy (D), digoxin and flecainide combination therapy (DF), flecainide monotherapy (F), digoxin and sotalol combination therapy (DS), and sotalol monotherapy (S) (some drugs such as verapamil and DFS combination were discarded because they were not sufficient to produce results). As for combination therapy, to ensure that the evaluation of the efficacy of the drug is put first in this meta-analysis, considering that many doctors would promptly add drugs when a single drug is ineffective, our definition of combination drugs would be described as: if a second drug was started before the third day of treatment, it was defined as combination therapy; 3) the cardioversion success rate was assessed as the primary outcome, which was defined as the reversion to a normal sinus rhythm during or after the administration of therapeutic drugs using a transplacental approach, without recurrence or relapse until birth; 4) and the use of an appropriate cohort study design. Cardioversions that were successfully controlled during the fetal stage were considered successful, even if tachycardia recurred postnatally. The intrauterine death ratio was as used as a secondary endpoint in the treatment arms, while postpartum deaths were not included. The exclusion criteria included 1) AET and PJRT, due to inadequate sample size; 2) failure to report the primary outcomes of interest; 3) the initial diagnosis occurring after 37 gestational weeks; 4) the study did not distinguish between specific types of fetal tachycardia among the enrolled cases.

Non-treatment and placebo treatments are clinically unfavorable for patients and are ethically disallowed for consistent fetal tachycardia; therefore, every regimen was used as the reference baseline for all network meta-analysis comparisons for the results of each set of two-by-two comparisons can be clearly described. In this analysis, we only evaluated the therapeutic efficacy of first-line transplacental medication administration on fetal tachycardia and assessed the associated safety of each treatment option. We also collected the publication year of the study, the dosage of treatment for existing CHD comorbidities, and the occurrence of both maternal and neonatal adverse events.

Study Quality Assessment

The risks of bias and article quality were assessed with the Newcastle–Ottawa Scale (NOS) (Wells et al., 2014) according to 3 aspects: Selection (4 stars), Comparability (2 stars), Outcome (3 stars). Publication bias was assessed using funnel plots in Stata, version 16.

Data Assessment and Statistical Analysis

Five therapeutic regimens were evaluated, including monotherapies and combination therapies, namely D monotherapy, DF combination therapy, F monotherapy, DS combination therapy, and S monotherapy, data of which were then fed into the model (see **Supplementary Materials**). The sotalol and flecainide combination therapy, amiodarone monotherapy, and verapamil monotherapy were excluded from the meta-analysis due to inadequate data availability. Five subgroups were established according to the type of tachycardia, including total (means all the included patients), SVT, AF, hydrops, and non-hydrops groups. We used a Bayesian hierarchical model to establish a network analysis framework and used Markov Chain Monte Carlo (MCMC) simulation procedures to generate posterior distributions by R package (gemtc) (Harrer et al., 2021).

In Bayesian statistics, we calculated credible intervals (Crl) rather than confidence intervals around our estimates, and a 95% credible interval indicating that 'there is a 95% probability that the true value of the parameter falls within our interval' rather than 'of every confidence interval calculated with each data set, 95% of the credible intervals (Crl) contain the true values of the parameters' in frequentist statistics. The Bayesian hierarchical model uses uninformative priors on the effect in every comparison which doesn't have a big impact on the posterior results, and the effects of a multi-arm study stem came from a multivariate (normal) distribution (Harrer et al., 2021). The model considered the replaceability of each treatment and used collected direct and indirect data based on uninformative priors, in order to obtain a relative accurate posterior result. The MCMC sampling allows to estimate the posterior distributions of our parameters, and thus generate the results of network meta-analysis (Harrer et al., 2021). Due to the inability to control for variables during observational studies, we did not assume that each study is an estimator of the same true effect size, but there are "study-specific" true effects estimated by each observed effect size and they are part of an overarching distribution of true effect sizes, and the variation between studies was estimated using a random-effects model.

A network plot was generated for the primary endpoint in each group using a random-effects model, using four Markov chains, 5,000 burn-in iterations, and 20,000 simulation iterations, where the parameters were considered enough for sensitive and well-converging network models, details on parameters could be accessed by command: *?mtc.run* of R package (gemtc) (Harrer et al., 2021). Convergence assessments were performed using Gelman–Rubin plots. This plot used the term Potential Scale Reduction Factor (PSRF) to compare the variation within each chain to the variation between chains, and how both develop over time. The PRSF would gradually shrink down with increasing numbers of iterations, and should at least be below 1.05 in the end for an enough converged model. We also evaluated the consistency of our network model using the node-split method. Using the network meta-analysis results, we calculated the probability of a treatment being the first, second, third, fourth, or fifth ranking treatment; presented the comparison in one cumulative probability plot; data of each set of two-by-two comparisons would be arranged in a table. We only represented forest plot by comparing with D monotherapy. The same approach was used to assess the secondary outcome of mortality across the whole population.

We integrated the safety and efficacy data in the total tachycardia population by introducing a metric we referred

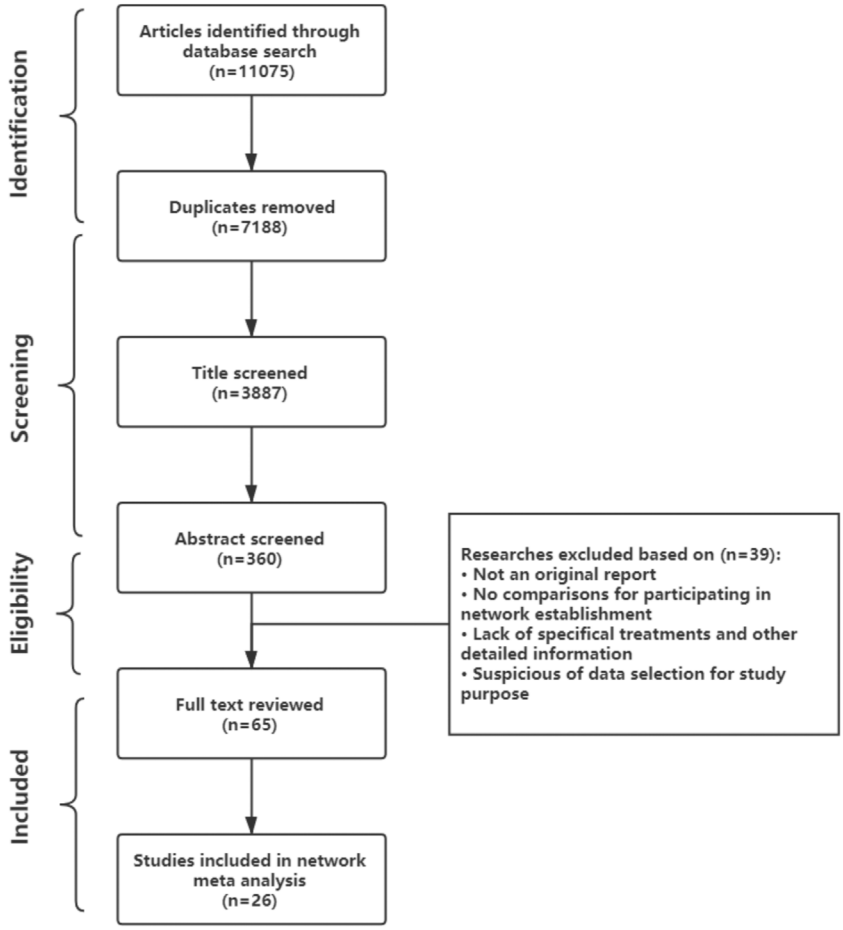

FIGURE 1 | Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart describing study selection.

to as the term *safety index*, which was calculated as the number of deaths divided by the number of successful cardioversions, to simultaneously assess the risks and benefits for each therapeutic regimen. It was derived from a pharmacologic concept——therapeutic index, which evaluates the safety of a drug and calculated as the median lethal dose divided by median effective dose. We hypothesized that a higher safety index value indicates reduced benefits of a regimen, because there were more death events with the same recovery events. Different with the comparison barely on death rate and cardioversion rate, safety index may complement the analysis of the data. For example:

-In study A, 20 of D group for first-line treatment and 10 of F, 12 of D for cardioversion and 7 of F, and 1 of D for death and 1 of F.
-In study B, also 20 of D group for first-line treatment and 10 of F, but 11 of D for cardioversion and 8 of F, and 4 of D for death and 2 of F.
-For cardioversion, the rate shows 23/40 of D < 15/20 of F, but for death, 5/40 of D < 2/20 of F, we may tell F is more efficiency but more unsecure.
-But as for safety index, 5/23 of D > 3/15 of F, we find that D would have more death events with the same recovery events. Two conclusions are in conflict in this example.

From our point of view, the difference may have relation with existence of the unrecovered but also alive population after first-line therapy, causing some data hidden behind. So safety index does provide another aspect for explaining the results.

The R (gemtc) package (van Valkenhoef et al., 2012) and Stata were used to perform statistical calculations for this network meta-analysis (Salanti, 2012; Shim et al., 2017; Shim et al., 2019; Harrer et al., 2021). We have put the R code and collected data in **Supplementary Materials** for convenience of replication.

RESULTS

Study Inclusion and Data Extraction

After the database search, 11,075 publications were identified, and 26 observational studies satisfied the established inclusion and exclusion criteria after screening and eligibility procedures were applied (shown in **Figure 1**). Any discrepancies were resolved

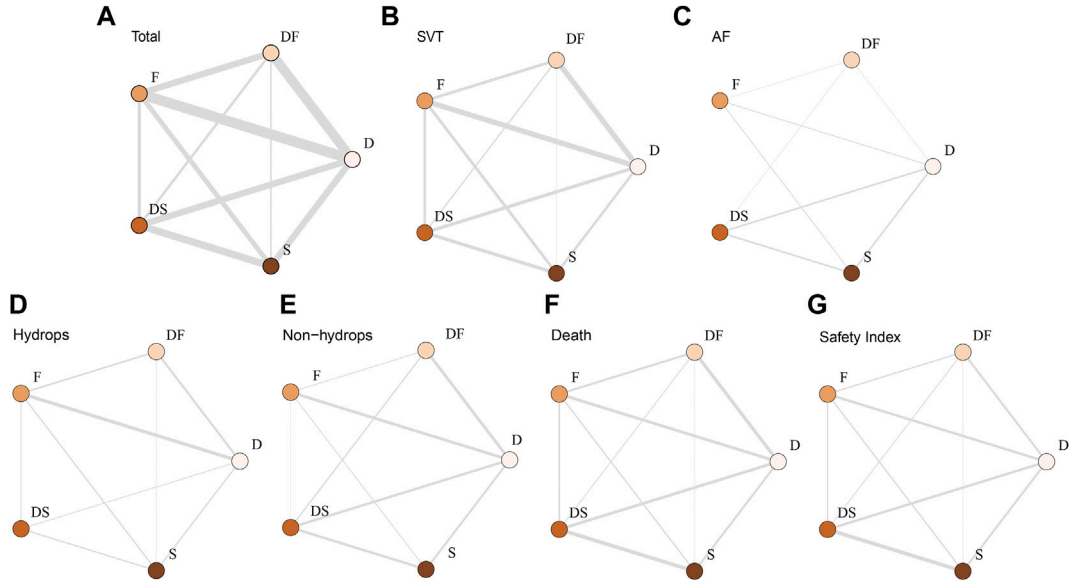

FIGURE 2 | Network plot of the enrolled analyses. The network plots represent comparison sizes between two regimens, with edge thickness representing the number of related studies. **(A)** Total group. **(B)** SVT group. **(C)** AF group (lacking any direct comparisons between F and DS). **(D)** Hydrops group (lacking any direct comparisons between DF and DS). **(E)** Non-hydrops group (lacking any direct comparisons between DF and DS). **(F)** Death rate in the total group. **(G)** Safety index in the total group. D, digoxin monotherapy; DF, digoxin and flecainide combination therapy; DS, digoxin and sotalol combination therapy; F, flecainide monotherapy; S, sotalol monotherapy; SVT, supraventricular tachycardia; AF atrial flutter.

between two reviewers by discussion until consensus was reached. Among the 26 included studies, one was a prospective study, and 25 were retrospective studies (van Engelen et al., 1994; Frohn-Mulder et al., 1995; Naumburg et al., 1997; Lisowski et al., 2000; Oudijk et al., 2000; Ebenroth et al., 2001; Jouannic et al., 2002; Krapp et al., 2002; Boldt et al., 2003; Jouannic et al., 2003; Oudijk et al., 2003; D'Alto et al., 2008; Pézard et al., 2008; Lulic Jurjevic et al., 2009; Hahurij et al., 2011; Shah et al., 2012; Uzun et al., 2012; van der Heijden et al., 2013; Ekman-Joelsson et al., 2015; Sridharan et al., 2016; Strizek et al., 2016; Ekiz et al., 2018; Karmegeraj et al., 2018; Miyoshi et al., 2019; O'Leary et al., 2020; Tunca Sahin et al., 2021). Only one randomized controlled trial (RCT; www.fasttherapytrial.com) (Edgar Jaeggi, MD, etc.) was retrieved during this search, but this RCT has not finished; therefore, it was treated as gray literature. Detailed reasons for the exclusion of identified articles are provided in **Figure 1**. The characteristics of the 26 included studies were summarized in **Supplementary Table S1**. 21 studies were conducted in Europe with 7 in Netherlands, and 3 studies were established in United States, 1 in Japan, 1 in India. Included gestational age at birth or when outcome recorded was ranged from 23 to 42 weeks but concentrated in 38 weeks. Quantitative data were organized into five groups according to treatment (D, DF, F, DS, and S). The maternal, neonatal and follow-up adverse events data were extracted regardless of the tachycardia type and specific regimens, because this information was not always reported in detail. The data set of extracted results that were used for statistical analyses are presented in the **Supplementary Materials**.

Combination Therapy of Digoxin and Flecainide Revealed Superiority for Cardioversion

For the primary outcomes, the data volumes and model stability were assessed for each of the five groups. The network plots represented the data size and the comparison size between two regimens, and the edge thickness corresponded to the numbers of studies included for comparisons. Total, SVT, and hydrops groups were found to form a complete network structure, with relationships identified across all treatment regimens; by contrast, some direct and indirect comparisons were lacking for the AF and non-hydrops groups, including F vs. DS and DF vs. S, suggesting that more attention should be paid when interpreting the results for these two groups (**Figures 2A–E**). Gelman–Rubin plots indicated that the shrink factor was stably maintained below 1.05 for all five groups (**Supplementary Figures S1–S5**), indicating enough converged models being applied. To assess inconsistencies, the node-split forest plots (**Supplementary Tables S2–S8**) revealed the effects of various comparisons when using only direct, only indirect, or all available evidence. Inconsistency was identified in all five groups due to one or more comparisons with $p < 0.05$, which indicated that the studies included systematically different populations. As shown in

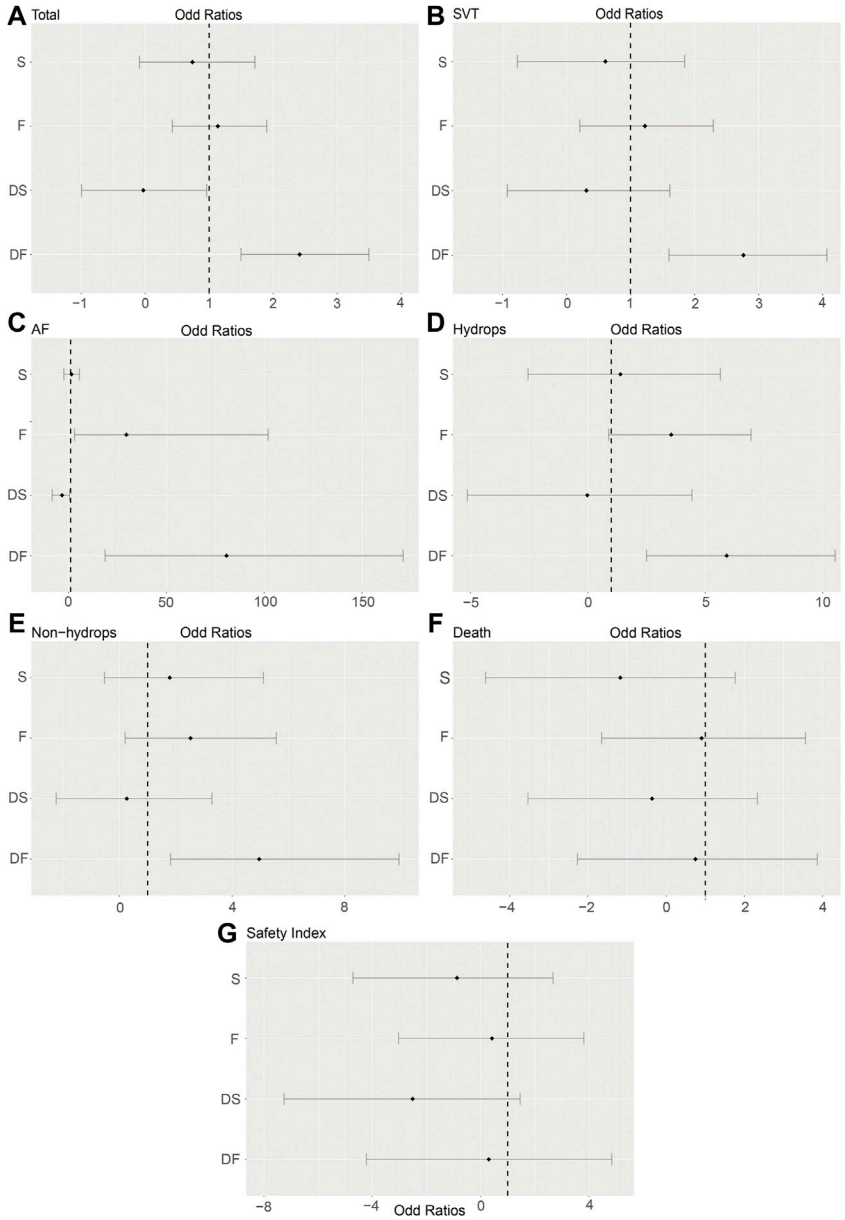

FIGURE 3 | Forest plots of the enrolled analyses. The forest plots showed odds ratios (ORs) and 95% credible intervals (95% CrI) comparing the effectiveness and risks among five regimens, using D as the baseline reference (OR = 1). **(A)** Total group. **(B)** SVT group. **(C)** AF group. **(D)** Hydrops group. **(E)** Non-hydrops group. **(F)** Death rate in the total group. **(G)** Safety index in the total group. D, digoxin monotherapy; DF, digoxin and flecainide combination therapy; DS, digoxin and sotalol combination therapy; F, flecainide monotherapy; S, sotalol monotherapy; SVT, supraventricular tachycardia; AF atrial flutter.

Figures 3A–E, **4A–E**, the top two best-ranked regimens were consistently identified as DF and F. The odds ratio (OR) and 95% credible interval (95% CrI) when compared with D as the reference therapy was significantly different across all groups for DF: Total, 2.44 (95% CrI: 1.59, 3.52); SVT, 2.77 (95% CrI: 1.59, 4.07); AF, 67.85 (95% CrI: 14.25, 168.68); hydrops, 6.03 (95% CrI: 2.54, 10.68); and non-hydrops, 5.06 (95% CrI: 1.87, 9.88). Other therapies showed significant effects for specific groups when compared with different regimens, details were shown in **Table 1**. The results indicated there wasn't one regimen having all statistically significant differences when comparing to the other therapies. Relatively, DF and F administrations demonstrated better efficacy for controlling fetal heart rate, regardless of the tachycardia type or the presence of hydrops, indicating the superiority of the two treatment protocols. DF demonstrated superiority on D in all five subgroups. In total group and AF group, DF had better effectiveness than DS. In AF group, superiority of F regimen could also be observed. Majority of comparisons showed no significant differences.

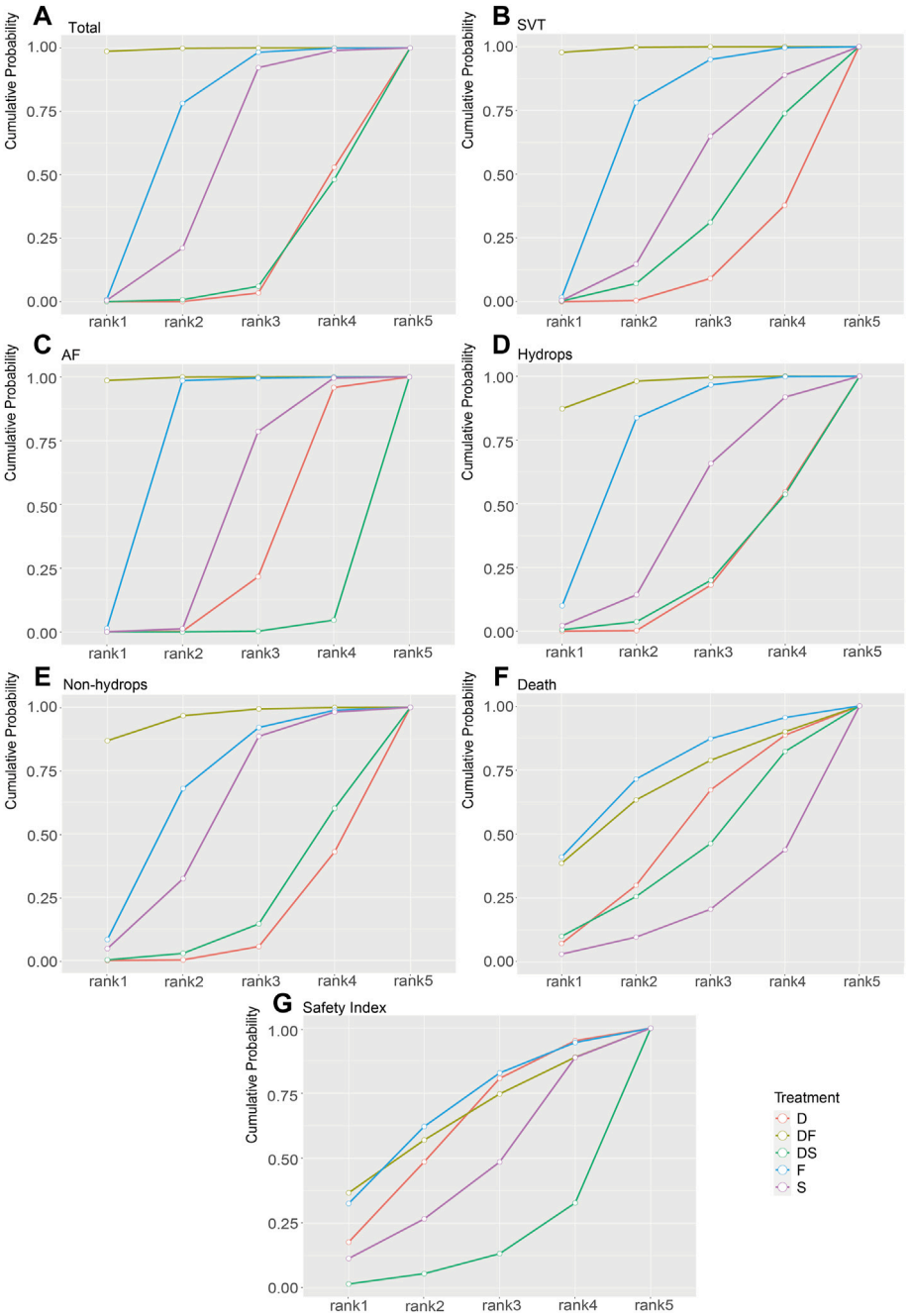

FIGURE 4 | Rank-line plots of the enrolled analyses. The cumulative probability of superiority is shown as a line chart, and the area under the curve (AUC) represents the rankings, with a larger AUC indicating a higher rank. **(A)** Total group. **(B)** SVT group. **(C)** AF group. **(D)** Hydrops group. **(E)** Non-hydrops group. **(F)** Death rate in the total group. **(G)** Safety index in the total group. D, digoxin monotherapy; DF, digoxin and flecainide combination therapy; DS, digoxin and sotalol combination therapy; F, flecainide monotherapy; S, sotalol monotherapy; SVT, supraventricular tachycardia; AF atrial flutter.

Intrauterine Death

To assess the secondary outcome of death rates, a network plot (**Figure 2F**), Gelman–Rubin plot (**Supplementary Figure S6**), and inconsistency test (**Supplementary Table S7**) were used to examine intrauterine death. The results demonstrated no significant differences, indicating no differences in intrauterine death outcomes across the examined treatment regimens (**Figure 3F** and **Table 2**). As shown in **Figure 4F**, the two treatments found to be the most efficacious, DF and F, were also the top-ranked regimens for the death rate assessment. Therefore, we attempted to further assess the benefits and risks of each treatment regimen by analyzing the safety index.

TABLE 1 | OR (95% CI) data from the total, SVT, AF, hydrops, and non-hydrops groups.

OR (95% CrI)-column vs. row	D	DF	F	DS	S
Total group					
D		#	#	0.02 (−0.99–1.01)	#
DF	2.44 (1.51–3.52)		1.29 (0.23–2.48)	2.47 (1.19–3.86)	1.68 (0.41–2.97)
F	1.15 (0.42–1.93)	#		1.17 (0.04–2.32)	0.39 (−0.71–1.38)
DS	#	#	#		#
S	0.76 (−0.1–1.76)	#	#	0.79 (−0.19–1.84)	
SVT group					
D		#	#	#	#
DF	2.77 (1.59–4.07)		1.53 (0.13–3.04)	2.46 (0.8–4.15)	2.17 (0.56–4.01)
F	1.22 (0.21–2.31)	#		0.92 (−0.57–2.38)	0.63 (−0.73–2.14)
DS	0.31 (−0.96–1.64)	#	#		#
S	0.61 (−0.78–1.84)	#	#	0.29 (−1.24–1.63)	
AF group					
D		#	#	3.4 (−0.51–8.4)	#
DF	67.85 (14.25–168.68)		32.39 (0.95–130.4)	71.54 (17.57–172.23)	66.32 (12.66–167.25)
F	26.75 (2.05–96.94)	#		30.36 (5.19–100.28)	25.31 (0.5–95.34)
DS	#	#	#		#
S	1.44 (−2.51–5.49)	#	#	4.87 (1.06–9.78)	
Hydrops group					
D		#	#	#	#
DF	6.03 (2.54–10.68)		2.33 (−1.45–6.87)	6.03 (0.83–12.98)	4.56 (−0.25–10.31)
F	3.64 (0.9–7.11)	#		3.63 (−0.88–9.52)	2.19 (−2.18–7.11)
DS	0.01 (−5.17–4.52)	#	#		#
S	1.43 (−2.58–5.75)	#	#	1.43 (−2.43–6.12)	
Non-hydrops group					
D		#	#	#	#
DF	5.06 (1.87–9.88)		2.51 (−1.24–7.35)	4.8 (0.89–9.86)	3.24 (−1–8.21)
F	2.53 (0.24–5.5)	#		2.29 (−1.11–5.92)	0.73 (−2.84–4.08)
DS	0.26 (−2.23–3.22)	#	#		#
S	1.79 (−0.48–5.03)	#	#	1.55 (−0.86–4.38)	

**To clearly observe and interpretate the results, we use '#' to replace the opposite and negative OR (95% CrI) in the cell that corresponds to another cell with a positive OR (95% CrI). For example, in Total group, (1, 2) or (D, DF) equal to 2.44 (1.51, 3.52) meant patients with DF (column) are 2.44 times more likely to obtain the reversion to a normal sinus rhythm than D (row), with a confidence interval at (1.51, 3.52). However, in network meta-analysis, (2, 1) or (DF, D) equal to -2.44 (−1.51, −3.52) exactly, so '#' was left to replace and avoid confusion. A red background emphasized statistically significant result.*

*All network meta-analysis comparisons are represented in table but only Digoxin as baseline in forest plot (**Figure 3**). As shown in **Table 1**, DF demonstrated superiority on D in all five subgroups. In total group and AF group, DF had better effectiveness than DS. In AF group, superiority of F regimen could also be observed. Majority of comparisons showed no significant differences.*

OR, odds ratio; CrI, credible interval; D, digoxin monotherapy; DF, digoxin and flecainide combination therapy; DS, digoxin and sotalol combination therapy; F, flecainide monotherapy; S, sotalol monotherapy; SVT, supraventricular tachycardia; AF, atrial flutter.

Safety Index

It was necessary to mention that only the data of cardioversion must be no zero to assess a safety index, studies with substandard data were removed, resulting in a loss of data volume. For example, data of Jouannic, J, M. et al. in **Supplementary Materials** as we provided online, the cardioversion for total were 0 in D and DS groups, so we had to remove both data for code running. **Figure 2G** and **Supplementary Figure S7** show acceptable results, but the inconsistency assessment (**Supplementary Table S8**) indicated an increase in the number of inconsistent comparisons, reducing the credibility of the cumulative probability plot. **Table 2** showed the absence of significant results, preventing the determination of absolute ranking among these five regimens (**Figures 3G**, **4G**). To summarize, the DF and F regimens demonstrated relatively superior efficacy for terminating fetal tachycardias, regardless of the tachycardia type or the presence of hydrops, with no significant difference in the associated risks such as death rate.

Quality Evaluation and Publication Bias

Supplementary Table S9 indicated that a low risk of bias was determined for all 26 studies included in this network meta-

TABLE 2 | OR (95% CI) data from the total group for the death rate and safety index according to treatment group.

OR (95% CrI)-column vs. row					
Death rate group					
	D	**DF**	**F**	**DS**	**S**
D		#	#	0.35 (–2.34, 3.45)	1.15 (–1.84, 4.56)
DF	0.77 (–2.25, 3.89)		2.35 (–1.31, 7.44)	1.15 (–2.57, 5.27)	1.96 (–2, 6.27)
F	0.9 (–1.63, 3.61)	0.11 (–3.22, 3.62)		1.27 (–1.96, 4.96)	2.07 (–1.38, 6.01)
DS	#	#	#		0.79 (–1.88, 3.49)
S	#	#	#	#	
Safety index group					
	D	**DF**	**F**	**DS**	**S**
D		#	#	2.53 (–1.35, 7.39)	0.89 (–2.6, 4.79)
DF	0.37 (–4.17, 4.84)		#	2.92 (–2.5, 9.09)	1.26 (–3.97, 6.76)
F	0.47 (–2.97, 3.84)	0.02 (–4.44, 4.99)		3 (–1.55, 8.46)	1.35 (–2.9, 5.95)
DS	#	#	#		#
S	#	#	#	1.66 (–1.71, 5.44)	

*All network meta-analysis comparisons are represented in table but only Digoxin as baseline in forest plot (**Figure 3**). As shown in **Table 2**, no significant difference existed in death rate group, but S appeared to be more safe though less efficacious. After considering both effectiveness and safety in the safety index group, no significant differences were observed in all five regimens.*

OR, odds ratio; CrI, credible interval; D, digoxin monotherapy; DF, digoxin and flecainide combination therapy; DS, digoxin and sotalol combination therapy; F, flecainide monotherapy; S, sotalol monotherapy; SVT, supraventricular tachycardia; AF, atrial flutter.

analysis (all studies obtained stars ≥7 (9 stars in total)) The publication bias of primary endpoints in the five groups is shown in **Supplementary Figure S8**, and no evident bias was observed for the total, SVT, hydrops, or safety index subgroups.

DISCUSSION

Oudijk et al. (2002) raised it very early that digoxin was the most common drug used but with effectiveness needing discussion, and flecainide was very effective in the control of fetal SVT based on their previous work and contribution. Jaeggi et al. (2011) established the response rate curve for digoxin monotherapy, sotalol monotherapy, and flecainide monotherapy over time, proposing associations between the fetal response to placental treatment and the fetal status, tachycardia type, and the type of anti-arrhythmia drug. Two meta-analyses reported by Hill et al. (2017) and Alsaied et al. (2017) both reached the same conclusion that flecainide monotherapy appears to be more effective as first-line therapy for fetal tachycardias than digoxin. In our results, after dividing the sample into four subgroups, the characteristics of the overall population were evaluated using network meta-analysis methods, which revealed that DF and F performed better than D, DS, and S in all five groups. In the AF group, significant differences were observed for multiple therapies. As secondary outcomes represented, no significance were observed among five regimens. Our results support the superiority of flecainide, particularly when combined with digoxin. As data showed, we suggested flecainide as the first-line regimen for balance between curative effect and side effects from monotherapy, digoxin should be promptly supplemented when flecainide monotherapy is ineffective to control fetal tachycardia as early as possible. Sotalol is considered for treatment alone or combined with digoxin when patients do not respond to above regimens. For AF group, DF and F come first, then S, finally DS and D.

We developed the safety index used in this study based on a pharmacology concept known as the therapeutic index, which is equal to the median lethal dose divided by the median effective dose. We borrowed the implication that a larger safety index value indicates reduced safety. Consequently, no differences were observed in results, which means there are similar amount of death events with the same amount of cardioversion events. However, due to the lack of detailed safety results provided by many of the studies included in this meta-analysis, inconsistencies were observed during the safety index analysis, suggesting that these outcomes should be considered with caution.

In addition, according to the **Supplementary Materials** on structural heart malformations we collected, in 26 inclusions, 3 studies didn't mention whether they had included fetuses with malformations, 4 studies mentioned but lacked of data, 10 studies clarified exclusion of structurally impaired patients, and 9 studies provided data for malformations. Generally, there could been identified that 34 patients with malformations included in our network meta-analysis, accounting for 4.25% of total amount of data (799 patients). Because most of the literatures did not provide a detailed description of the treatment groups to which each structurally impaired patients belonged, we could not exclude in the raw data of the meta-analysis and therefore inform here. Existence of cases of cardiac malformations may affect the results and adverse events associated with these regimens in newborns.

Other extracted data included maternal, neonatal and follow-up adverse events. Reporting of the maternal adverse events were less than that of neonatal and follow-up (**Supplementary Materials**). We found that, all mothers' safety were guaranteed with priority and most mothers could tolerate treatment. Apart from common side effects such as nausea, headache, or transient blurred visions, serious

events like cardiac electrophysiological abnormalities could be resolved after dose reduction or change of treatment, however, which could have an influence on the continuity of treatment of the fetus. Also of interest, it seemed maternal side effects were less in flecainide than digoxin. As dosage data we collected in **Supplementary Materials**, most researchers were strict of the dosage, and were flexible after receiving feedback on the effect and adverse reactions of medication. Although the dosage is importantly affecting the effect of fetal treatment, due to consideration on the tolerance of the mothers, we could not obtain clear relationship between the dosage and the efficacy. The neonatal and follow-up adverse events have been also collected: relapses of tachycardia were the most common events in alive infants with or without successful cardioversion, such as Wolff-Parkinson-White syndrome, permanent junctional reciprocating tachycardias, ectopic atrial tachycardia, etc. They were receiving antiarrhythmic drugs either for recurrence and prophylaxis, and most recovered or had been stably controlled. But for patients companied with severe hydrops or other complex complications, though receiving therapies such as drug regimen (furosemide, isoprenaline, dopamine, digoxin, propafenone, propranolol, verapamil or adenosine, etc.), electric cardioversion, radiofrequency catheter ablation or even surgical cardiac repair according to the individual condition, part of infants could not survive of severe heart failure or may suffer from central nervous system complications for a life-long time, but our meta-analysis only took intrauterine death into account to avoid mixing fetal therapy and neonatal regimens. Based on the textual descriptions of the neonatal situation and follow-up records collected in various articles, we found that over time, the number of treatments available to children in the postnatal period increased, adverse events other than the nervous system were mostly controlled, and the age for all-cause death was increasingly concentrated in the neonatal period or related with premature birth which may due to incomplete development of the fetus, while neonatal death patients often had obvious comorbidities. Children who passed the neonatal period seemed to have better outcomes. This suggested that we are in need of more experiences, on the one hand, to ensure that pregnant fetuses can safely survive the developmental period *in utero*, and the other, to take precautions against and control neonatal comorbidities.

The etiological implications of SVT and AF are commonly thought to be associated with abnormalities of the sinus node, the atrioventricular junction, or the ventricles. Atrioventricular reentrant tachycardia (AVRT) accounts for 90% of feta tachycardias (Jaeggi et al., 2011), originating from an accessory pathway between the atria and ventricles that creates an extra electrical circuit. Typically, AVRT has a characteristic ventricular-atrial (VA) interval, with an atrial-ventricular ratio <1, indicating a short VA tachycardia. Most hydrops is associated with AVRT, which could affect treatment. Digoxin cannot pass completely through the placenta; therefore, even when the maternal dose approaches toxic levels, the fetal drug level may not be sufficient; therefore, flecainide and sotalol are recommended for the treatment of hydrops. AF, however, frequently develops during late pregnancy (Jaeggi et al., 2011) due to premature atrial contraction (Wacker-Gussmann et al., 2016). In AF patients, atrial rates are commonly much faster (300–500 bpm) and twice the ventricular rates because of a physiological block at the atrioventricular node, but the degree of blockage varies. Moreover, AF can also coexist with AVRT in the same fetus (Jaeggi et al., 2011). Although hydrops is a significant lethality factor, the effects of hydrops on drug transformation and distribution and the changes in the electrophysiological characteristics of drug responses require more in-depth study. The primary effect of flecainide is the significant inhibition of sodium ion influx. When used in large doses, flecainide acts as a β-receptor blocker that can block calcium ion channels. Digoxin can inhibit the sodium–potassium pump and increase the frequency of sodium–calcium exchange. By reducing the autonomy of the sinus node, digoxin slows the heart rate. When digoxin and flecainide are combined, they simultaneously reduce the heart rate through mutually beneficial antagonistic effects. Sotalol blocks β1 receptors and reduces sympathetic excitability, with little direct influence on cardiomyocytes, which may explain its relative safety and lack of strong curative effects. However, this article was unable to infer any clear relationships between the observed results and the pharmacological effects of these drugs from a deeper perspective.

In clinical practice, depending on the emergency level after assessing the tachycardia type, hemodynamic consequences, fetal development, and maternal choice, doctors can choose no intervention, drug therapy, or delivery. However, pharmacological therapy and delivery are not mutually exclusive options. Treatment is recommended until the delivery of a full-term baby, if permitted, with a strong correlation observed between postnatal SVT and later gestational age at fetal SVT diagnosis (Hinkle et al., 2017). In our data of neonatal and follow-up adverse events, premature infants often related with worse outcomes. However, the presence of significant polyhydramnios may have to give birth for safety or mother (Carvalho, 2019).

Currently, network meta-analyses are developing methods to result in higher accuracy. Some researchers indicated that the precise classification of scientific questions in network meta-analyses would help identify specific statistical methods and more customized model options for addressing medical questions. For example, in a network meta-analysis of drug efficacy, if the dose data is collected, a dose–response network meta-analysis can be performed to obtain a more reliable interpretation. However, we did not discuss this relationship in detail because dosage details are often difficult to collect.

Future RCTs could continue to apply Bayesian hierarchical models and verify the outcomes of this article. The existing model can also be optimized to facilitate the combine the two research designs. Future RCT studies that provide individual patient data can be used for meta-regression analyses that evaluate the intervention effects according to more individualized data rather than average values.

Strengths and Limitations

The use of Bayesian model analysis can prevent the second-class errors that ordinary meta-analyses are prone to producing when assessing multiple comparisons. In addition, our model incorporates a larger samples size (799 patients in total group, subgroups data could be accessed in **Supplementary Materials**) than previous meta-analyses.

Our included samples were limited to cases of SVT or AF, and other rarer manifestations were not included. In addition, Inconsistency identified in all seven data set indicated that the studies included systematically different populations, which were likely due to various causes, including differences in the severity of

tachycardia, the gestation period, drug doses, and administration routes other than placental administration, such as muscular injection, umbilical vein injection, or oral administration, which were difficult to unify or control across these 26 observational studies. It should be noted that most of the samples in this paper have normal heart structure, so the conclusions in this paper are more suitable for children with normal heart structure. Whether the included retrospective studies applied similar practical treatment conditions was difficult to determine, and retrospective observation data is unable to meet randomization requirements. Prospective studies and ongoing RCT studies would provide more methodological control. However, Efthimiou et al. (2017) proposed that the inclusion of evidence from non-random studies (observational studies) can improve the accuracy of the results obtained from RCT-based network meta-analyses; therefore, this article can provide supplemental data for any follow-up studies. Due to the complexity of the Bayesian model, which requires similar distributions by default, the results may be influenced by non-similar distributions.

CONCLUSION

We conducted a network meta-analysis of first-line treatments used to manage fetal tachycardia using a Bayesian model. The superior effectiveness was remarkable for digoxin and flecainide combination therapy and flecainide monotherapy across all subgroup analyses (total, SVT, AF, hydrops, and non-hydrops). No significant differences in safety risks were identified among these therapies. Thus, the flecainide monotherapy and combination of digoxin and flecainide should be considered the most superior therapeutic strategies for fetal tachycardia. For details, it is suggested that flecainide acts as the first-line regimen for balance between curative effect and side effects from monotherapy, digoxin should be promptly supplemented when flecainide monotherapy is ineffective to control fetal tachycardia as early as possible. Sotalol is considered for treatment alone or combined with digoxin when patients do not respond to above regimens.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

YH and YL conceived the idea and the conception of the study. JQ and ZD contributed significantly to literature collection, data analysis, and draft of manuscript. CT and YZ helped perform the organization of research and the data analysis with constructive discussions. RH and JL contributed to the selection of studies, data extraction, and assessment of methodological quality. YH and YL supervised the project and contributed equally to the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.935455/full#supplementary-material

Supplementary Figure S1 | Gelman Runbin plot of Total group. Gelman Runbin plots represented the model fitting results between each comparison, it indicated active results if the line's shrink factor is stably below 1.05 as iterations increase. All Gelman Runbin plot (**Supplementary Figures S1–S7**) showed active results.

Supplementary Figure S2 | Gelman Runbin plot of SVT group.

Supplementary Figure S3 | Gelman Runbin plot of AF group.

Supplementary Figure S4 | Gelman Runbin plot of Hydrops group.

Supplementary Figure S5 | Gelman Runbin plot of Non-hydrops group.

Supplementary Figure S6 | Gelman Runbin plot of Death group.

Supplementary Figure S7 | Gelman Runbin plot of Safety Index group.

Supplementary Figure S8 | Funnel plots of enrolled analyses. The funnel plots represented the publication bias of results in 7 groups, no significant bias were found in Total, SVT, AF, Death and Safety Index groups. Hydrops and Non-hydrops groups may have risks of publication bias. **(A)** Total group; **(B)** SVT group; **(C)** AF group; **(D)** Hydrops group; **(E)** Non-hydrops group; **(F)** Death rate in Total group; **(G)** Safety Index in Total group. D, Digoxin; DF, Digoxin and Flecainide; DS, Digoxin and Sotalol; F, Flecainide; S, Sotalol.

REFERENCES

Alsaied, T., Baskar, S., Fares, M., Alahdab, F., Czosek, R. J., Murad, M. H., et al. (2017). First-Line Antiarrhythmic Transplacental Treatment for Fetal Tachyarrhythmia: A Systematic Review and Meta-Analysis. *J. Am. Heart Assoc.* 6, 1–9. doi:10.1161/JAHA.117.007164

Boldt, T., Eronen, M., and Andersson, S. (2003). Long-term Outcome in Fetuses with Cardiac Arrhythmias. *Obstet. Gynecol.* 102, 1372–1379. doi:10.1016/j.obstetgynecol.2003.08.019

Brugada, J., Katritsis, D. G., Arbelo, E., Arribas, F., Bax, J. J., Blomström-Lundqvist, C., et al. (2020). 2019 ESC Guidelines for the Management of Patients with Supraventricular tachycardiaThe Task Force for the Management of Patients with Supraventricular Tachycardia of the European Society of Cardiology (ESC). *Eur. Heart J.* 41, 655–720. doi:10.1093/eurheartj/ehz467

Carvalho, J. S. (2019). Fetal Dysrhythmias. *Best. Pract. Res. Clin. Obstet. Gynaecol.* 58, 28–41. doi:10.1016/j.bpobgyn.2019.01.002

Carvalho, J. S., Prefumo, F., Ciardelli, V., Sairam, S., Bhide, A., and Shinebourne, E. A. (2007). Evaluation of Fetal Arrhythmias from Simultaneous Pulsed Wave Doppler in Pulmonary Artery and Vein. *Heart* 93, 1448–1453. doi:10.1136/hrt.2006.101659

Crosson, J. E., and Scheel, J. N. (1996). Fetal Arrhythmias: Diagnosis and Current Recommendations for Therapy. *Prog. Pediatr. Cardiol.* 5, 141–147. doi:10.1016/1058-9813(95)00159-x

D'Alto, M., Russo, M. G., Paladini, D., Di Salvo, G., Romeo, E., Ricci, C., et al. (2008). The Challenge of Fetal Dysrhythmias: Echocardiographic Diagnosis

and Clinical Management. *J. Cardiovasc Med. Hagerst.* 9, 153–160. doi:10.2459/JCM.0b013e3281053bf1

Ebenroth, E. S., Cordes, T. M., and Darragh, R. K. (2001). Second-line Treatment of Fetal Supraventricular Tachycardia Using Flecainide Acetate. *Pediatr. Cardiol.* 22, 483–487. doi:10.1007/s002460010279

Efthimiou, O., Mavridis, D., Debray, T. P., Samara, M., Belger, M., Siontis, G. C., et al. (2017). Combining Randomized and Non-randomized Evidence in Network Meta-Analysis. *Stat. Med.* 36, 1210–1226. doi:10.1002/sim.7223

Ekiz, A., Kaya, B., Bornaun, H., Acar, D. K., Avci, M. E., Bestel, A., et al.(2018). Flecainide as First-Line Treatment for Fetal Supraventricular Tachycardia. *J. Matern. Fetal Neonatal Med.* 31, 407–412. doi:10.1080/14767058.2017.1286317

Ekman-Joelsson, B. M., Mellander, M., Lagnefeldt, L., and Sonesson, S. E. (2015). Foetal Tachyarrhythmia Treatment Remains Challenging Even if the Vast Majority of Cases Have a Favourable Outcome. *Acta Paediatr.* 104, 1090–1097. doi:10.1111/apa.13111

Frohn-Mulder, I. M., Stewart, P. A., Witsenburg, M., Den Hollander, N. S., Wladimiroff, J. W., and Hess, J. (1995). The Efficacy of Flecainide versus Digoxin in the Management of Fetal Supraventricular Tachycardia. *Prenat. Diagn* 15, 1297–1302. doi:10.1002/pd.1970151309

Hahurij, N. D., Blom, N. A., Lopriore, E., Aziz, M. I., Nagel, H. T., Rozendaal, L., et al. (2011). Perinatal Management and Long-Term Cardiac Outcome in Fetal Arrhythmia. *Early Hum. Dev.* 87, 83–87. doi:10.1016/j.earlhumdev.2010.11.001

Harrer, M., Cuijpers, P., Furukawa, T. A., and Ebert, D. D. (2021). *Doing Meta-Analysis With R: A Hands-On Guide*. Boca Raton, FL and London: Chapman & Hall/CRC Press.

Hill, G. D., Kovach, J. R., Saudek, D. E., Singh, A. K., Wehrheim, K., and Frommelt, M. A. (2017). Transplacental Treatment of Fetal Tachycardia: A Systematic Review and Meta-Analysis. *Prenat. Diagn* 37, 1076–1083. doi:10.1002/pd.5144

Hinkle, K. A., Peyvandi, S., Stiver, C., Killen, S. A. S., Weng, H. Y., Etheridge, S. P., et al. (2017). Postnatal Outcomes of Fetal Supraventricular Tachycardia: a Multicenter Study. *Pediatr. Cardiol.* 38, 1317–1323. doi:10.1007/s00246-017-1662-1

Hutton, B., Salanti, G., Caldwell, D. M., Chaimani, A., Schmid, C. H., Cameron, C., et al. (2015). The PRISMA Extension Statement for Reporting of Systematic Reviews Incorporating Network Meta-Analyses of Health Care Interventions: Checklist and Explanations. *Ann. Intern Med.* 162, 777–784. doi:10.7326/M14-2385

Jaeggi, E. T., Carvalho, J. S., De Groot, E., Api, O., Clur, S. A., Rammeloo, L., et al. (2011). Comparison of Transplacental Treatment of Fetal Supraventricular Tachyarrhythmias with Digoxin, Flecainide, and Sotalol: Results of a Nonrandomized Multicenter Study. *Circulation* 124, 1747–1754. doi:10.1161/CIRCULATIONAHA.111.026120

Jouannic, J. M., Delahaye, S., Fermont, L., Le Bidois, J., Villain, E., Dumez, Y., et al. (2003). Fetal Supraventricular Tachycardia: a Role for Amiodarone as Second-Line Therapy? *Prenat. Diagn* 23, 152–156. doi:10.1002/pd.542

Jouannic, J. M., Le Bidois, J., Fermont, L., Villain, E., Mahieu-Caputo, D., Dumez, Y., et al. (2002). Prenatal Ultrasound May Predict Fetal Response to Therapy in Non-hydropic Fetuses with Supraventricular Tachycardia. *Fetal Diagn Ther.* 17, 120–123. doi:10.1159/000048021

Karmegeraj, B., Namdeo, S., Sudhakar, A., Krishnan, V., Kunjukutty, R., and Vaidyanathan, B. (2018). Clinical Presentation, Management, and Postnatal Outcomes of Fetal Tachyarrhythmias: A 10-year Single-Center Experience. *Ann. Pediatr. Cardiol.* 11, 34–39. doi:10.4103/apc.APC_102_17

Kerenyi, T. D., Gleicher, N., Meller, J., Brown, E., Steinfeld, L., Chitkara, U., et al. (1980). Transplancental Cardioversion of Intrauterine Supraventricular Tachycardia with Digitalis. *Lancet* 2, 393–394. doi:10.1016/s0140-6736(80)90441-9

Krapp, M., Baschat, A. A., Gembruch, U., Geipel, A., and Germer, U. (2002). Flecainide in the Intrauterine Treatment of Fetal Supraventricular Tachycardia. *Ultrasound Obstet. Gynecol.* 19, 158–164. doi:10.1046/j.0960-7692.2001.00562.x

Krapp, M., Kohl, T., Simpson, J. M., Sharland, G. K., Katalinic, A., and Gembruch, U. (2003). Review of Diagnosis, Treatment, and Outcome of Fetal Atrial Flutter Compared with Supraventricular Tachycardia. *Heart* 89, 913–917. doi:10.1136/heart.89.8.913

Lisowski, L. A., Verheijen, P. M., Benatar, A. A., Soyeur, D. J., Stoutenbeek, P., Brenner, J. I., et al. (2000). Atrial Flutter in the Perinatal Age Group: Diagnosis, Management and Outcome. *J. Am. Coll. Cardiol.* 35, 771–777. doi:10.1016/s0735-1097(99)00589-6

Lulic Jurjevic, R., Podnar, T., and Vesel, S. (2009). Diagnosis, Clinical Features, Management, and Post-natal Follow-Up of Fetal Tachycardias. *Cardiol. Young* 19, 486–493. doi:10.1017/S1047951109990497

Miyoshi, T., Maeno, Y., Hamasaki, T., Inamura, N., Yasukochi, S., Kawataki, M., et al. (2019). Antenatal Therapy for Fetal Supraventricular Tachyarrhythmias: Multicenter Trial. *J. Am. Coll. Cardiol.* 74, 874–885. doi:10.1016/j.jacc.2019.06.024

Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., et al. (2015). Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 Statement. *Syst. Rev.* 4, 1. doi:10.1186/2046-4053-4-1

Naumburg, E., Riesenfeld, T., and Axelsson, O. (1997). Fetal Tachycardia: Intrauterine and Postnatal Course. *Fetal Diagn Ther.* 12, 205–209. doi:10.1159/000264469

Nii, M., Hamilton, R. M., Fenwick, L., Kingdom, J. C., Roman, K. S., and Jaeggi, E. T. (2006). Assessment of Fetal Atrioventricular Time Intervals by Tissue Doppler and Pulse Doppler Echocardiography: Normal Values and Correlation with Fetal Electrocardiography. *Heart* 92, 1831–1837. doi:10.1136/hrt.2006.093070

O'Leary, E. T., Alexander, M. E., Bezzerides, V. J., Drogosz, M., Economy, K. E., Friedman, K. G., et al. (2020). Low Mortality in Fetal Supraventricular Tachycardia: Outcomes in a 30-year Single-Institution Experience. *J. Cardiovasc Electrophysiol.* 31, 1105–1113. doi:10.1111/jce.14406

Oudijk, M. A., Michon, M. M., Kleinman, C. S., Kapusta, L., Stoutenbeek, P., Visser, G. H., et al. (2000). Sotalol in the Treatment of Fetal Dysrhythmias. *Circulation* 101, 2721–2726. doi:10.1161/01.cir.101.23.2721

Oudijk, M. A., Ruskamp, J. M., Ambachtsheer, B. E., Ververs, T. F., Stoutenbeek, P., Visser, G. H., et al. (2002). Drug Treatment of Fetal Tachycardias. *Paediatr. Drugs* 4, 49–63. doi:10.2165/00128072-200204010-00006

Oudijk, M. A., Ruskamp, J. M., Ververs, F. F., Ambachtsheer, E. B., Stoutenbeek, P., Visser, G. H., et al. (2003). Treatment of Fetal Tachycardia with Sotalol: Transplacental Pharmacokinetics and Pharmacodynamics. *J. Am. Coll. Cardiol.* 42, 765–770. doi:10.1016/s0735-1097(03)00779-4

Pézard, P. G., Boussion, F., Sentilhes, L., Lépinard, C., Couvreur, M. H., Victor, J., et al. (2008). Fetal Tachycardia: a Role for Amiodarone as First- or Second-Line Therapy? *Arch. Cardiovasc Dis.* 101, 619–627. doi:10.1016/j.acvd.2008.08.012

Riley, R. D., Moons, K. G. M., Snell, K. I. E., Ensor, J., Hooft, L., Altman, D. G., et al. (2019). A Guide to Systematic Review and Meta-Analysis of Prognostic Factor Studies. *BMJ* 364, k4597. doi:10.1136/bmj.k4597

Salanti, G. (2012). Indirect and Mixed-Treatment Comparison, Network, or Multiple-Treatments Meta-Analysis: Many Names, Many Benefits, Many Concerns for the Next Generation Evidence Synthesis Tool. *Res. Synth. Methods* 3, 80–97. doi:10.1002/jrsm.1037

Shah, A., Moon-Grady, A., Bhogal, N., Collins, K. K., Tacy, T., Brook, M., et al. (2012). Effectiveness of Sotalol as First-Line Therapy for Fetal Supraventricular Tachyarrhythmias. *Am. J. Cardiol.* 109, 1614–1618. doi:10.1016/j.amjcard.2012.01.388

Shim, S., Yoon, B. H., Shin, I. S., and Bae, J. M. (2017). Network Meta-Analysis: Application and Practice Using Stata. *Epidemiol. Health* 39, e2017047. doi:10.4178/epih.e2017047

Shim, S. R., Kim, S. J., Lee, J., and Rücker, G. (2019). Network Meta-Analysis: Application and Practice Using R Software. *Epidemiol. Health* 41, e2019013. doi:10.4178/epih.e2019013

Simpson, J. M. (2006). Fetal Arrhythmias. *Ultrasound Obstet. Gynecol.* 27, 599–606. doi:10.1002/uog.2819

Sridharan, S., Sullivan, I., Tomek, V., Wolfenden, J., Škovránek, J., Yates, R., et al. (2016). Flecainide versus Digoxin for Fetal Supraventricular Tachycardia: Comparison of Two Drug Treatment Protocols. *Heart rhythm.* 13, 1913–1919. doi:10.1016/j.hrthm.2016.03.023

Strizek, B., Berg, C., Gottschalk, I., Herberg, U., Geipel, A., and Gembruch, U. (2016). High-dose Flecainide Is the Most Effective Treatment of Fetal Supraventricular Tachycardia. *Heart rhythm.* 13, 1283–1288. doi:10.1016/j.hrthm.2016.01.029

Tunca Sahin, G., Lewis, M., and Uzun, O. (2021). Association of Fetal Atrial Flutter with Neonatal Atrioventricular Re-entry Tachycardia Involving Accessory Pathway: A Link to Be Remembered. *Pediatr. Cardiol.* 42, 849–856. doi:10.1007/s00246-021-02549-6

Uzun, O., Babaoglu, K., Sinha, A., Massias, S., and Beattie, B. (2012). Rapid Control of Foetal Supraventricular Tachycardia with Digoxin and Flecainide Combination Treatment. *Cardiol. Young* 22, 372–380. doi:10.1017/S1047951111001272

van der Heijden, L. B., Oudijk, M. A., Manten, G. T. R., ter Heide, H., Pistorius, L., and Freund, M. W. (2013). Sotalol as First-Line Treatment for Fetal Tachycardia and Neonatal Follow-Up. *Ultrasound Obstet. Gynecol.* 42, 285–293. doi:10.1002/uog.12390

van Engelen, A. D., Weijtens, O., Brenner, J. I., Kleinman, C. S., Copel, J. A., Stoutenbeek, P., et al. (1994). Management Outcome and Follow-Up of Fetal Tachycardia. *J. Am. Coll. Cardiol.* 24, 1371–1375. doi:10.1016/0735-1097(94)90122-8

van Valkenhoef, G., Lu, G., de Brock, B., Hillege, H., Ades, A. E., and Welton, N. J. (2012). Automating Network Meta-Analysis. *Res. Synth. Methods* 3, 285–299. doi:10.1002/jrsm.1054

Veduta, A., Panaitescu, A. M., Ciobanu, A. M., Neculcea, D., Popescu, M. R., Peltecu, G., et al. (2021). Treatment of Fetal Arrhythmias. *J. Clin. Med.* 10, 5-11–16-22. doi:10.3390/jcm10112510

Wacker-Gussmann, A., Strasburger, J. F., Srinivasan, S., Cuneo, B. F., Lutter, W., and Wakai, R. T. (2016). Fetal Atrial Flutter: Electrophysiology and Associations with Rhythms Involving an Accessory Pathway. *J. Am. Heart Assoc.* 5. doi:10.1161/JAHA.116.003673

Wells, G., Shea, B., and O'Connell, J. (2014). *The Newcastle-Ottawa Scale (NOS) for Assessing The Quality of Nonrandomised Studies in Meta-Analyses*, 7. Ottawa: Health Research Institute Web site.

Yuan, S. M. (2020). Fetal Arrhythmias: Diagnosis and Treatment. *J. Matern. Fetal Neonatal Med.* 33, 2671–2678. doi:10.1080/14767058.2018.1555804

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Association of LEPTIN and other inflammatory markers with preeclampsia: A systematic review

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Background: Preeclampsia is a serious pregnancy complication that affects 5%–10% of the obstetric population.

Objective: To study inflammatory markers associated with preeclampsia.

Search Strategy: Searches of articles on the topic published over a 10-year period (2009–2019) were performed in three databases (PubMed, Cochrane, and Embase) using the keywords preeclampsia and inflammatory markers. The PubMed search using 10 years and humans as filters retrieved 124 articles. Using an advanced search strategy, 0 articles were identified in Embase and 10 articles in Cochrane. After screening and eligibility assessment, 13 articles were included in the systematic review and meta-analysis. Meta-analysis and quality assessment of the studies were performed using the Review Manager 5.3 program.

Results: For meta-analysis, women with preeclampsia were compared to control women, i.e., pregnancies without arterial hypertension. Leptin levels were significantly higher ($p < 0.0002$) in women with preeclampsia compared to controls. Total cholesterol was also significantly elevated in women with preeclampsia ($p < 0.0001$). There was no significant difference in HDL between groups, but women with preeclampsia had significantly increased LDL ($p < 0.01$). The same was observed for triglycerides, which were significantly increased in women with preeclampsia ($p < 0.04$) compared to controls. Analysis of TNF-alpha, an important inflammatory marker, showed higher levels in women with preeclampsia ($p < 0.03$) compared to controls. The same was observed for another important inflammatory marker, interleukin 6, which was significantly increased in women with preeclampsia ($p < 0.0002$). There was a significant increase of C-reactive protein in women with preeclampsia ($p < 0.003$) compared to controls.

Conclusion: Women with preeclampsia have increased levels of inflammatory markers compared to control women.

KEYWORDS
preeclaimpsia, inflammatory, markers, C reaction protein, HDL

Introduction

Hypertensive disorders are one of the most common complications of pregnancy worldwide and account for about 20% of deaths of pregnant women in Latin America according to data from a study by the World Health Organization published in 2014. These disorders are serious conditions whose prevalence ranges from 3% to 9% (Brown et al., 2018). Among hypertensive disorders, preeclampsia is a matter of concern because of its impact on maternal and neonatal health. Preeclampsia is a leading cause of maternal and perinatal morbidity and mortality, affecting approximately 5% of all pregnancies worldwide (Jeyabalan, 2013; Brown et al., 2018; Wang et al., 2021).

Preeclampsia is a disorder of pregnant women that occurs after 20 weeks of gestation, although it can present as late as 4–6 weeks postpartum. The clinical manifestations include hypertension and proteinuria in 24 h > 0.3 g/L, with or without edema, but the disease may even affect all organ systems (Rana et al., 2019).

Increased maternal inflammatory status and oxidative stress associated with excess adipose tissue are considered the main biological triggers of abnormal early placentation among obese subjects. Placental defects can lead to increased resistance in the maternal-fetal circulation, triggering the development of preeclampsia. (Mayrink et al., 2018).

Adiponectin and leptin are adipokines, hormones produced mainly by adipose tissue, that are responsible for the regulation of lipid metabolism, placental angiogenesis, insulin sensitivity, inflammatory processes, and trophoblast invasion (Thagaard et al., 2019a; Kaze et al., 2021; Bhat et al., 2022). Adipokines appear to be involved in the complex mechanisms of early pregnancy and implantation and may therefore play a potential role in the development of preeclampsia. In early pregnancy, maternal leptin concentration correlates strongly with pre-pregnancy body mass index. Subsequently, placental production of leptin contributes to the increase in maternal leptin concentration during pregnancy (Thagaard et al., 2019a; Kaze et al., 2021; Bhat et al., 2022). Beneventi et al., 2020 investigated maternal and fetal plasma levels of leptin in pregnancies complicated by obesity and preeclampsia. The authors found that pregnant women with obesity had higher serum leptin levels than normal-weight subjects with and without hypertension or normotensive subjects with obesity (Larsen et al., 2019; Beneventi et al., 2020; Abraham and Romani, 2022).

From a practical and clinical point of view, maternal serum adiponectin and leptin can be used as markers to identify women with a predisposition to developing hypertension during pregnancy and thus can permit early detection. The importance of further studies on these adipokines lies in the fact that these proteins may be useful in the future not only as metabolic predictors but also for the prevention of arterial hypertension, diabetes, and diabetes. Atherogenesis as has been shown experimentally (Farkhondeh et al., 2020; Kim et al., 2022).

The objective of this study was to investigate inflammatory markers such as adiponectin and leptin associated with preeclampsia.

Methods

The search strategy followed the recommendations of Berstock et al., 2019 (Berstock and Whitehouse, 2019). Articles published from January 2009 to November 2019 in PubMed, Embase, and Cochrane were eligible. First, we selected keywords from related articles. Medical Subject Headings (MeSH) were then used to find more related keywords with similar meanings: ("inflammatory markers" [MeSH Terms] OR ("preeclampsia" [All Fields] AND ("inflammatory markers and preeclampsia") [MeSH Terms] [All Fields]. Searches were performed in the three databases. PubMed searches using 10 years and humans as filters resulted in 124 potential articles. Searching only the title in Embase resulted in 0 articles and the Cochrane database search retrieved 10 articles, all involving humans. After a first selection, 84 articles were not selected because they were unrelated to the topic of the systematic review, 3 articles involved animals, 4 articles were reviews, and 4 articles were duplicates. Thirty-five articles remained for abstract reading; of these, 22 were also unrelated to the topic of the systematic review and were excluded. Finally, 13 articles were included in the systematic review and meta-analysis (Figure 1). This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines following the PICO framework (P = patients, I = intervention, C = comparator group, and O = outcome) (Eriksen and Frandsen, 2018).

Two researchers with experience in compelling systematic reviews (E.C.V. and _KBS_) independently and blindly retrieved the articles and evaluated the titles and abstracts following the inclusion and exclusion criteria according to the PICO components (Eriksen and Frandsen, 2018). The selected articles were then critically evaluated for inclusion in the review or exclusion. Disagreements regarding the selection of studies were resolved by a third reviewer (HK).

The following data were extracted from the studies selected for the systematic review and entered into a table: author names and year of publication, study design, the definition of preeclampsia (systolic blood pressure, diastolic

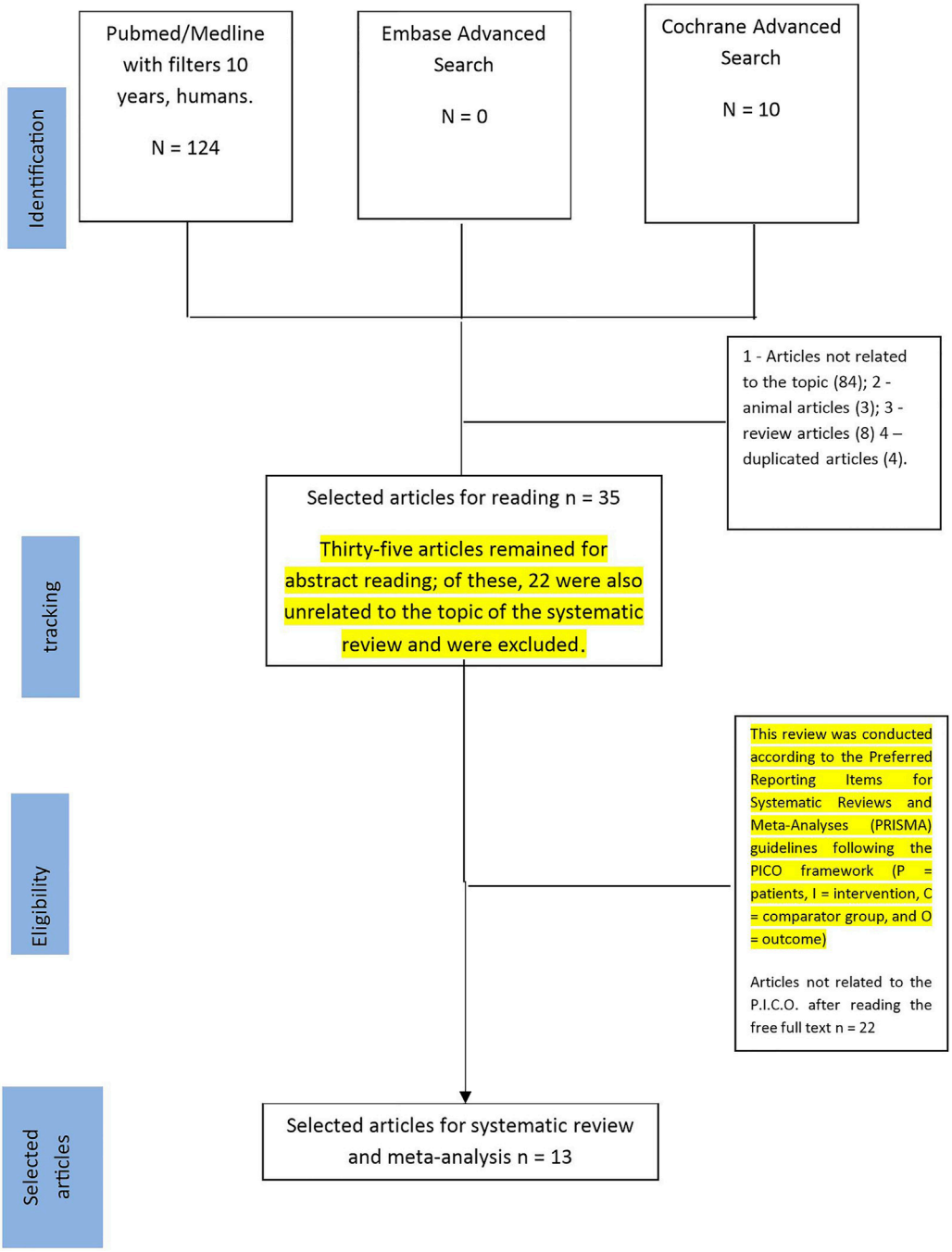


FIGURE 1
Flowchart for selection of studies.

blood pressure, and/or diabetes), number of participants in the study, maternal age (years), inflammatory markers study and outcome (Table 1). The RevMan version 5.3 program (Cochrane Collaboration, Oxford, United Kingdom) was used for meta-analysis. A random-effect model was used to estimate heterogeneity.

Statistical analysis

In the statistics of the preeclampsia groups, the means, standard deviations and the total number of women were described, and in the control groups also each author. And also the mean difference, randomized with a confidence interval of 95%.Meta-analysis was

TABLE 1 Characteristics of studies on women with preeclampsia.

Author, publication year	Country	Study design	Definition of PE: SBP/DBP or diabetes	No of participants in the study	Maternal age (years)	Inflammatory markers studied	Outcome
Valencia-Ortega et al., 2018	Mexico	Cross-sectional study	142/87 mmHg	50 PE/50 control	28.5 PE/ 28 control	TNF-α, IL-6, IL-8, IL-10, IL-1RA, ICAM-1, VCAM-1	PE is associated with a pro-inflammatory placental state
Mouse et al., 2017	Australia	Randomized controlled trial	Overweight type 1 or 2 diabetes	102	31.9	Triglycerides, total cholesterol, HDL, LDL, vitamin D, adiponectin, IL-6, MCP1	Vitamin D in obese pregnant women is associated with an increase in cardiovascular risk during pregnancy and this association is mediated by adiponectin
Kharb et al., 2017	India	Cross-sectional study	140/90 mmHg	50	—	Triglycerides, total cholesterol, HDL, LDL, leptin, IGF-1	Alterations in biochemical markers of growth and obesity occur in mothers and fetuses and modifications in the uterine environment can contribute to prevent future cardiovascular risk
Perichart-Pereira et al., 2017	Mexico	Prospective cohort	140/90 mmHg	177	27	Insulin, total cholesterol, HDL, LDL, triglycerides, IL-1β, leptin, adiponectin	Maternal weight status affected the concentrations of insulin, leptin, adiponectin, triglycerides and C-reactive protein throughout pregnancy
Gauster et al., 2017	Austria	Cross-sectional study	diabetes	17	31	TNF-α, HSP70, HO1	Diabetes increases placental cellular stress in the first trimester
Bashir et al., 2017	Saudi Arabia/ Egypt	Cross-sectional study	140/90 mmHg	158	27	Leptin, TNF-α, SOD, NO, IL-6	The combination of PE and high altitude residence resulted in significantly elevated maternal serum leptin
Ferguson et al., 2016	United States	Prospective birth cohort	140/90 mmHg	441	20–40	C-reactive protein, IL-1β, IL-6, IL-10, TNF-α	Demonstration of significant associations between biomarkers of inflammation and oxidative stress and PE
Estensen et al., 2015	Norway	Longitudinal study	140/90 mmHg	95	32	STNFR1, sVCAM.	Preeclamptic pregnancies are characterized by increased circulating levels of systemic and vascular inflammatory markers
Udenze et al., 2015	Nigeria	Case-control study	160/110 mmHg	100	32	IL-6, CRP, TNF-α	The inflammatory cytokines IL-6, TNF-α and C-reactive protein are elevated in severe PE

(Continued on following page)

TABLE 1 (*Continued*) Characteristics of studies on women with preeclampsia.

Author, publication year	Country	Study design	Definition of PE: SBP/DBP or diabetes	No of participants in the study	Maternal age (years)	Inflammatory markers studied	Outcome
Drost et al., 2014	The Netherlands	Retrospective cohort	130/90 mmHg	671	39	Adiponectin, Leptin, sVCAM.	The authors demonstrated an independent association of preeclampsia with SE-selectin and PAPPA, which may contribute to future cardiovascular events in women post-PE
Du et al., 2013	United States	Cross-sectional study	PE with diabetes	66	30	C-reactive protein, IL-1ra	In pregnant women with diabetes, elevated C-reactive protein and IL-1ra were associated with subsequent PE
Babu et al., 2012	India	Case-control study	140/90 mmHg	90	23	C-reactive protein	Oxidative stress and the inflammatory response are greater in women with PE compared to pregnant women with gestational hypertension
Can et al., 2011	Turkey	Cross-sectional study	140/90 mmHg	104	30	C-reactive protein	The results confirm that inflammatory reactions are closely associated with PE

PE, preeclampsia; SBP, systolic blood pressure; DBP, diastolic blood pressure.

carried out with the Review Manager 5.3 software program (Cochrane Collaboration, Oxford, United Kingdom) by comparing the means and standard deviations of the preeclampsia and the control groups. The random-effects model was used in the case of heterogeneity (DerSimonian and Kacker, 2007; von Hippel, 2015).

Results

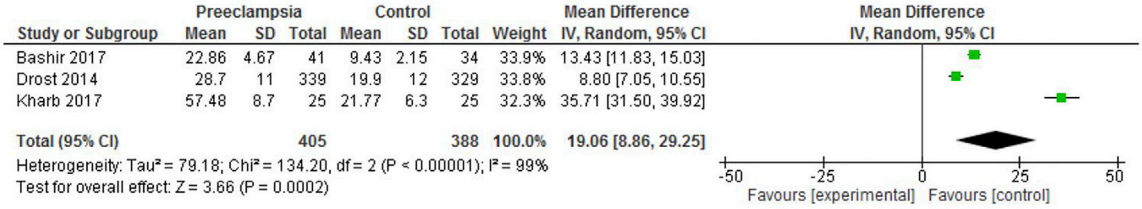
In the meta-analyses, we first evaluated leptin whose levels were significantly increased ($p < 0.0002$) in the group of women with preeclampsia compared to the control group (Figure 2A). Total cholesterol was also elevated in the group with preeclampsia ($p < 0.0001$) compared to the control group (Figure 2B). There was no difference in HDL between groups ($p = 0.66$), probably because of the high standard deviation in one of the articles analyzed (Figure 2C); however, LDL was significantly increased in women with preeclampsia ($p < 0.01$) compared to control (Figure 2D). There was also a difference in triglycerides ($p < 0.04$) between the experimental group and the control group (Figure 3A). When we analyzed other inflammatory markers such as tumor necrosis factor alpha (TNF-α), we observed significantly increased levels of this marker in the group of women with preeclampsia ($p < 0.03$) compared to women with normal pregnancies (Figure 3B). The same was observed for interleukin 6 (IL-6) (Figure 3C), with this marker being significantly increased in the group of women with preeclampsia ($p < 0.002$). There was a significant increase of C-reactive protein in women with preeclampsia ($p < 0.003$) compared to pregnant women without hypertension (Figure 3D).

The following results of the risk of bias assessment should be highlighted. All articles were classified as low risk of bias for selective reporting. When we analyzed incomplete outcome data and other bias in the 13 articles, 12 were rated as low risk of bias and only one as unclear risk of bias (Figure 4). Regarding random sequence generation, most articles (10 articles or 76.92%) were at low risk of bias and only 3 articles were at unclear risk of bias. When we analyzed blinding of participants and personnel and blinding of outcome assessment, slightly less than half of the articles (6 articles) were rated as low risk of bias and the remaining 7 articles as unclear risk of bias. Most articles had an unclear risk of bias only for allocation concealment, with only one article showing low risk of bias (Figure 5).

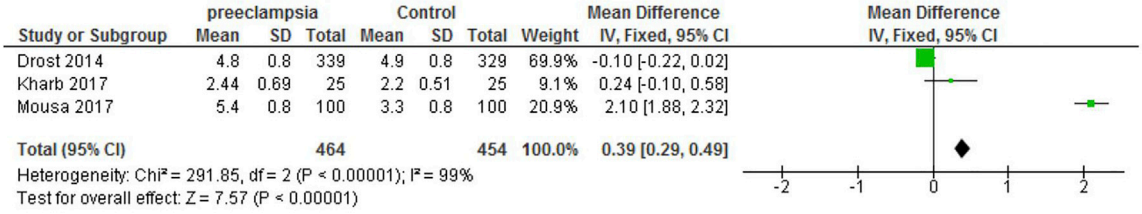
Discussion

The main finding of this study was that women with preeclampsia had increased levels of inflammatory markers

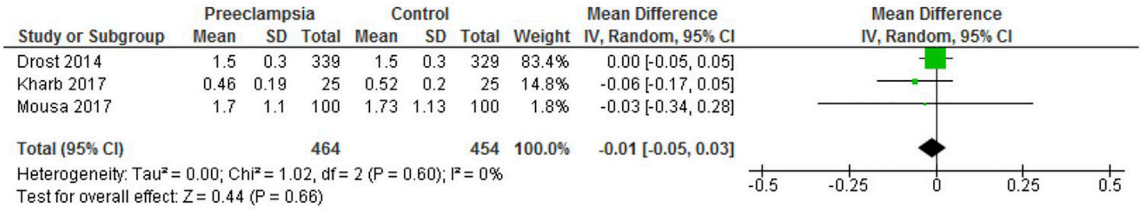
A Meta-analyses of Leptin (ng/mL) among preeclampsia (woman pregnant with preeclampsia) and control (woman pregnant without preeclampsia).



B Meta-analyses of Total cholesterol (mg/dL) among preeclampsia (woman pregnant with preeclampsia) and control (woman pregnant without preeclampsia).



C Meta-analyses of HDL (mg/dL) among preeclampsia (woman pregnant with preeclampsia) and control (woman pregnant without preeclampsia).



D Meta-analysis of LDL (mg/dL) among preeclampsia (woman pregnant with preeclampsia) and control (woman pregnant without preeclampsia).

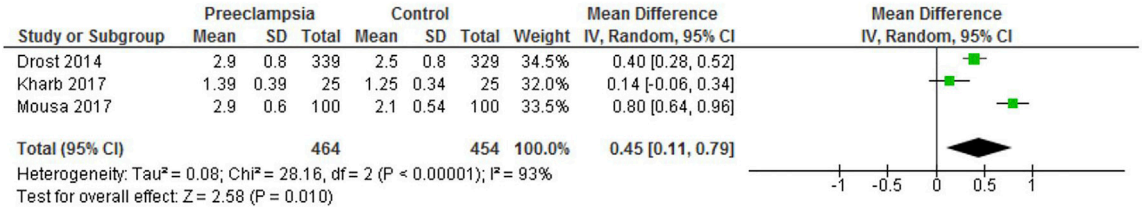
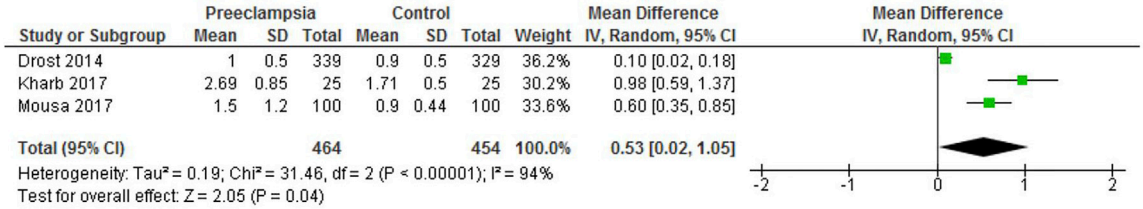
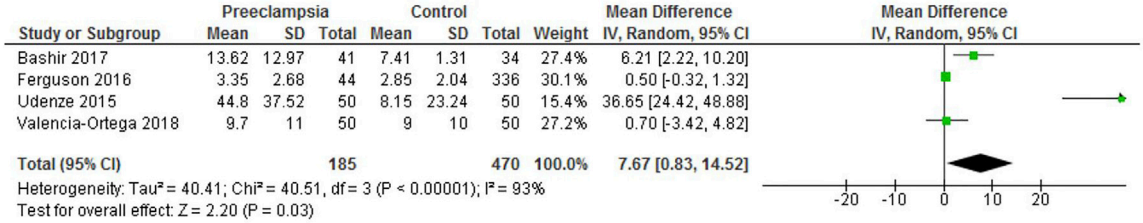


FIGURE 2
Meta-analyses of inflammatory markers among pregnant women with preeclampsia and control pregnant women (control), that is, without preeclampsia. **(A)**—Meta-analyses of Leptin (ng/ml) among preeclampsia (woman pregnant with preeclampsia) and control (woman pregnant without preeclampsia). **(B)**—Meta-analyses of Total cholesterol (mg/dl) among preeclampsia (woman pregnant with preeclampsia) and control (woman pregnant without preeclampsia). **(C)**—Meta-analyses of HDL (mg/dl) among preeclampsia (woman pregnant with preeclampsia) and control (woman pregnant without preeclampsia). **(D)**—Meta-analysis of LDL (mg/dl) among preeclampsia (woman pregnant with preeclampsia) and control (woman pregnant without preeclampsia).

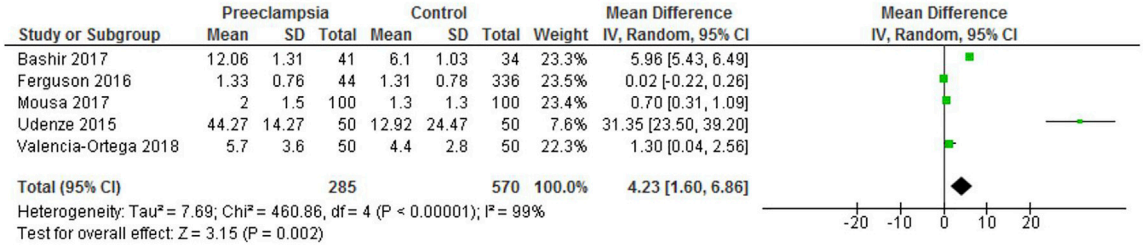
A Meta-analysis of Triglycerides (mg/dL) among preeclampsia (woman pregnant with preeclampsia) and control (woman pregnant without preeclampsia).



B Meta-analysis of TNFα (pg/mL) among preeclampsia (woman pregnant with preeclampsia) and control (woman pregnant without preeclampsia).



C Meta-analysis of IL6 (pg/mL) among preeclampsia (woman pregnant with preeclampsia) and control (woman pregnant without preeclampsia).



D Meta-analysis of C-reactive protein (mg/dL) among preeclampsia (woman pregnant with preeclampsia) and control (woman pregnant without preeclampsia).

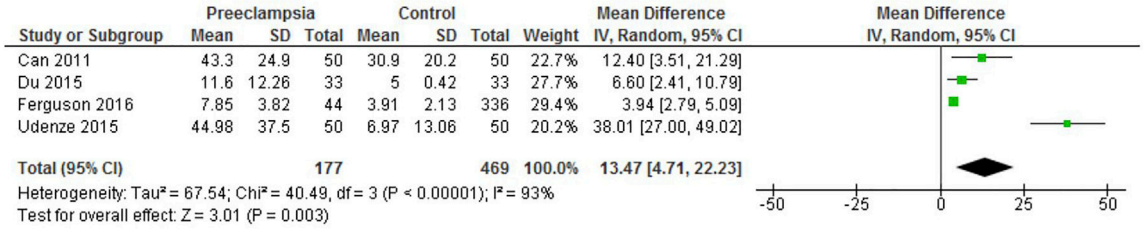


FIGURE 3
Meta-analyses of inflammatory markers among pregnant women with preeclampsia and control pregnant women (control), that is, without preeclampsia. **(A)**—Meta-analysis of Triglycerides (mg/dl) among preeclampsia (woman pregnant with preeclampsia) and control (woman pregnant without preeclampsia). **(B)**—Meta-analysis of TNFα (pg/ml) among preeclampsia (woman pregnant with preeclampsia) and control (woman pregnant without preeclampsia). **(C)**—Meta-analysis of IL6 (pg/ml) among preeclampsia (woman pregnant with preeclampsia) and control (woman pregnant without preeclampsia). **(D)**—Meta-analysis of C-reactive protein (mg/dl) among preeclampsia (woman pregnant with preeclampsia) and control (woman pregnant without preeclampsia).

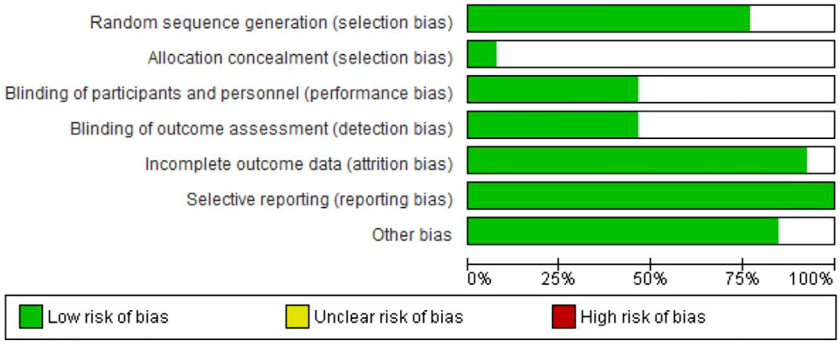


FIGURE 4
Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

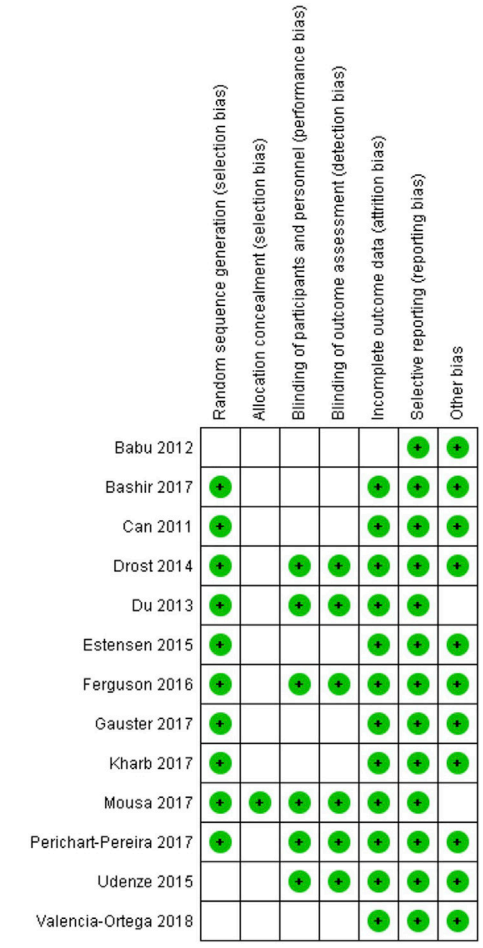


FIGURE 5
Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

compared to pregnant women without this condition. The inflammatory markers showing a significant difference were leptin, TNF-α, IL-6, and C-reactive protein.

Adiponectin is an anti-inflammatory factor, that is, associated with the physiopathology of preeclampsia. Several authors have studied the association between adiponectin and preeclampsia and found conflicting results. Most studies report an increase of adiponectin in the third trimester of gestation in patients with preeclampsia compared to control (Pérez-Pérez et al., 2020; Pheiffer et al., 2021; Lara-Barea et al., 2022), while others found the Other adipokines such as resistin, visfatin and vaspin in gestational diabetes and preeclampsia have been described but their physiological role has yet to be established and work has been more descriptive regarding these other adipokines (Miehle et al., 2012). In pregnant women plasma levels of resistin are elevated compared to non-pregnant women, however in gestational diabetes there are inconsistent data in the literature with some studies demonstrating elevation of resistin while others found decreased levels. Visfatin has anti-apoptotic properties and recombinant human visfatin treatment of human fetal membranes causes a significant increase in inflammatory cytokines, but its role in preeclampsia and gestational diabetes remains contradictory. In the case of vaspin, serum levels are not associated with markers of insulin resistance in pregnant patients. Recent studies associate resistin and visfatin as predictors of gestational diabetes mellitus and also that these adipolines are found in tissues such as adipose, subcutaneous adipose, placenta and cord blood (Bawah et al., 2019; Valencia-Ortega et al., 2022). Meta-analysis of the adiponectin results was not possible in our study because the selected articles did not report these results.

Leptin is an adipokine expressed in adipose tissue, that is, involved in energy expenditure and the modulation of insulin resistance (Thagaard et al., 2019b). This hormone is also produced by trophoblastic cells of the placenta (Beneventi et al., 2020). A recent study suggests that high preconceptual leptin levels may be a body mass index-independent risk factor for gestational diabetes mellitus and also a body mass index-dependent risk factor for hypertensive pregnant women (Peltokorpi et al., 2022).

Some studies measuring leptin in the second and third trimesters of gestation found an increase in leptin levels in pregnant women with preeclampsia compared to the control group (Thagaard et al., 2019b; de Knegt et al., 2021). Our meta-analysis supports these results since leptin was also found to be increased in patients with preeclampsia. Another study suggested that leptin may be a predictor in the obese population and elevated leptin levels have also been associated with cardiovascular disease (Nzelu et al., 2020; Stefańska et al., 2021).

The systematic review of Black et al., 2018 (Black and Horowitz, 2018) examined 73 studies published between 1998 and 2016 and revealed that some inflammatory markers such as IL-6, IL-8, TNF-α, and C-reactive protein may be useful for identifying women at risk of developing preeclampsia, in agreement with the results of our meta-analysis. Likewise, two other systematic reviews (30,31) also identified elevated levels of TNF-α, IL-6, and IL-10 in studies on preeclampsia.

With respect to IL-6, studies have demonstrated a significant increase of this cytokine in pregnant women with preeclampsia complications when compared to pregnant controls (Nzelu et al., 2020; Stefańska et al., 2021). Another studied used inflammatory marker is TNF-α. The results are contradictory, with most studies reporting an increase of this marker in preeclampsia, including the present study in which meta-analysis showed a significant difference in TNF-α levels compared to control. On the other hand, some studies did not find a significant difference between pregnant women with preeclampsia and pregnant controls (Lau et al., 2013; Black and Horowitz, 2018).

C-reactive protein is an acute-phase protein of inflammation and is frequently studied as a marker of preeclampsia. Our results also showed an increase in this protein, in agreement with the literature. In one systematic review, most studies (18 studies) showed elevated levels of C-reactive protein in women with preeclampsia compared to the control groups, while a minority of 10 studies found that C-reactive protein levels did not differ from controls (Black and Horowitz, 2018).

Abnormal lipid profiles have also been linked to both preeclampsia and impaired metabolic health in obese people. High levels of LDL have been observed in patients with preeclampsia in the third trimester of gestation compared to normotensive controls (Alahakoon et al., 2020). Our results revealed no significant difference in LDL between the preeclampsia and control groups. There was also no difference in HDL between the groups analyzed. Other studies found low HDL levels and high triglyceride levels in patients with preeclampsia when compared to controls (Dong et al., 2021; Zhou et al., 2021). In our study, triglyceride levels were higher in patients with preeclampsia than in the control group. Furthermore, high triglyceride and LDL levels and low HDL levels have been associated with impaired cardiovascular health (Soppert et al., 2020).

Future perspectives for this topic should include more clinical and experimental research on inflammatory markers in preeclampsia and gestational diabetes mellitus with a substantial number of women studied and further research is needed on these more common cardiovascular markers and others less common cardiovascular markers.

Conclusion

We concluded that some inflammatory markers in our meta-analysis such as leptin, total cholesterol, triglycerides, TNFα and C-reactive protein were increased in pregnant women with preeclampsia compared to pregnant control women, so inflammatory markers are important markers of preeclampsia.

Data availability statement

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author.

Author contributions

ECAV—Project Administration, Data Curation, Formal Analysis, Writing—Original Draft, Writing—Review and; Editing HAK—Original Draft, Writing—Review and; Editing KBS—Original Draft, Writing—Review and; Editing RCC—Project Administration, Data Curation, Formal Analysis, Writing—Original Draft, Writing—Review and; Editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abraham, T., and Romani, A. M. P. (2022). The relationship between obesity and pre-eclampsia: Incidental risks and identification of potential biomarkers for pre-eclampsia. *Cells* 11. doi:10.3390/cells11091548

Alahakoon, T. I., Medbury, H. J., Williams, H., and Lee, V. W. (2020). Lipid profiling in maternal and fetal circulations in preeclampsia and fetal growth restriction-A prospective case control observational study. *BMC Pregnancy Childbirth* 20, 61. doi:10.1186/s12884-020-2753-1

Bawah, A. T., Seini, M. M., Abaka-Yawason, A., Alidu, H., and Nanga, S. (2019). Leptin, resistin and visfatin as useful predictors of gestational diabetes mellitus. *Lipids Health Dis.* 18, 221. doi:10.1186/s12944-019-1169-2

Beneventi, F., Locatelli, E., de Amici, M., Cavagnoli, C., Bellingeri, C., de Maggio, I., et al. (2020). Maternal and fetal Leptin and interleukin 33 concentrations in pregnancy complicated by obesity and preeclampsia. *J. Maternal-Fetal Neonatal Med.* 33, 3942–3948. doi:10.1080/14767058.2019.1593359

Berstock, J. R., and Whitehouse, M. R. (2019). How to prepare and manage a systematic review and meta-analysis of clinical studies. *EFORT Open Rev.* 4, 213–220. doi:10.1302/2058-5241.4.180049

Bhat, A., Bhat, H., Bhat, J. A., Hussain Bhat, M., Rashid, M., Jan, R., et al. (2022). Leptin in obesity and hypertension. 26, 26–31. doi: doi:10.5603/AH.a2022.0003

Black, K. D., and Horowitz, J. A. (2018). Inflammatory markers and preeclampsia: A systematic review. *Nurs. Res.* 67, 242–251. doi:10.1097/NNR.0000000000000285

Brown, M. A., Magee, L. A., Kenny, L. C., Karumanchi, S. A., McCarthy, F. P., Saito, S., et al. (2018). The hypertensive disorders of pregnancy: ISSHP classification, diagnosis & management recommendations for international practice. *Pregnancy Hypertens.* 13, 291–310. doi:10.1016/j.preghy.2018.05.004

de Knegt, V. E., Hedley, P. L., Kanters, J. K., Thagaard, I. N., Krebs, L., Christiansen, M., et al. (2021). The role of leptin in fetal growth during pre-eclampsia. *Int. J. Mol. Sci.* 22, 4569. doi:10.3390/ijms22094569

DerSimonian, R., and Kacker, R. (2007). Random-effects model for meta-analysis of clinical trials: An update. *Contemp. Clin. Trials* 28, 105–114. doi:10.1016/j.cct.2006.04.004

Dong, J., Wang, M., Gao, J., Liu, J., and Chen, Y. (2021). Association between the levels of CGI-58 and lipoprotein lipase in the placenta of patients with preeclampsia. *Exp. Ther. Med.* 22, 1129. doi:10.3892/etm.2021.10563

Eriksen, M. B., and Frandsen, T. F. (2018). The impact of patient, intervention, comparison, outcome (pico) as a search strategy tool on literature search quality: A systematic review. *J. Med. Libr. Assoc.* 106, 420–431. doi:10.5195/jmla.2018.345

Farkhondeh, T., Llorens, S., Pourbagher-Shahri, A. M., Ashrafizadeh, M., Talebi, M., Shakibaei, M., et al. (2020). An overview of the role of adipokines in cardiometabolic diseases. *Molecules* 25, E5218. doi:10.3390/molecules25215218

Jeyabalan, A. (2013). Epidemiology of preeclampsia: Impact of obesity. *Nutr. Rev.* 71, S18–S25. doi:10.1111/nure.12055

Kaze, A. D., Musani, S. K., Bidulescu, A., Correa, A., Bertoni, A. G., Ahima, R. S., et al. (2021). Plasma leptin and blood pressure progression in blacks: The jackson heart study. *Hypertension* 77, 1069–1075. doi:10.1161/HYPERTENSIONAHA.120.16174

Kim, J. E., Kim, J. S., Jo, M. J., Cho, E., Ahn, S. Y., Kwon, Y. J., et al. (2022). The roles and associated mechanisms of adipokines in development of metabolic syndrome. *Molecules* 27, 334. doi:10.3390/molecules27020334

Lara-Barea, A., Sánchez-Lechuga, B., Campos-Caro, A., Córdoba-Doña, J. A., de la Varga-Martínez, R., Arroba, A. I., et al. (2022). Angiogenic imbalance and inflammatory biomarkers in the prediction of hypertension as well as obstetric and perinatal complications in women with gestational diabetes mellitus. *J. Clin. Med.* 11, 1514. doi:10.3390/jcm11061514

Larsen, J. B., Andersen, A. S., Hvas, C. L., Thiel, S., Lassen, M. R., Hvas, A. M., et al. (2019). Lectin pathway proteins of the complement system in normotensive pregnancy and pre-eclampsia. *Am. J. Reprod. Immunol.* 81, e13092. doi:10.1111/aji.13092

Lau, S. Y., Guild, S. J., Barrett, C. J., Chen, Q., Mccowan, L., Jordan, V., et al. (2013). Tumor necrosis factor-alpha, interleukin-6, and interleukin-10 levels are altered in preeclampsia: A systematic review and meta-analysis. *Am. J. Reprod. Immunol.* 70, 412–427. doi:10.1111/aji.12138

Mayrink, J., Costa, M. L., and Cecatti, J. G. (2018). Preeclampsia in 2018: Revisiting concepts, physiopathology, and prediction. *ScientificWorldJournal.* 2018, 6268276. doi:10.1155/2018/6268276

Miehle, K., Stepan, H., and Fasshauer, M. (2012). Leptin, adiponectin and other adipokines in gestational diabetes mellitus and pre-eclampsia. *Clin. Endocrinol.* 76, 2–11. doi:10.1111/j.1365-2265.2011.04234.x

Nzelu, D., Dumitrascu-Biris, D., Karampitsakos, T., Nicolaides, K. K., and Kametas, N. A. (2020). First trimester inflammatory mediators in women with chronic hypertension. *Acta Obstet. Gynecol. Scand.* 99, 1198–1205. doi:10.1111/aogs.13857

Peltokorpi, A., Irina, L., Liisa, V., and Risto, K. (2022). Preconceptual leptin levels in gestational diabetes and hypertensive pregnancy. *Hypertens. Pregnancy* 41, 70–77. doi:10.1080/10641955.2022.2033763

Pérez-Pérez, A., Vilariño-García, T., Guadix, P., Dueñas, J. L., and Sánchez-Margalet, V. (2020). Leptin and nutrition in gestational diabetes. *Nutrients* 12, E1970. doi:10.3390/nu12071970

Pheiffer, C., Dias, S., Jack, B., Malaza, N., and Adam, S. (2021). Adiponectin as a potential biomarker for pregnancy disorders. *Int. J. Mol. Sci.* 22, 1326. doi:10.3390/ijms22031326

Rana, S., Lemoine, E., Granger, J., and Karumanchi, S. A. (2019). Preeclampsia: Pathophysiology, challenges, and perspectives. *Circ. Res.* 124, 1094–1112. doi:10.1161/CIRCRESAHA.118.313276

Soppert, J., Lehrke, M., Marx, N., Jankowski, J., and Noels, H. (2020). Lipoproteins and lipids in cardiovascular disease: From mechanistic insights to therapeutic targeting. *Adv. Drug Deliv. Rev.* 159, 4–33. doi:10.1016/j.addr.2020.07.019

Stefańska, K., Zieliński, M., Jankowiak, M., Zamkowska, D., Sakowska, J., Adamski, P., et al. (2021). Cytokine imprint in preeclampsia. *Front. Immunol.* 12, 667841. doi:10.3389/fimmu.2021.667841

Thagaard, I. N., Hedley, P. L., Holm, J. C., Lange, T., Larsen, T., Krebs, L., et al. (2019a). Leptin and Adiponectin as markers for preeclampsia in obese pregnant women, a cohort study. *Pregnancy Hypertens.* 15, 78–83. doi:10.1016/j.preghy.2018.12.002

Thagaard, I. N., Hedley, P. L., Holm, J. C., Lange, T., Larsen, T., Krebs, L., et al. (2019b). Leptin and Adiponectin as markers for preeclampsia in obese pregnant women, a cohort study. *Pregnancy Hypertens.* 15, 78–83. doi:10.1016/j.preghy.2018.12.002

Valencia-Ortega, J., González-Reynoso, R., Ramos-Martínez, E. G., Ferreira-Hermosillo, A., Peña-Cano, M. I., Morales-Ávila, E., et al. (2022). New insights into adipokines in gestational diabetes mellitus. *Int. J. Mol. Sci.* 23, 6279. doi:10.3390/ijms23116279

von Hippel, P. T. (2015). The heterogeneity statistic I2 can be biased in small meta-analyses. *BMC Med. Res. Methodol.* 15, 35. doi:10.1186/s12874-015-0024-z

Wang, W., Xie, X., Yuan, T., Wang, Y., Zhao, F., Zhou, Z., et al. (2021). Epidemiological trends of maternal hypertensive disorders of pregnancy at the global, regional, and national levels: A population-based study. *BMC Pregnancy Childbirth* 21, 364. doi:10.1186/s12884-021-03809-2

Zhou, J., Bai, J., Guo, Y., Fu, L., and Xing, J. (2021). Higher levels of triglyceride, fatty acid translocase, and toll-like receptor 4 and lower level of HDL-C in pregnant women with GDM and their close correlation with neonatal weight. *Gynecol. Obstet. Invest.* 86, 48–54. doi:10.1159/000510032

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Mesenchymal stem cells-derived exosomes as a promising new approach for the treatment of infertility caused by polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a multifactorial metabolic and most common endocrine disorder that its prevalence, depending on different methods of evaluating PCOS traits, varies from 4% to 21%. Chronic low-grade inflammation and irregular apoptosis of granulosa cells play a crucial role in the pathogenesis of PCOS infertility. Mesenchymal stem cells (MSCs)-derived exosomes and extracellular vesicles (EVs) are lipid bilayer complexes that act as a means of intercellular transferring of proteins, lipids, DNA and different types of RNAs. It seems that this nanoparticles have therapeutic effects on the PCOS ovary such as regulating immunity response, anti-inflammatory (local and systemic) and suppress of granulosa cells (GCs) apoptosis. Although there are few studies demonstrating the effects of exosomes on PCOS and their exact mechanisms is still unknown, in the present study we reviewed the available studies of the functions of MSC-derived exosome, EVs and secretome on apoptosis of granulosa cells and inflammation in the ovary. Therefore, the novel cell-free therapeutic approaches for PCOS were suggested in this study.

KEYWORDS

mesenchymal stem cell, exosome, extracellular vesicle, apoptosis, chronic inflammation

Introduction

Polycystic ovary syndrome (PCOS) is a multifactorial metabolic disease and common endocrine condition that leads to increased production of androgens, decreased production of the estrogens and progesterone and consequences of infertility (Xu and Qiao, 2022). Common biochemical hallmarks of polycystic ovary syndrome are the

absence of ovulation with high levels of androgens, luteinizing hormone (LH), luteinizing hormone/follicle-stimulating hormone ratios while follicle-stimulating hormone (FSH) remains normal or low (Eid et al., 2005). The global prevalence of this disorder varies from 4% to 21% depending on different methods of evaluating PCOS traits and diagnostic criteria (Lizneva et al., 2016), which can be recognized as the most common cause of infertility or failed birth in recent years (Rotterdam, 2004; Lizneva et al., 2016). It seems the principal ovarian consequences of PCOS are growth arrest in the early antral follicles and abnormal folliculogenesis (Franks et al., 2008). Although the current treatments include various gonadotropin (Artini et al., 1996), clomiphene citrate (Legro et al., 2007) and metformin (Sam and Dunaif, 2003), but, it has been pointed out that each of these treatments have various advantages and disadvantages (Elnashar et al., 2006; Legro et al., 2007). Therefore, alternative and non-invasive treatments improving follicle growth, resumption of oocyte maturation and different leading factors of PCOS are needed. There is evidence that mesenchymal stem cells (MSCs) have anti-inflammatory, fibrogenesis inhibiting, antioxidant, and regenerative effects (Zhao et al., 2019). These roles can give to the MSCs a potential therapeutic application in various abnormalities such as the female reproductive disorders (Mutlu et al., 2015; He et al., 2018).

In addition to intercellular interactions such as autocrine, paracrine or endocrine signaling, recently, extracellular vesicles (EVs) as a new tool for intercellular communication has attracted the attention of researchers. Although, some researchers consider the secretion of EVs as a mechanism of the cell to dispose of useless molecules (Van der Pol et al., 2012). But using the extracellular vesicles, various active biomolecules including nucleic acids, proteins and lipids can be transferred from origin cells to target cells (György et al., 2011; Koniusz et al., 2016). Precise characterization of the EVs content has opened up their promising applications in diagnosis and therapy, as well as the development of innovative drug delivery systems (Barile and Vassalli, 2017). According to their biosynthesis mechanism and size, those can be divided in to microvesicles (50–3000 nm), exosomes (40–100 nm) and apoptotic bodies (800–5000 nm) (Yamamoto et al., 2016). Origin-based contents, genetic materials and ability to content shuttling to other cells make exosomes as an attractive research subject for manipulating the functions of different cells locally and/or remotely (Han et al., 2016). In various physiological and pathological processes including reproduction, gametogenesis, embryogenesis and differentiation, exosomes are secreted by most cell types into the extracellular environment and have been detected in various body fluids (Raposo and Stoorvogel, 2013; Machtinger et al., 2016) so that they act as a means of transferring proteins, lipids, DNA and diversity of RNA species between cells (Barile and Vassalli, 2017). The presence of EVs in reproductive bio-fluids such as follicular fluid and ovarian fluid shows their role in the intercellular communication necessary for the proper functioning of the reproductive system (Machtinger et al., 2016). Considering the positive role of MSC-derived exosomes, the goal of this study is to review the available reports on their role in treatments of the various reproductive processes and present a potential role of the exosome in *in vitro* maturation of oocyte and the improve of infertility in PCOS women.

Mesenchymal stem cells-derived exosomes

Growing evidence from a various experimental and clinical trials support the effectiveness of MSCs on treating different diseases such as renal fibrosis, cardiovascular disorders, neurological diseases and female reproductive disorders (Du and Taylor, 2009; Goradel et al., 2018; Liang et al., 2018; Liu et al., 2018; Sneddon et al., 2018); these cells can be harvested from the varieties of tissues including bone marrow, umbilical cord, adipose tissue, placental tissue, menstrual blood and dental pulp (Priester et al., 2020).

In spite of the therapeutic potential of MSCs, large-scale MSC expansion for clinical use is limited owing to the cells' capacity to divide in culture for a limited number of passages. Also, the cells could be associated with some challenges including difficulty of their transportation, transplant rejection and commercialization (Mendt et al., 2019). Therefore, in the recent decades, great efforts have been taken to find alternatives to reducing problems of MSCs usage while preserving their positive properties.

MSCs are a massive source for exosome production and are used in various research fields due to their greater availability and high proliferative ability (Cheng et al., 2017; Cheng et al., 2021). Exosomes, which are lipid bilayer nanoparticles that secrete into the microenvironment from various types of cells especially mesenchymal stem cells that offer promising therapeutic potential. In addition to other bioactive molecules that we have detailed in our previous study (Izadi et al., 2021), exosomes have various types of signaling molecules such as mRNA and miRNA (Valadi et al., 2007). Higher biological stability, easier storage, easier penetration into target tissues and low immunogenicity are some of the considerable advantages that make exosomes more useful compared to their source cells for medical applications (El Andaloussi et al., 2013; Zhang et al., 2016). Exosomes secreted from different cells have almost similar protein molecules with biological activities including immune modulation, regeneration, and tissue repair and angiogenesis promotion. Exhibiting the same activities in all MSC-derived exosomes may be related to the existence of a common protein signature (van Balkom et al., 2019). Additionally, some types of MSCs secrete exosomes with unique characteristics (Tang et al., 2021). Rising evidence suggests that MSCs-derived

exosomes have immunomodulation, anti-inflammatory (Urbanelli et al., 2015; Izadi et al., 2021) and anti-apoptosis effects (Fu et al., 2020; Wen et al., 2020), therapeutic potential of female reproductive disorders (Liao et al., 2021; Zohrabi et al., 2022).

Potential applications of MSCs-derived exosomes in PCOS patient

Many studies have shown that chronic low-grade, increase in pro-inflammatory cytokines, decrease of anti-inflammatory cytokines, insulin resistance, hypersensitivity of Helper T-cells (Th1); Th1-type immunity and the ratio of Th1 to Th2 cells, as well as Th1 cytokines such as IFN-γ and IL-2 are increased during immune reactions in PCOS patients (Qin et al., 2016) and hyperandrogenism play a crucial roles in PCOS pathogenesis (González et al., 2014a). Moreover, it has been reported that chronic inflammation in PCOS can lead to poor oocyte quality, ovarian dysfunction, disrupts oocyte development, and affect endometrial receptivity (Velez et al., 2021).

Few recent studies have reported that human umbilical cord mesenchymal stem cells (huMSCs) therapy can improve ovarian dysfunction by the systemic immunomodulation and local immune response in the ovary of PCOS patients (Xie et al., 2019). In a letrozole-induced PCOS mouse model, the beneficial effect of human bone marrow derived mesenchymal stem cells (BM-hMSCs)on the partial restoration of ovaries, the number of corpora lutea, and antral follicles has been reported (Chugh et al., 2021a). Notably, an increasing number of studies have discovered that the communication between MSCs and target tissue such as ovarian microenvironment is through the exosomes and secretome (Harrell et al., 2019; Xu et al., 2019). Also, another study reported that huMSCs--derived exosomes ameliorates the granulosa cells immune response through the inhibition of NF-κB signaling pathway in the PCOS (Zhao et al., 2022).

Recently, a study showed that MSCs-derived exosomes cause the decreased concentration of IL-1β and TNF-α, while the secretion of TGF-β increased in *in vitro* culture of mononuclear cells. Also, it demonstrated that MSCs-derived exosomes can increase Th2 (Th2-related anti-inflammatory cytokine such as IL-10 that is reduced in PCOS patients) and Treg and decrease Th1 (Chen et al., 2016). Apoptosis plays the key role in follicular atresia and cyclic growth and regression of follicles in the human ovary (Tilly, 1996). It has been reported that factors involved in the induction of apoptosis in the ovaries (Jansen et al., 2004) and also the number of atretic follicles increase in PCOS patients (Laven et al., 2001). EVs derived from huMSCs have also shown anti-apoptotic and fertility recovery effects and promoted secretive functions of granulosa cells in induced POI mice (Liu et al., 2020). Also it can reduce ovarian damage and protect GCs through anti-apoptotic and anti-inflammatory effects and improve ovarian function in chemotherapy-induced POF mice (Deng et al., 2021).

Therefore, exosome as a novel cell-free therapeutic strategy can be used promisingly in diseases of inflammatory origin by maintaining the immune balance (Chen et al., 2016).

The effects of bioactive compounds in the MSC-derived exosomes and secretome

Although recently there have been many studies on exosomes as a novel avenue for female infertility treatment, precise mechanisms of MSCs-derived exosomes on female reproductive diseases are also unclear. Given that chronic inflammation is associated with the pathogenesis of PCOS, there is also a positive feedback loop between inflammation, androgen production and metabolic disorders in PCOS (González et al., 2014b; Fox et al., 2019); Since, near to 50% of PCOS patients show high secretion of androgens (Marti et al., 2017; McAllister et al., 2019), therefore, the main strategy to treat PCOS can be suppression of androgen secretion (McAllister et al., 2019).

It has been reported that cytokine IL-10 that is found in secretome improves fertility through the suppressing androgen secretion by ovarian theca cells and reducing inflammation (Chugh et al., 2021b). Bone morphogenetic proteins (BMPs) are multifunctional growth factors that play an important role in folliculogenesis and female fertility; these proteins are secreted by BM-hMSCs (Yoshino et al., 2011). The theca cells in the ovary proliferate rapidly and increased androgen production in PCOS (Bremer, 2010; Zhang et al., 2012), it has been reported that BMP-2 can inhibit the proliferation of different cells *in vitro* (Hardwick et al., 2004; Chen et al., 2012; Zhang et al., 2012). Another study showed that BMP-2 can treat hyperandrogenemia in PCOS by suppressing steroidogenesis (Chugh et al., 2021a). Therefore, BMP-2 may improve the hyper-androgenemia in PCOS.

In PCOS and other ovarian disorders, the effect of exosome therapy has been reported to affect apoptosis by delivering genetic material such as miR-323-3p miR-146a and miR-10a (Xiao et al., 2016; Zhao et al., 2019), miR-664-5p (Sun et al., 2019), and miR-21(90).

Mesenchymal stem cells have the ability to secrete a large number of growth factors such as fibroblast growth factor (FGF), insulin-like growth factor-1 (IGF-1), VEGF, TGF-β, and EGF (Labouyrie et al., 1999; Izumida et al., 2005; Yoon et al., 2010) which may have the effect of reinitiate meiosis and improve oocyte maturation (Ling et al., 2008). A clinical trial in which the retrograde injection method was used to transplant MSCs based on a collagen scaffold into the ovaries of patients with some ovarian disorders suggests that EVs can be transferred by intra-ovarian injection (Ding et al., 2018). The biologically active

TABLE 1 Therapeutic potential of MSC-derived secretome in ovarian and *in vitro* culture.

Secretome type	Bioactive compounds	Source cell type	Target cells or tissue	Effects	Ref. No.
Exosome	miR-323-3p	AMSCs	cumulus cells	Inhibit apoptosis in GCs, regulation of steroidogenesis	Zhao et al. (2019)
	miR-146a, miR-10a	AFSCs	GCs	Inhibit apoptosis in GCs	Xiao et al. (2016)
	miR-664-5p	BMSC	GCs	Inhibit apoptosis in GCs	Sun et al. (2019)
Conditioned media	IL-10	BM-hMSCs	intra-ovarian injection	Reduce inflammation and androgen secretion	Chugh et al. (2021b)
	BMP-2	BM-hMSCs	H295R cells*	Reduce Steroidogenesis	Chugh et al. (2021a)
	EGF, IGF-1	MSCs	Oocyte	Improve oocyte maturation	Ling et al. (2008)
Extracellular vesicle	miR-21	AFMSCs	GCs	Inhibit apoptosis in GCs	Thabet et al. (2020)

BMP, Bone morphogenetic proteins; GCs, granulosa, cells; BM-hMSCs, Human bone marrow mesenchymal stem cells; BMSC, Bone mesenchymal stem cell; AMSCs, Adipose mesenchymal stem cells; AFSCs, Amniotic fluid stem cells; AFMSCs, Amniotic fluid mesenchymal stem cells.
**In vitro* cell culture model for androgen production.

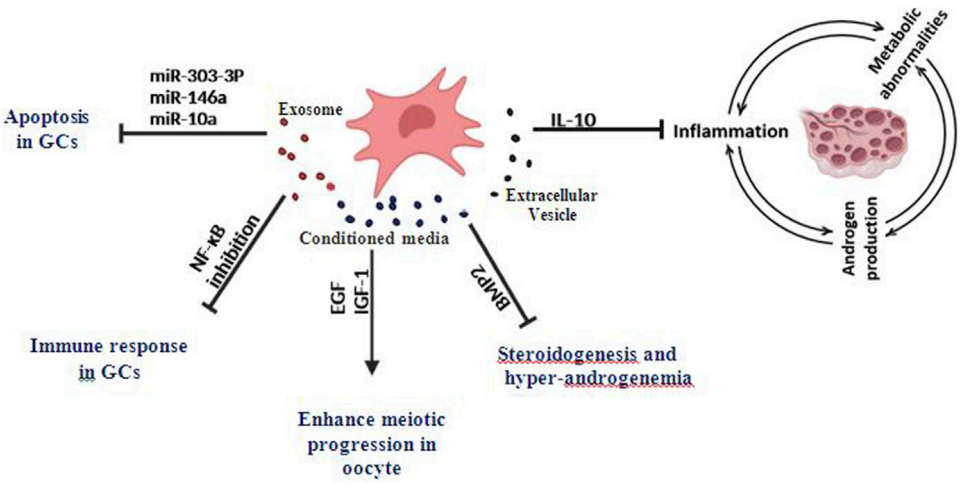

FIGURE 1
Effects of MSCs-derived exosomes, EVs, and Conditioned media on inflammation and granulosa cells apoptosis in PCOS and various ovarian disorders.

molecules and their effects are summarized in Table 1 and Figure 1.

Potential applications of MSCs-derived exosomes in enrichment IVM culture medium

Although *in vitro* fertilization (IVF) is an effective treatment for infertility in PCOS women, it is also associated with an increased risk of ovarian hyperstimulation syndrome (OHSS) (Shalom-Paz et al., 2012). Therefore, minimizing the risk of ovarian stimulation while providing an acceptable fertility success rate should be the focus of treatment efforts. Currently, to prevent OHSS, immature oocytes are collected from small antral follicles within unstimulated or very little stimulated ovaries then these oocytes are matured *in vitro*. Patients with PCOS could potentially benefit from IVM, as it reduces the risk of OHSS as well as costs (Shalom-Paz et al., 2012; Ho et al., 2019). But since the IVM and success rate of fertilization of oocytes matured *in vitro* is not satisfactory, therefore, to overcome these limitations faced by IVM, several studies have been conducted that focus on effects of cultural media containing various additives for improving oocyte quality (Jee et al., 2008; Ben-Ami et al., 2011; Blanco et al., 2011; Ishizuka et al., 2013; Sánchez et al., 2015). Primarily, an optimal culture medium is needed to increase the efficiency of IVM, which can

be achieved by better understanding the molecular events that trigger oocyte maturation (Chian et al., 2004). Before ovulation, the LH surge triggers a cascade of cellular and molecular events in the ovarian follicle including resumption of oocyte meiosis, cumulus expansion, follicular wall rupture, and cumulus-oocyte mass extrusion (Richards et al., 2002). Despite mural granulosa cells and external theca cells expressing high LH receptors, oocyte and cumulus cells express little or no LH receptors and therefore do not respond to LH exposure *in vitro* (PENG et al., 1991). Therefore, it seems the effects of LH on cumulus-oocytes may be through the release of the paracrine mediators from granulosa cells (Conti et al., 2006). Also, recently, it was reported that LH stimulation of isolated human granulosa cells causes the increase of EGF-like growth factors (Ben-Ami et al., 2006; Ben-Ami et al., 2009). Several experimental studies in animals and cell culture have demonstrated that EGF and IGF-1 can improve maturation in cumulus surrounded (Sakaguchi et al., 2000; Sakaguchi et al., 2002) and denuded oocytes as well as *in vitro* which is similar to what happens *in vivo* (Das et al., 1991; Lonergan et al., 1996).

Given that each oocyte is surrounded by cumulus granulosa, mural granulosa, theca cells and follicular fluid to form ovarian follicles as reproductive units, therefore, the oocyte can be affected by each of these components (Di Pietro, 2016). However, new exosome-based therapeutic approaches in PCOS are few. Recently, regulation of steroidogenesis, promotion of cell growth and inhibition of apoptosis in the cumulus cells by exosomal miR-323-3p has been reported in the women with PCOS (Zhao et al., 2019). Also, animal studies revealed beneficial effects of EVs (Liao et al., 2021), exosomes derived from amniotic fluid stem cells (Xiao et al., 2016) and bone mesenchymal stem cells (Sun et al., 2019) on various ovarian disorders and fertility recovery. These nanoparticles inhibit apoptosis in the damaged granulosa cells through the delivery of miR-146a, miR-10a (Xiao et al., 2016), miR-664-5p (Sun et al., 2019), and miR-21(90). Studies have shown that exosomes derived from huMSCs can increased of Bcl-2 and caspase-3 whereas decreased the expression of Bax, cleaved caspase-3, and cleaved poly (ADP-ribose) polymerase (PARP) to attenuation of cisplatin-induced ovarian granulosa cell apoptosis *in vitro* (Sun et al., 2017; Zhang et al., 2020). In addition, BM-hMSCs conditioned media could regulate the steroidogenesis, inhibit androgen secretion and suppress inflammatory pathways in a cellular model (Chugh et al., 2021a; Chugh et al., 2021b). Moreover, it has been reported that *in vitro* maturation of mouse oocytes with or without cumulus cells can be improved by its co-culture with conditioned medium of MSCs (Ling et al., 2008). Therefore, According to the beneficial effects of MSCs-derived exosomes, EVs and secretome can have the potential to optimize the culture media for oocyte maturation in PCOS.

Conclusion

Although the pathogenesis of PCOS is still controversial and remains unclear, several studies implicate chronic inflammation in the pathogenesis of PCOS and others implicate irregular granulosa cell apoptosis in PCOS infertility. In this study, we present a promising opportunity to develop novel cell-free therapy approaches to restore fertility in PCOS condition. According to the recent studies, MSCs-derived exosomes, EVs and secretomes inhibit inflammation and apoptosis, regulate steroidogenesis and inhibit androgen production in *in vitro* as well as *in vivo*. Consequently, it is worthwhile to challenge the effectiveness and efficiency of the exosomes in enriched culture media for improving oocyte development as well as PCOS treatment.

Author contributions

MI: study design, investigation, and writing original draft. MR: helping on writing the manuscript and validation of data and revising. AA: helping on writing the first draft of the manuscript. MK: helping on writing the first draft of the manuscript. BA: supervisor, validation of data, and revising the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Artini, P. G., De Micheroux, A., and D'Ambrogio, G. (1996). Growth hormone cotreatment with gonadotropins in ovulation induction. *J. Endocrinol. Invest.* 19 (11), 763–779. doi:10.1007/BF03347881

Barile, L., and Vassalli, G. (2017). Exosomes: Therapy delivery tools and biomarkers of diseases. *Pharmacol. Ther.* 174, 63–78. doi:10.1016/j.pharmthera.2017.02.020

Ben-Ami, I., Armon, L., Freimann, S., Strassburger, D., Ron-El, R., and Amsterdam, A. (2009). EGF-like growth factors as LH mediators in the human corpus luteum. *Hum. Reprod.* 24 (1), 176–184. doi:10.1093/humrep/den359

Ben-Ami, I., Freimann, S., Armon, L., Dantes, A., Strassburger, D., Friedler, S., et al. (2006). PGE2 up-regulates EGF-like growth factor biosynthesis in human granulosa cells: New insights into the coordination between PGE2 and LH in ovulation. *Mol. Hum. Reprod.* 12 (10), 593–599. doi:10.1093/molehr/gal068

Ben-Ami, I., Komsky, A., Bern, O., Kasterstein, E., Komarovsky, D., and Ron-El, R. (2011). *In vitro* maturation of human germinal vesicle-stage oocytes: Role of epidermal growth factor-like growth factors in the culture medium. *Hum. Reprod.* 26 (1), 76–81. doi:10.1093/humrep/deq290

Blanco, M., Demyda, S., Moreno, M. M., and Genero, E. (2011). Developmental competence of *in vivo* and *in vitro* matured oocytes: A review. *Biotechnol. Mol. Biol. Rev.* 6 (7), 155–165.

Bremer, A. A. (2010). Polycystic ovary syndrome in the pediatric population. *Metab. Syndr. Relat. Disord.* 8 (5), 375–394. doi:10.1089/met.2010.0039

Chen, A., Wang, D., Liu, X., He, S., Yu, Z., and Wang, J. (2012). Inhibitory effect of BMP-2 on the proliferation of breast cancer cells. *Mol. Med. Rep.* 6 (3), 615–620. doi:10.3892/mmr.2012.962

Chen, W., Huang, Y., Han, J., Yu, L., Li, Y., Lu, Z., et al. (2016). Immunomodulatory effects of mesenchymal stromal cells-derived exosome. *Immunol. Res.* 64 (4), 831–840. doi:10.1007/s12026-016-8798-6

Cheng, L., Zhang, K., Wu, S., Cui, M., and Xu, T. (2017). Focus on mesenchymal stem cell-derived exosomes: Opportunities and challenges in cell-free therapy. *Stem Cells Int.* 2017, 6305295. doi:10.1155/2017/6305295

Cheng, L., Zhang, K., Wu, S., Cui, M., and Xu, T. (2021). Focus on mesenchymal stem cell-derived exosomes: Opportunities and challenges in cell-free therapy focus on mesenchymal stem cell-derived exosomes: Opportunities and challenges in cell-free therapy. *Stem Cells Int.*

Chian, R-C., Buckett, W. M., and Tan, S-L. (2004). *In-vitro* maturation of human oocytes. *Reprod. Biomed. Online* 8 (2), 148–166. doi:10.1016/s1472-6483(10)60511-1

Chugh, R. M., Park, H.-s., El Andaloussi, A., Elsharoud, A., Esfandyari, S., Ulin, M., et al. (2021). Mesenchymal stem cell therapy ameliorates metabolic dysfunction and restores fertility in a PCOS mouse model through interleukin-10. *Stem Cell. Res. Ther.* 12 (1), 1–19. doi:10.1186/s13287-021-02472-w

Chugh, R. M., Park, H-s., Esfandyari, S., Elsharoud, A., Ulin, M., and Al-Hendy, A. (2021). Mesenchymal stem cell-conditioned media regulate steroidogenesis and inhibit androgen secretion in a PCOS cell model via BMP-2. *Int. J. Mol. Sci.* 22 (17), 9184. doi:10.3390/ijms22179184

Conti, M., Hsieh, M., Park, J-Y., and Su, Y-Q. (2006). Role of the epidermal growth factor network in ovarian follicles. *Mol. Endocrinol.* 20 (4), 715–723. doi:10.1210/me.2005-0185

Das, K., Stout, L. E., Hensleigh, H. C., Tagatz, G. E., Phipps, W. R., and Leung, B. S. (1991). Direct positive effect of epidermal growth factor on the cytoplasmic maturation of mouse and human oocytes. *Fertil. Steril.* 55 (5), 1000–1004. doi:10.1016/s0015-0282(16)54313-1

Deng, T., He, J., Yao, Q., Wu, L., Xue, L., Wu, M., et al. (2021). Human umbilical cord mesenchymal stem cells improve ovarian function in chemotherapy-induced premature ovarian failure mice through inhibiting apoptosis and inflammation via a paracrine mechanism. *Reprod. Sci.* 28 (6), 1718–1732. doi:10.1007/s43032-021-00499-1

Di Pietro, C. (2016). Exosome-mediated communication in the ovarian follicle. *J. Assist. Reprod. Genet.* 33 (3), 303–311. doi:10.1007/s10815-016-0657-9

Ding, L., Yan, G., Wang, B., Xu, L., Gu, Y., Ru, T., et al. (2018). Transplantation of UC-MSCs on collagen scaffold activates follicles in dormant ovaries of POF patients with long history of infertility. *Sci. China. Life Sci.* 61 (12), 1554–1565. doi:10.1007/s11427-017-9272-2

Du, H., and Taylor, H. S. (2009). Reviews: Stem cells and female reproduction. *Reprod. Sci.* 16 (2), 126–139. doi:10.1177/1933719108329956

Eid, G. M., Cottam, D. R., Velcu, L. M., Mattar, S. G., Korytkowski, M. T., Gosman, G., et al. (2005). Effective treatment of polycystic ovarian syndrome with Roux-en-Y gastric bypass. *Surg. Obes. Relat. Dis.* 1 (2), 77–80. doi:10.1016/j.soard.2005.02.008

El Andaloussi, S., Mäger, I., Breakefield, X. O., and Wood, M. J. (2013). Extracellular vesicles: Biology and emerging therapeutic opportunities. *Nat. Rev. Drug Discov.* 12 (5), 347–357. doi:10.1038/nrd3978

Elnashar, A., Abdelmageed, E., Fayed, M., and Sharaf, M. (2006). Clomiphene citrate and dexamethazone in treatment of clomiphene citrate-resistant polycystic ovary syndrome: A prospective placebo-controlled study. *Hum. Reprod.* 21 (7), 1805–1808. doi:10.1093/humrep/del053

Fox, C. W., Zhang, L., Sohni, A., Doblado, M., Wilkinson, M. F., Chang, R. J., et al. (2019). Inflammatory stimuli trigger increased androgen production and shifts in gene expression in theca-interstitial cells. *Endocrinology* 160 (12), 2946–2958. doi:10.1210/en.2019-00588

Franks, S., Stark, J., and Hardy, K. (2008). Follicle dynamics and anovulation in polycystic ovary syndrome. *Hum. Reprod. Update* 14 (4), 367–378. doi:10.1093/humupd/dmn015

Fu, D., Jiang, H., Li, C., Gao, T., Liu, M., and Li, H. (2020). MicroRNA-338 in MSCs-derived exosomes inhibits cardiomyocyte apoptosis in myocardial infarction. *Eur. Rev. Med. Pharmacol. Sci.* 24 (19), 10107–10117. doi:10.26355/eurrev_202010_23230

González, F., Sia, C. L., Bearson, D. M., and Blair, H. E. (2014). Hyperandrogenism induces a proinflammatory TNFα response to glucose ingestion in a receptor-dependent fashion. *J. Clin. Endocrinol. Metab.* 99 (5), E848–E854. doi:10.1210/jc.2013-4109

González, F., Sia, C. L., Shepard, M. K., Rote, N. S., and Minium, J. (2014). The altered mononuclear cell-derived cytokine response to glucose ingestion is not regulated by excess adiposity in polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.* 99 (11), E2244–E2251. doi:10.1210/jc.2014-2046

Goradel, N. H., Hour, F. G., Negahdari, B., Malekshahi, Z. V., Hashemzehi, M., Masoudifar, A., et al. (2018). Stem cell therapy: A new therapeutic option for cardiovascular diseases. *J. Cell. Biochem.* 119 (1), 95–104. doi:10.1002/jcb.26169

György, B., Szabó, T. G., Pásztói, M., Pál, Z., Misják, P., Aradi, B., et al. (2011). Membrane vesicles, current state-of-the-art: Emerging role of extracellular vesicles. *Cell. Mol. Life Sci.* 68 (16), 2667–2688. doi:10.1007/s00018-011-0689-3

Han, C., Sun, X., Liu, L., Jiang, H., Shen, Y., Xu, X., et al. (2016). Exosomes and their therapeutic potentials of stem cells. *Stem Cells Int.* 2016, 7653489. doi:10.1155/2016/7653489

Hardwick, J. C., Van Den Brink, G. R., Bleuming, S. A., Ballester, I., Van Den Brande, J. M., Keller, J. J., et al. (2004). Bone morphogenetic protein 2 is expressed by, and acts upon, mature epithelial cells in the colon. *Gastroenterology* 126 (1), 111–121. doi:10.1053/j.gastro.2003.10.067

Harrell, C. R., Fellabaum, C., Jovicic, N., Djonov, V., Arsenijevic, N., and Volarevic, V. (2019). Molecular mechanisms responsible for therapeutic potential of mesenchymal stem cell-derived secretome. *Cells* 8 (5), 467. doi:10.3390/cells8050467

He, Y., Chen, D., Yang, L., Hou, Q., Ma, H., and Xu, X. (2018). The therapeutic potential of bone marrow mesenchymal stem cells in premature ovarian failure. *Stem Cell. Res. Ther.* 9 (1), 263–267. doi:10.1186/s13287-018-1008-9

Ho, V. N., Braam, S. C., Pham, T. D., Mol, B. W., and Vuong, L. N. (2019). The effectiveness and safety of *in vitro* maturation of oocytes versus *in vitro* fertilization in women with a high antral follicle count. *Hum. Reprod.* 34 (6), 1055–1064. doi:10.1093/humrep/dez060

Ishizuka, Y., Nishimura, M., Matsumoto, K., Miyashita, M., Takeo, T., Nakagata, N., et al. (2013). The influence of reduced glutathione in fertilization medium on the fertility of *in vitro*–matured C57BL/6 mouse oocytes. *Theriogenology* 80 (5), 421–426. doi:10.1016/j.theriogenology.2013.07.002

Izadi, M., Marvast, L. D., Rezvani, M. E., Zohrabi, M., Aliabadi, A., Mousavi, S. A., et al. (2021). Mesenchymal stem-cell derived exosome therapy as a potential future approach for treatment of male infertility caused by Chlamydia infection. *Front. Microbiol.* 12, 785622. doi:10.3389/fmicb.2021.785622

Izumida, Y., Aoki, T., Yasuda, D., Koizumi, T., Suganuma, C., Saito, K., et al. (2005). Hepatocyte growth factor is constitutively produced by donor-derived bone marrow cells and promotes regeneration of pancreatic β-cells. *Biochem. Biophys. Res. Commun.* 333 (1), 273–282. doi:10.1016/j.bbrc.2005.05.100

Jansen, E., Laven, J. S., Dommerholt, H. B., Polman, J., van Rijt, C., van den Hurk, C., et al. (2004). Abnormal gene expression profiles in human ovaries from polycystic ovary syndrome patients. *Mol. Endocrinol.* 18 (12), 3050–3063. doi:10.1210/me.2004-0074

Jee, B. C., Han, S. H., Moon, J. H., Suh, C. S., Kim, S. H., and Group SnucoMARTS (2008). Influence of well defined protein source on *in vitro* maturation of human

oocyte: Human follicular fluid versus human serum albumin. *Fertil. Steril.* 89 (2), 348–352. doi:10.1016/j.fertnstert.2007.02.052

Koniusz, S., Andrzejewska, A., Muraca, M., Srivastava, A. K., Janowski, M., and Lukomska, B. (2016). Extracellular vesicles in physiology, pathology, and therapy of the immune and central nervous system, with focus on extracellular vesicles derived from mesenchymal stem cells as therapeutic tools. *Front. Cell. Neurosci.* 10, 109. doi:10.3389/fncel.2016.00109

Labouyrie, E., Dubus, P., Groppi, A., Mahon, F. X., Ferrer, J., Parrens, M., et al. (1999). Expression of neurotrophins and their receptors in human bone marrow. *Am. J. Pathol.* 154 (2), 405–415. doi:10.1016/S0002-9440(10)65287-X

Laven, J. S., Imani, B., Eijkemans, M. J., de Jong, F. H., and Fauser, B. C. (2001). Absent biologically relevant associations between serum inhibin B concentrations and characteristics of polycystic ovary syndrome in normogonadotrophic anovulatory infertility. *Hum. Reprod.* 16 (7), 1359–1364. doi:10.1093/humrep/16.7.1359

Legro, R. S., Barnhart, H. X., Schlaff, W. D., Carr, B. R., Diamond, M. P., Carson, S. A., et al. (2007). Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. *N. Engl. J. Med.* 356 (6), 551–566. doi:10.1056/NEJMoa063971

Liang, N., Trujillo, C. A., Negraes, P. D., Muotri, A. R., Lameu, C., and Ulrich, H. (2018). Stem cell contributions to neurological disease modeling and personalized medicine. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 80, 54–62. doi:10.1016/j.pnpbp.2017.05.025

Liao, Z., Liu, C., Wang, L., Sui, C., and Zhang, H. (2021). Therapeutic role of mesenchymal stem cell-derived extracellular vesicles in female reproductive diseases. *Front. Endocrinol.* 12, 665645. doi:10.3389/fendo.2021.665645

Ling, B., Feng, D., Zhou, Y., Gao, T., Wei, H., and Tian, Z. (2008). Effect of conditioned medium of mesenchymal stem cells on the *in vitro* maturation and subsequent development of mouse oocyte. *Braz. J. Med. Biol. Res.* 41, 978–985. doi:10.1590/s0100-879x2008005000053

Liu, B., Ding, F., Hu, D., Zhou, Y., Long, C., Shen, L., et al. (2018). Human umbilical cord mesenchymal stem cell conditioned medium attenuates renal fibrosis by reducing inflammation and epithelial-to-mesenchymal transition via the TLR4/NF-κB signaling pathway *in vivo* and *in vitro*. *Stem Cell. Res. Ther.* 9 (1), 7–14. doi:10.1186/s13287-017-0760-6

Liu, C., Yin, H., Jiang, H., Du, X., Wang, C., Liu, Y., et al. (2020). Extracellular vesicles derived from mesenchymal stem cells recover fertility of premature ovarian insufficiency mice and the effects on their offspring. *Cell. Transpl.* 29, 0963689720923575. doi:10.1177/0963689720923575

Lizneva, D., Suturina, L., Walker, W., Brakta, S., Gavrilova-Jordan, L., and Azziz, R. (2016). Criteria, prevalence, and phenotypes of polycystic ovary syndrome. *Fertil. Steril.* 106 (1), 6–15. doi:10.1016/j.fertnstert.2016.05.003

Lonergan, P., Carolan, C., Van Langendonckt, A., Donnay, I., Khatir, H., Mermillod, P., et al. (1996). Role of epidermal growth factor in bovine oocyte maturation and preimplantation embryo development *in vitro*. *Biol. Reprod.* 54 (6), 1420–1429. doi:10.1095/biolreprod54.6.1420

Machtinger, R., Laurent, L. C., and Baccarelli, A. A. (2016). Extracellular vesicles: Roles in gamete maturation, fertilization and embryo implantation. *Hum. Reprod. Update* 22 (2), 182–193. doi:10.1093/humupd/dmv055

Marti, N., Bouchoucha, N., Sauter, K-S., and Flück, C. E. (2017). Resveratrol inhibits androgen production of human adrenocortical H295R cells by lowering CYP17 and CYP21 expression and activities. *PloS one* 12 (3), e0174224. doi:10.1371/journal.pone.0174224

McAllister, J. M., Han, A. X., Modi, B. P., Teves, M. E., Mavodza, G. R., Anderson, Z. L., et al. (2019). miRNA profiling reveals miRNA-130b-3p mediates DENND1A variant 2 expression and androgen biosynthesis. *Endocrinology* 160 (8), 1964–1981. doi:10.1210/en.2019-00013

Mendt, M., Rezvani, K., and Shpall, E. (2019). Mesenchymal stem cell-derived exosomes for clinical use. *Bone Marrow Transpl.* 54 (2), 789–792. doi:10.1038/s41409-019-0616-z

Mutlu, L., Hufnagel, D., and Taylor, H. S. (2015). The endometrium as a source of mesenchymal stem cells for regenerative medicine. *Biol. Reprod.* 92 (6), 138. doi:10.1095/biolreprod.114.126771

Peng, X-R., Hsueh, A. J., Lapolt, P. S., Bjersing, L., and Ny, T. (1991). Localization of luteinizing hormone receptor messenger ribonucleic acid expression in ovarian cell types during follicle development and ovulation. *Endocrinology* 129 (6), 3200–3207. doi:10.1210/endo-129-6-3200

Priester, C., MacDonald, A., Dhar, M., and Bow, A. (2020). Examining the characteristics and applications of mesenchymal, induced pluripotent, and embryonic stem cells for tissue engineering approaches across the germ layers. *Pharmaceuticals* 13 (11), 344. doi:10.3390/ph13110344

Qin, L., Xu, W., Li, X., Meng, W., Hu, L., Luo, Z., et al. (2016). Differential expression profile of immunological cytokines in local ovary in patients with polycystic ovarian syndrome: Analysis by flow cytometry. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 197, 136–141. doi:10.1016/j.ejogrb.2015.12.003

Raposo, G., and Stoorvogel, W. (2013). Extracellular vesicles: Exosomes, microvesicles, and friends. *J. Cell. Biol.* 200 (4), 373–383. doi:10.1083/jcb.201211138

Richards, J. S., Russell, D. L., Ochsner, S., and Espey, L. L. (2002). Ovulation: New dimensions and new regulators of the inflammatory-like response. *Annu. Rev. Physiol.* 64 (1), 69–92. doi:10.1146/annurev.physiol.64.081501.131029

Rotterdam, E. (2004). Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil. Steril.* 81, 19–25. doi:10.1016/j.fertnstert.2003.10.004

Sakaguchi, M., Dominko, T., Leibfried-Rutledge, M., Nagai, T., and First, N. (2000). A combination of EGF and IGF-I accelerates the progression of meiosis in bovine follicular oocytes *in vitro* and fetal calf serum neutrali zes the acceleration effect. *Theriogenology* 54 (8), 1327–1342. doi:10.1016/s0093-691x(00)00439-8

Sakaguchi, M., Dominko, T., Yamauchi, N., Leibfried-Rutledge, M., Nagai, T., and First, N. (2002). Possible mechanism for acceleration of meiotic progression of bovine follicular oocytes by growth factors *in vitro*. *Reproduction* 123 (1), 135–142. doi:10.1530/rep.0.1230135

Sam, S., and Dunaif, A. (2003). Polycystic ovary syndrome: Syndrome XX? *Trends Endocrinol. Metab.* 14 (8), 365–370. doi:10.1016/j.tem.2003.08.002

Sánchez, F., Romero, S., De Vos, M., Verheyen, G., and Smitz, J. (2015). Human cumulus-enclosed germinal vesicle oocytes from early antral follicles reveal heterogeneous cellular and molecular features associated with *in vitro* maturation capacity. *Hum. Reprod.* 30 (6), 1396–1409. doi:10.1093/humrep/dev083

Shalom-Paz, E., Holzer, H., Son, W-Y., Levin, I., Tan, S. L., and Almog, B. (2012). PCOS patients can benefit from *in vitro* maturation (IVM) of oocytes. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 165 (1), 53–56. doi:10.1016/j.ejogrb.2012.07.001

Sneddon, J. B., Tang, Q., Stock, P., Bluestone, J. A., Roy, S., Desai, T., et al. (2018). Stem cell therapies for treating diabetes: Progress and remaining challenges. *Cell. stem Cell.* 22 (6), 810–823. doi:10.1016/j.stem.2018.05.016

Sun, B., Ma, Y., Wang, F., Hu, L., and Sun, Y. (2019). miR-644-5p carried by bone mesenchymal stem cell-derived exosomes targets regulation of p53 to inhibit ovarian granulosa cell apoptosis. *Stem Cell. Res. Ther.* 10 (1), 360–369. doi:10.1186/s13287-019-1442-3

Sun, L., Li, D., Song, K., Wei, J., Yao, S., Li, Z., et al. (2017). Exosomes derived from human umbilical cord mesenchymal stem cells protect against cisplatin-induced ovarian granulosa cell stress and apoptosis *in vitro*. *Sci. Rep.* 7 (1), 1–13. doi:10.1038/s41598-017-02786-x

Tang, Y., Zhou, Y., and Li, H-J. (2021). Advances in mesenchymal stem cell exosomes: A review. *Stem Cell. Res. Ther.* 12 (1), 71–12. doi:10.1186/s13287-021-02138-7

Thabet, E., Yusuf, A., Abdelmonsif, D. A., Nabil, I., Mourad, G., and Mehanna, R. A. (2020). Extracellular vesicles miRNA-21: A potential therapeutic tool in premature ovarian dysfunction. *Mol. Hum. Reprod.* 26 (12), 906–919. doi:10.1093/molehr/gaaa068

Tilly, J. L. (1996). Apoptosis and ovarian function. *Rev. Reprod.* 1 (3), 162–172. doi:10.1530/ror.0.0010162

Urbanelli, L., Buratta, S., Sagini, K., Ferrara, G., Lanni, M., and Emiliani, C. (2015). Exosome-based strategies for diagnosis and therapy. *Recent Pat. CNS Drug Discov.* 10 (1), 10–27. doi:10.2174/1574889810666150702124059

Valadi, H., Ekström, K., Bossios, A., Sjöstrand, M., Lee, J. J., and Lötvall, J. O. (2007). Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell. Biol.* 9 (6), 654–659. doi:10.1038/ncb1596

van Balkom, B. W., Gremmels, H., Giebel, B., and Lim, S. K. (2019). Proteomic signature of mesenchymal stromal cell-derived small extracellular vesicles. *Proteomics* 19 (1-2), 1800163. doi:10.1002/pmic.201800163

Van der Pol, E., Böing, A. N., Harrison, P., Sturk, A., and Nieuwland, R. (2012). Classification, functions, and clinical relevance of extracellular vesicles. *Pharmacol. Rev.* 64 (3), 676–705. doi:10.1124/pr.112.005983

Velez, L. M., Seldin, M., and Motta, A. B. (2021). Inflammation and reproductive function in women with polycystic ovary syndrome. *Biol. Reprod.* 104 (6), 1205–1217. doi:10.1093/biolre/ioab050

Wen, Z., Mai, Z., Zhu, X., Wu, T., Chen, Y., Geng, D., et al. (2020). Mesenchymal stem cell-derived exosomes ameliorate cardiomyocyte apoptosis in hypoxic conditions through microRNA144 by targeting the PTEN/AKT pathway. *Stem Cell. Res. Ther.* 11 (1), 36–17. doi:10.1186/s13287-020-1563-8

Xiao, G-Y., Cheng, C-C., Chiang, Y-S., Cheng, W. T-K., Liu, I., and Wu, S-C. (2016). Exosomal miR-10a derived from amniotic fluid stem cells preserves ovarian follicles after chemotherapy. *Sci. Rep.* 6 (1), 1–12. doi:10.1038/srep23120

Xie, Q., Xiong, X., Xiao, N., He, K., Chen, M., Peng, J., et al. (2019). Mesenchymal stem cells alleviate DHEA-induced polycystic ovary syndrome (PCOS) by inhibiting inflammation in mice. *Stem Cells Int.* 2019, 9782373. doi:10.1155/2019/9782373

Xu, S., Liu, C., and Ji, H-L. (2019). Concise review: Therapeutic potential of the mesenchymal stem cell derived secretome and extracellular vesicles for radiation-induced lung injury: Progress and hypotheses. *Stem Cells Transl. Med.* 8 (4), 344–354. doi:10.1002/sctm.18-0038

Xu, Y., and Qiao, J. (2022). Association of insulin resistance and elevated androgen levels with polycystic ovarian syndrome (PCOS): A review of literature. *J. Healthc. Eng.*, 9240569. doi:10.1155/2022/9240569

Yamamoto, S., Azuma, E., Muramatsu, M., Hamashima, T., Ishii, Y., and Sasahara, M. (2016). Significance of extracellular vesicles: Pathobiological roles in disease. *Cell. Struct. Funct.* 41, 137–143. doi:10.1247/csf.16014

Yoon, B. S., Moon, J-H., Jun, E. K., Kim, J., Maeng, I., Kim, J. S., et al. (2010). Secretory profiles and wound healing effects of human amniotic fluid–derived mesenchymal stem cells. *Stem Cells Dev.* 19 (6), 887–902. doi:10.1089/scd.2009.0138

Yoshino, O., Shi, J., Osuga, Y., Harada, M., Nishii, O., Yano, T., et al. (2011). The function of bone morphogenetic proteins in the human ovary. *Reprod. Med. Biol.* 10 (1), 1–7. doi:10.1007/s12522-010-0072-3

Zhang, B., Yeo, R. W. Y., Tan, K. H., and Lim, S. K. (2016). Focus on extracellular vesicles: Therapeutic potential of stem cell-derived extracellular vesicles. *Int. J. Mol. Sci.* 17 (2), 174. doi:10.3390/ijms17020174

Zhang, J., Ge, Y., Sun, L., Cao, J., Wu, Q., Guo, L., et al. (2012). Effect of bone morphogenetic protein-2 on proliferation and apoptosis of gastric cancer cells. *Int. J. Med. Sci.* 9 (2), 184–192. doi:10.7150/ijms.3859

Zhang, J., Yin, H., Jiang, H., Du, X., and Yang, Z. (2020). The protective effects of human umbilical cord mesenchymal stem cell-derived extracellular vesicles on cisplatin-damaged granulosa cells. *Taiwan. J. Obstet. Gynecol.* 59 (4), 527–533. doi:10.1016/j.tjog.2020.05.010

Zhao, Y., Pan, S., and Wu, X. (2022). Human umbilical cord mesenchymal stem cell-derived exosomes inhibit ovarian granulosa cells inflammatory response through inhibition of NF-κB signaling in polycystic ovary syndrome. *J. Reprod. Immunol.* 152, 103638. doi:10.1016/j.jri.2022.103638

Zhao, Y., Tao, M., Wei, M., Du, S., Wang, H., and Wang, X. (2019). Mesenchymal stem cells derived exosomal miR-323-3p promotes proliferation and inhibits apoptosis of cumulus cells in polycystic ovary syndrome (PCOS). *Artif. Cells Nanomed. Biotechnol.* 47 (1), 3804–3813. doi:10.1080/21691401.2019.1669619

Zohrabi, M., Dehghan Marvast, L., Izadi, M., Mousavi, S. A., and Aflatoonian, B. (2022). Potential of mesenchymal stem cell-derived exosomes as a novel treatment for female infertility caused by bacterial infections. *Front. Microbiol.* 12, 785649. doi:10.3389/fmicb.2021.785649

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Tissue concentration of vascular endothelial growth factor is not related to the depth of trophoblastic invasion in ampullary pregnancies—A pilot study

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Introduction: The factors that modulate trophoblastic invasion into the tubal wall remain uncertain. Moreover, it is known that the concentration of vascular endothelial growth factor (VEGF) is increased in cases of deeper trophoblastic invasion in the fallopian tubes.

Objective: This study aimed to assess if there is a correlation between VEGF tissue expression and the depth of trophoblastic infiltration into the tubal wall in patients with ampullary pregnancy.

Methods: A cross-sectional study was conducted in patients with a diagnosis of tubal pregnancy in the ampullary region who underwent salpingectomy. Inclusion criteria were spontaneously conceived singleton pregnancies, diagnosis of tubal pregnancy in the ampullary region, and radical surgical treatment. A lack of agreement regarding the location of the tubal pregnancy and impossibility of either anatomopathological or tissue VEGF analysis were the exclusion criteria. Histologically, trophoblastic invasion into the tubal wall was classified as grade I when limited to the tubal mucosa, grade II when it reached the muscle layer, and grade III when it comprised the full thickness of the tubal wall. A total of 42 patients fulfilled the inclusion criteria and were selected to participate in the study. Eight patients were excluded. After surgery, tissue VEGF expression was measured by immunohistochemistry and the point counting technique.

Results: Histological analysis revealed that eight patients had stage I tubal infiltration, seven had stage II, and 19 had stage III. The difference between the percentage of VEGF expression in the trophoblastic tissue was not significant in relation to the degree of trophoblastic invasion (p = 0.621) (ANOVA). Trophoblastic tissue VEGF showed no statistical difference for

prediction of both degrees of trophoblastic invasion (univariate multinomial regression).

Conclusion: The depth of trophoblastic penetration into the tubal wall in ampullary pregnancies is not associated with tissue VEGF expression.

KEYWORDS

first trimester hemorrhage, VEGF, vascular endothelial growth factor, ampullary pregnancy, trophoblast, pregnancy ectopic, tissue expression

1 Introduction

Ectopic pregnancy (EP) is an obstetric complication in which the fertilized ovum implants outside the intrauterine cavity (Cunningham et al., 2022). The most common site of ectopic implantation is the fallopian tube (approximately 95% of cases), but other sites such as the ovaries and abdomen can be involved too (Tang et al., 2022). Seventy percent of fallopian tube pregnancies are located in the ampullary segment (Cunningham et al., 2022; Tang et al., 2022). EP is the most common cause of maternal mortality in the first trimester of pregnancy, justifying its clinical relevance (Creanga et al., 2017; Lisonkova et al., 2019). Its incidence has increased over the last decades and can be explained by the increased incidence of its risk factors, including pelvic inflammatory disease, the use of emergency contraceptive methods, and pregnancies conceived by assisted reproductive treatments (ACOG Practice Bulletin No, 2019).

Some potential predictors of trophoblastic invasion of the fallopian tubes in cases of EP, such as serum beta-hCG, transvaginal ultrasound, and maternal serum concentrations of vascular endothelial growth factor (VEGF), have been reported. Serum concentration of beta-hCG has high sensitivity and specificity in predicting trophoblastic tubal invasion and could be used to choose the best treatment for these patients (Cabar et al., 2010; Erol et al., 2015).

VEGF participates in the processes of implantation and placentation (Leung et al., 1989) and cellular VEGF production is increased in hypoxic conditions (Ladoux and Frelin, 1993). The implantation environment in the oviduct is very different from that of the well-vascularized endometrium, and production and secretion of VEGF seem to be elevated in EP in an attempt to acclimatize to an unfavorable environment (Zou et al., 2013; Zarezade et al., 2015).

The mechanisms that facilitate the trophoblastic invasion in the wall of the fallopian tube are unknown. We hypothesize that since there is a greater expression of VEGF in the EP implantation site, VEGF trophoblastic tissue concentration would be correlated with the depth of trophoblastic invasion into the wall of the oviduct.

Thus, the objective of the present study was to verify the correlation between trophoblastic tissue expression of VEGF in ampullary pregnancies and the depth of trophoblastic invasion into the tubal wall.

2 Materials and methods

A prospective study was conducted on patients with a diagnosis of tubal pregnancy in the ampullary region who underwent salpingectomy. Inclusion criteria were spontaneously conceived singleton pregnancies, diagnosis of tubal pregnancy in the ampullary region, and radical surgical treatment (salpingectomy). Cases in which there was no agreement regarding the location of the tubal pregnancy upon surgical description and histological analysis were excluded. Assessment of gestational age was made based on the last menstrual period. Institutional Review Board approval was obtained and informed consent was also obtained from each patient before participation in the study.

A total of 63 consecutive cases of EP were recorded during the study period. Of these, 21 patients were not included for different reasons: in one case, it was not possible to obtain the informed consent, and 20 patients were treated by a conservative approach (clinical or surgical). Forty-two patients fulfilled the inclusion criteria and were selected to participate in the study. Eight patients were excluded: in three patients, the tubal implantation site could not be identified, two showed no trophoblastic tissue in histological analysis, and in three, it was not possible to identify trophoblastic tissue VEGF.

To confirm the diagnosis, patients were routinely subjected to a serum beta-hCG determination; a transvaginal ultrasound was also performed. After diagnostic confirmation, if the patient could be enrolled in the protocol, informed consent was obtained.

After surgery, the fallopian tubes were immediately fixed in 10% formalin and sectioned serially for light-microscopic analysis. An average of 10 sections stained with hematoxylin-eosin was analyzed. To facilitate the identification of trophoblastic tissue invaded by the trophoblast, histological material was also stained with Masson's trichrome to identify muscular fibers. Histological assessment was performed by a single well-experienced pathologist who was blinded to the clinical and laboratory characteristics of the patients.

Ampullary pregnancies were classified histologically according to the depth of trophoblastic infiltration into the tubal wall (Natale et al., 2003). In stage I, trophoblastic infiltration was limited to the tubal mucosa; in stage II, trophoblastic infiltration extended to the tubal muscularis; and in stage III, complete tubal wall infiltration with or without rupture of the serosa was observed. Immunohistochemical staining for human placental lactogen or cytokeratin 7 (hPL or CK7) was performed to identify trophoblastic cells and determine the depth of trophoblastic invasion in the tubal wall.

To analyze VEGF tissue expression, immunohistochemical reactions were performed on histological sections of the uterine tube fragments and studied by the biotin-streptavidin peroxidase method.

Histological sections of 3-µm thickness were obtained and collected on glass slides previously treated with 2% organo-silane adhesive solution (Sigma Aldrich Co., St. Louis, Missouri, United States). The slides were stained by hematoxylin-eosin for morphological analysis.

Next, the histological sections were deparaffinized in xylene at 60°C for 30 min, followed by two xylol baths at room temperature for 30 min and hydrated in a series of decreasing concentrations of ethanol, running water, and distilled water. After being washed in Tris-saline buffer (20 mM TBS and pH 7.4), the samples underwent the following protocol:

Rescue of the antigenic sites was performed with a steam cooker by immersing the slides in citrate (pH 6.0) for 1 min at 125°C. After this, the slides were washed with running water, distilled water, and PBS/Tween (Phosphate buffered saline-Twen® Tablets).

The slides were then incubated with monoclonal mouse anti-human VEGF (clone VG-1) (Santa Cruz, Biotechnology, Santa Cruz, CA, United States). Immunoglobulin G (IgG) from normal mice was used as control monoclonal antibodies. Tissues known to be positive were used as a positive control and omitted primary antibody as a negative control. (Positive control: Human tonsil, kidney, skin; negative control: omit primary antibody, isotype control, absorption control. The dilution used for all antibodies was 1: 800, carried out in a diluent solution, and applied over the cuts of the tissue. The slides were incubated overnight.

The slides were then washed in PBS/Tween and incubated in an oven at 37°C with Novolink Polymer secondary antibodies (Leica Biosystems Newcastle Ltd.).

After this step, the slides were washed in PBS/Tween and followed by the development of the chromogen 3, three diaminobenzidine (DAB) (Sigma Chemical Corporation code: D5637, St Louis, Missouri, United States). The slides were washed extensively in tap water and distilled water and counterstained with Harris hematoxylin (Merck, Darmstadt, Germany). Then, they were washed in running water and in distilled water, dehydrated, diaphanized, and mounted with resin for Entellan microscopy (Merck, Darmstadt, Germany).

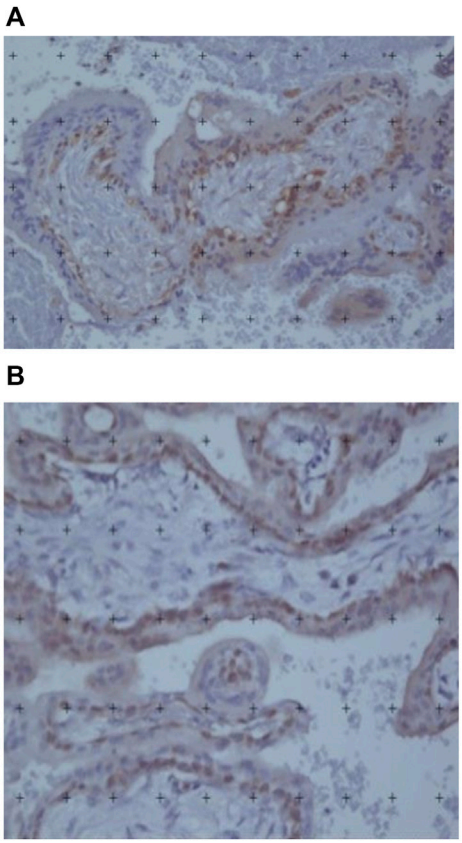

FIGURE 1
(A) and **(B)**: Histological images of two different fallopian tube segments affected by ectopic pregnancy after immunohistochemistry and microscopic view with tissue VEGF analysis using the point counting technique. (×400 magnification).

Evaluation of VEGF immunostaining in tubal tissue was performed by the stereological point-counting method (with modifications) based on Gundersen et al. (Gundersen et al., 1988) and using an image analysis system (Image-Pro Plus 6.0). (Fallopian tube tissues containing placental tissue were studied). Briefly, this system consists of a camera (Olympus Co., St Laurent, Quebec, Canada) coupled with a microscope (Olympus BX-51, Olympus Co., Tokyo, Japan), which captures images and sends them to a monitor with a scanning system (Oculus TCX, Coreco, Inc., St. Laurent, Quebec, Canada). A reticulum with 100 points was distributed over the orthogonally captured image. The assessment was performed by one observer, who was blinded, in 10 randomized fields of uterine tube tissue at increased magnification (×400). The percentage (p) of marked points in the reference compartment for VEGF was expressed according to the formula: $p = (Pi \times 100)/Pt$; where Pi is the number of points that matches the positive marking by

TABLE 1 Sociodemographic and clinical characteristics of patients associated to Trophoplastic invasion.

Covariates	Trophoblastic invasion			*p*
	GI (*n* = 8)	GII (*n* = 7)	GIII (*n* = 19)	
Age (average; SD)	30.6 (6.7)	26.3 (5.5)	30.8 (6.9)	0.297[1]
Ethnicity (*n*;%)				
White	6 (75.0)	4 (57.1)	14 (73.7)	0.774[2]
non white	2 (25.0)	3 (42.9)	5 (26.3)	
Marital status (*n*;%)				
with partner	6 (75.0)	1 (14.3)	5 (26.3)	0.053[2]
no partner	2 (25.0)	6 (85.7)	14 (73.7)	
Comorbidities (*n*;%)				
No	5 (62.5)	6 (85.7)	15 (78.9)	0.480[2]
Yes	3 (37.5)	1 (14.3)	4 (21.1)	
Previous delivery (*n*;%)				
No	1 (12.5)	5 (71.4)	12 (63.2)	0.041[2]
Yes	7 (87.5)	2 (28.6)	37 (36.8)	
Abortion (*n*;%)				
No	5 (62.5)	4 (57.1)	12 (63.2)	1.00[2]
Yes	3 (37.5)	3 (42.9)	7 (36.8)	
Ectopic pregnancy (*n*;%)				
No	6 (75.0)	7 (100)	16 (84.2)	0.783[2]
Yes	2 (25.0)	0 (0.0)	3 (15.8)	
VEGF tissue (average; SD)	20.8 (10.9)	19.7 (14.1)	16.5 (7.6)	0.547[1]

1.ANOVA; 2. Linear-by-Linear Association exact test.

immunohistochemistry and Pt is the total number of points analyzed. The percentage (*p*) of VEGF was calculated from the sum of the results of all fields analyzed for each sample. (Figures 1–A,B).

Qualitative data were described using absolute and relative frequency (percentage) and were compared using Linear-by-Linear Association exact test.

For a summary of quantitative variables, median, minimum, maximum, mean and standard deviation (SD) were used. The difference between groups of trophoblastic invasion with respect of trophoblastic tissue VEGF was tested by parametric ANOVA test.

Multinomial logistic regression was used to compare the performance of the trophoblastic tissue VEGF expression associated to outcome the degree of trophoblastic invasion into the tubal wall. This analysis was applied to estimate the beta values and the odds ratios (OR) with 95% confidence interval (CI95%). The degree GI of trophoblastic invasion was considering the reference category.

p <.05 was considered statistically significant; all tests were two-tailed.

All statistical analyses were performed on a personal computer with the Statistics for Macintosh 22.0 (IBM Corp., Armonk, NY).

3 Results

The final sample consisted of 34 patients. The age of the women ranged from 11 to 40 years (29.8 ± 6.6 years), and there was no significant difference in mean maternal ages among the three histological groups (p = 0,706). Twenty four patients (70.4%) were Caucasian, and 14 (29.4%) were non-Caucasian. With respect to obstetric history, 18 patients (52.9%) were nulliparous and five (14.7%) had a history of EP in the contralateral fallopian tube. Histological analysis showed that eight patients (23.5%) had stage I tubal infiltration, 7 (20.6%) had stage II, and 19 (55.9%) had stage III. The gestational age ranged from 4 to 13.6 weeks (7.6 ± 2.1 week, and there was no significant difference in mean gestational ages among the three histological groups (p = 0,604).

According to ANOVA test, the difference between the percentage of trophoblastic tissue VEGF was not significant in relation to the degree of trophoblastic invasion (p = 0.621) (Table 1).

Univariate multinomial regression analysis was performed and the variable trophoblastic tissue VEGF was included in order to compare its performance as a predictive factor of depth of trophoblastic invasion into oviduct wall. We observed that

TABLE 2 VEG tissue expression associated trophoplastic invasion, as determinated by univariate multinomial regression analysis.

Trophoblastic invasion		B(SE)	OR	95% CI		*p*
				Lower	Upper	
Group II	VEGF tissue (%)	−0.01 (0.05)	0,99	0,90	1,09	0,842
Group III	VEGF tissue (%)	−0.05 (0.04)	0,96	0,88	1,04	0,299
Reference category = Group I						

trophoblastic tissue VEGF showed no statistical difference for prediction of both degrees of trophoblastic invasion (Table 2).

4 Discussion

In the past, the objective of EP treatment was to remove all trophoblastic tissue implanted in the uterine tube to preserve the patient's life. Nowadays, due to the development of subsidiary tests, it has become possible to carry out more conservative treatments–both clinical and surgical.

Therefore, search for markers that can allow early diagnosis and selection of cases in which the tubal lesion is less deeper and does not compromise its function is of great interest.

In the studied population, 23.5% of patients had trophoblastic invasion limited to the mucosa, 20.6% had invasion limited to the muscle layer, and the remaining 55.9% had invasion throughout the thickness of the tubal wall. These findings are similar to the study by Cabar et al. (2006), which reported degrees of trophoblastic invasion as 27.6%, 28.6%, and 43.8%, respectively. Probably, the main explanation for this finding is the difficulty in early diagnosis of some cases of EP. There was any difference between groups regarding age, ethnicity, marital status, comorbidities, previous delivery, abortion nor ectopic pregnancies (Table 1).

It is known that VEGF expression is increased under unfavorable tissue conditions, including an hypoxic environment, such as occurs in the site where the embryo implants in the fallopian tube. In such cases, besides the increased levels of VEGF, an increase in the concentration of flt -1 receptor (VEGFR-2) is also observed, as shown by Vuorela et al. (Vuorela et al., 2000). Evans et al. (Evans et al., 1998) observed that the levels of VEGF and its receptor are elevated when choriocarcinoma cells are grown in a low-oxygen environment. Therefore, in addition to the increase in serum VEGF at the site of tubal implantation, its flt -1 receptor is also possibly increased, thereby lowering its free fraction.

Moreover, according to Evans et al. (1998), serum progesterone and beta-hCG concentrations are also increased in early stages of pregnancy and have an important relationship with the increase of VEGF, thus contributing to trophoblastic invasion. The authors stated that progesterone enhances VEGF production in epithelial cells of the retina and hCG increases VEGF in granulosa cells (Evans et al., 1998). Since the serum levels of progesterone and hCG are lower in EP, despite local hypoxia, VEGF production may remain unchanged due to the negative effect of the decreasing of other hormones.

Previous studies have shown that estrogen increases VEGF secretion in humans and animals (Torry et al., 1996; Torry and Torry, 1997). Similarly, with decreased serum levels of estrogen, progesterone, human chorionic gonadotropin, and possibly other cytokines and growth factors still unknown in EP could increase their concentrations *via* VEGF, but this conclusion is not supported by a statistically significant relationships.

We intended to associate the depth of trophoblastic invasion into the tubal wall and maternal trophoblastic tissue VEGF expression. From a study by Lam et al. (2004) showing higher VEGF concentrations in implantation sites of EP and according to the study of Cabar et al. (Cabar et al., 2010), who showed that serum VEGF is increased in cases of deeper trophoblastic invasion, our initial hypothesis was that higher trophoblastic tissue VEGF expression could lead to a deeper invasion of trophoblastic cells into the wall of the uterine tube. We believed that this information could bring better understanding about the factors that facilitate trophoblastic invasion into the fallopian tube.

However, the percentage of trophoblastic tissue VEGF was similar in the three degrees of trophoblastic invasion ($p = 0.621$) and did not attain statistical difference. This finding suggests that VEGF at the site of tubal implantation appears not to be related to trophoblastic invasion, and perhaps others cytokines and other still unknown growth factors may contribute to this process. (Table 2) Zarezade et al. (2015) found similar results when investigating the expression of VEGF mRNA and VEGF receptor 1 (VEGFR1) and 2 (VEGFR2) in women with EP compared to women who underwent hysterectomy. The authors concluded that the expression of VEGF and its receptors is lower in women with EP compared to women with normal tubes.

Through this study, we tried to understand the reason why trophoblastic tissue invasion finds favorable conditions in some cases and reaches the deepest layers of the fallopian tube. The mechanism that explains factors determining the depth of tissue invasion in ectopic pregnancies remains unknown. We believe

this information is important as it could help to understand the histopathological mechanism involved in this process, providing new fronts for the diagnosis and treatment of this pregnancy complication. To our knowledge, this information is not yet available in the literature.

As a conclusion, we found that, despite previous studies indicating that serum VEGF is related to trophoblast invasion in the tubal wall in EPs, the present investigation did not find this association between tissue expression of this molecule and the depth of trophoblast invasion in the tubal wall in EPs. The tissue expression of VEGF at EP implantation site may not be primarily responsible for local modifications conducive to the development of a trophoblast.

The principal limitation of the study was the sample size, since we did not have a great number of cases. We believe that further studies, with larger sample sizes, should be carried out in order to better understand this histopathological process.

Data availability statement

The datasets presented in this article are not readily available. All the data generated in this study are anonymous and there is no way to individualize or identify patients. In Brazil, by law, this data cannot be provided to third parties. Further inquiries can be sent to the corresponding author FC, fabio.cabar@hc.fm.usp.br.

Ethics statement

The studies involving human participants were reviewed and approved by Comissão de Ética para Análise de Projetos de Pesquisa do Hospital das Clínicas da Faculdade de Medicina da USP. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

DRKT—Planning, carrying out and writing, PPP—Planning RPVF—Planning, MD—Writing, RS—Analysing, LA—Analysing, FC—Planning, carrying out, writing and supervision.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

ACOG Practice Bulletin No (2019). Tubal ectopic pregnancy: Correction. *Obstet. Gynecol.Obstet. Gynecol.* 133, 1059.

Cabar, F. R., Pereira, P. P., Schultz, R., Francisco, R. P., and Zugaib, M. (2010). Vascular endothelial growth factor and β-human chorionic gonadotropin are associated with trophoblastic invasion into the tubal wall in ectopic pregnancy. *Fertil. Steril.* 94, 1595–1600. doi:10.1016/j.fertnstert.2009.10.036

Cabar, F. R., Pereira, P. P., Schultz, R., and Zugaib, M. (2006). Predictive factors of trophoblastic invasion into the ampullary region of the tubal wall in ectopic pregnancy. *Hum. Reprod.* 21, 2426–2431. doi:10.1093/humrep/del170

Creanga, A. A., Syverson, C., Seed, K., and Callaghan, W. M. (2017). Pregnancy-related mortality in the United States, 2011–2013. *Obstet. Gynecol.* 130, 366–373. doi:10.1097/AOG.0000000000002114

Cunningham, F., Leveno, K. J., Dash, J., Hoffman, B., and Spong, C. B. (2022). *Williams obstetrics*. Editor N. Iorque (McGraw-Hill), 26.

Erol, O., Süren, D., Karaca, M., and Sezer, C. (2015). Ultrasonography for the prediction of extension of trophoblastic infiltration into the tubal wall in ampullary pregnancy. *Ginekol. Pol.* 86, 16–20. doi:10.17772/gp/1893

Evans, P. W., Wheeler, T., Anthony, F. W., and Osmond, C. (1998). A longitudinal study of maternal serum vascular endothelial growth factor in early pregnancy. *Hum. Reprod.* 13, 1057–1062. doi:10.1093/humrep/13.4.1057

Gundersen, H. J., Bagger, P., Bendtsen, T. F., Evans, S. M., Korbo, L., Marcussen, N., et al. (1988). The new stereological tools: Disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *APMIS* 96, 857–881. doi:10.1111/j.1699-0463.1988.tb00954.x

Ladoux, A., and Frelin, C. (1993). Hypoxia is a strong inducer of vascular endothelial growth factor mRNA expression in the heart. *Biochem. Biophys. Res. Commun.* 195, 1005–1010. doi:10.1006/bbrc.1993.2144

Lam, P. M., Briton-Jones, C., Cheung, C. K., Leung, S. W., Cheung, L. P., and Haines, C. (2004). Increased messenger RNA expression of vascular endothelial growth factor and its receptors in the implantation site of the human oviduct with ectopic gestation. *Fertil. Steril.* 82, 686–690. doi:10.1016/j.fertnstert.2003.12.052

Leung, D. W., Cachianes, G., Kuang, W. J., Goeddel, D. V., and Ferrara, N. (1989). Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 246, 1306–1309. doi:10.1126/science.2479986

Lisonkova, S., Tan, J., Wen, Q., Abdellatif, L., Richter, L. L., Alfaraj, S., et al. (2019). Temporal trends in severe morbidity and mortality associated with ectopic pregnancy requiring hospitalisation in Washington state, USA: A population-based study. *BMJ Open* 9, e024353. doi:10.1136/bmjopen-2018-024353

Natale, A., Candiani, M., Merlo, D., Izzo, S., Gruft, L., and Busacca, M. (2003). Human chorionic gonadotropin level as a predictor of trophoblastic infiltration into

the tubal wall in ectopic pregnancy: A blinded study. *Fertil. Steril.* 79, 981–986. doi:10.1016/s0015-0282(02)04922-1

Tang, P., Li, X., Li, W., Li, Y., Zhang, Y., and Yang, Y. (2022). The trend of the distribution of ectopic pregnancy sites and the clinical characteristics of caesarean scar pregnancy. *Reprod. Health* 19, 182. doi:10.1186/s12978-022-01472-0

Torry, D. S., Holt, V. J., Keenan, J. A., Harris, G., Caudle, M. R., and Torry, R. J. (1996). Vascular endothelial growth factor expression in cycling human endometrium. *Fertil. Steril.* 66, 72–80. doi:10.1016/s0015-0282(16)58390-3

Torry, D. S., and Torry, R. J. (1997). Angiogenesis and the expression of vascular endothelial growth factor in endometrium and placenta. *Am. J. Reprod. Immunol.* 37, 21–29. doi:10.1111/j.1600-0897.1997.tb00189.x

Vuorela, P., Helske, S., Hornig, C., Alitalo, K., Weich, H., and Halmesmäki, E. (2000). Amniotic fluid-soluble vascular endothelial growth factor receptor-1 in preeclampsia. *Obstet. Gynecol.* 95, 353–357. doi:10.1016/s0029-7844(99)00565-7

Zarezade, N., Saboori Darabi, S., Ramezanali, F., Amirchaghmaghi, E., Khalili, G., Moini, A., et al. (2015). mRNA expression of VEGF and its receptors in fallopian tubes of women with ectopic pregnancies. *Int. J. Fertil. Steril.* 9, 55–64. doi:10.22074/ijfs.2015.4209

Zou, S., Li, X., Feng, Y., Sun, S., Li, J., Egecioglu, E., et al. (2013). Comparison of the diagnostic values of circulating steroid hormones, VEGF-A, PIGF, and ADAM12 in women with ectopic pregnancy. *J. Transl. Med.* 11, 44. doi:10.1186/1479-5876-11-44

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Research trends and frontiers on antiphospholipid syndrome: A 10-year bibliometric analysis (2012–2021)

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Objectives: A growing body of studies related to antiphospholipid syndrome (APS) have been published in recent years. Nevertheless, there is a lack of visualized and systematic analysis in the literature on APS. Hence, this study sought to conduct a bibliometric analysis to identify research status and discover frontiers in the field.

Methods: Articles and reviews concerning APS were acquired from the Web of Science Core Collection (WoSCC) database. CiteSpace, VOSviewer and a bibliometric online analysis platform were employed to conduct a visualization and knowledge-map analysis.

Results: A total of 1,390 publications regarding APS were identified. Globally, Italy contributed the most publications. The University of Padua was the most productive institution. Lupus ranked first in both the most published and most co-cited journals. Savino Sciascia and Spiros Miyakis were the most prolific and most co-cited authors, respectively. "Vitamin K antagonists (VKA)" and "immunoglobulin A (IgA)" were current research foci. Burst analysis of keywords suggested that "neutrophil extracellular trap (NET)," "direct oral anticoagulant (DOAC)," "open label," "outcome," "hydroxychloroquine (HCQ)," and "arterial thrombosis (AT)" were significant future research frontiers.

Conclusion: The scientific literature on APS has increased steadily in the past 10 years. The clinical studies on the treatment and mechanism research of APS

Abbreviations: aCL, anticardiolipin; AT, arterial thrombosis; APS, antiphospholipid syndrome; aβ2GPI, anti-beta two glycoprotein I; aPL, antiphospholipid antibodies; CAPS, catastrophic antiphospholipid syndrome; COVID-19, coronavirus disease 2019; DOAC, direct oral anticoagulant; GAPSS, global antiphospholipid syndrome score; HCQ, hydroxychloroquine; IF, impact factor; IgA, immunoglobulin A; INR, international normalized ratio; JCR, Journal Citation Reports; LA, lupus anticoagulant; LLR, log-likelihood ratio; NET, neutrophil extracellular trap; PT, prothrombin time; SLE, systemic lupus erythematosus; Q, modularity; S, silhouette; SCI-Expanded, Science Citation Index Expanded; TIA, transient ischaemic attack; TI, title; New Engl J Med, New England Journal of Medicine; VKA, vitamin K antagonists; VTE, venous thromboembolism; WoS, Web of Science; WoSCC, Web of Science Core Collection.

are recognized as promising research hotspots in the domain of APS. The research status and trends of APS publications from the bibliometric perspective can provide a practical guide and important reference for subsequent studies by researchers and physicians in the domain.

KEYWORDS

antiphospholipid syndrome, bibliometric, CiteSpace, hotspots, VOSviewer

Introduction

Antiphospholipid syndrome (APS), also known as Hughes syndrome, is a systemic autoimmune disorder characterized by vascular (arterial, venous, microvascular) thrombosis and/or obstetric morbidity (Cohen and Isenberg, 2021). APS was first described by Professor Graham Hughes in 1983 (Hughes, 1983). It may occur alone, when it is called primary APS, or coexist with another autoimmune condition [mainly systemic lupus erythematosus (SLE)], that is, secondary APS (Luigi Meroni et al., 2019). The incidence and prevalence of APS are estimated to be approximately 2.1/100,000 per year and 50/100,000, respectively (Duarte-Garcia et al., 2019). The last 4 decades have witnessed a prominent evolution in the understanding of APS, and diagnostic methods have changed correspondingly. Although the classification criteria are frequently said to be unutilized for diagnosis, they are often applied to confirm the diagnosis in the domain of APS (Petri, 2020). The most famous seminars on the classification of APS were the conferences held at Sapporo in 1998 (Wilson et al., 1999) and Sydney in 2004 (Miyakis et al., 2006), which eventually reached an international consensus on the APS classification standard. The classification criteria for APS based on the Sydney standard are met when at least one clinical criterion (thrombosis or pregnancy morbidity) and at least one laboratory criterion [lupus anticoagulant (LA), anticardiolipin (aCL), or anti-beta two glycoprotein I (aβ2GPI) antibodies] are present. The above three antibodies are also collectively referred to as antiphospholipid antibodies (aPL). It was reported that the prevalence of aPL in the population was approximately 1–5%, but only a small proportion would develop APS (Cervera, 2017).

Of note, the aPL have recently been discovered in a considerable number of individuals with acute coronavirus disease 2019 (COVID-19), particularly in severe patients (Zhang et al., 2020; Karahan et al., 2022; Trahtemberg et al., 2021; Borghi et al., 2020), but it is still controversial whether they play a direct role in thrombosis or their existence is only a symptom of the disease's main infectious proinflammatory state (Foret et al., 2021). A more clinically challenging situation is the presence of catastrophic APS (CAPS), which is characterized by rapidly progressive small vessel thrombosis in multisystem organs within 1 week. Although CAPS is extremely infrequent, developing in less than 1% of individuals with APS (Rodriguez-Pinto et al., 2018), treatment must be started immediately to combat a mortality rate of up to 30% (Cervera et al., 2018). The pathophysiology of CAPS remains unclear; however, it has been hypothesized that several triggering factors, such as infection, surgery, trauma, and malignancy, may cause endothelial injury, which leads to an overproduction of cytokines and a thrombotic storm in the microcirculation (Rodriguez-Pinto et al., 2016).

A substantial number of research on APS has been conducted over the last 10 years. Nevertheless, the continuously expanding amount of studies makes it challenging for scholars to maintain pace with the most recent findings. Although some literature reviews and meta-analyses can present summary findings, these means usually fail to achieve the capture of changing trends in publications, the evaluation of research contributions, and the prediction of research hotspots. Bibliometric analysis is a method that employs statistical and mathematical techniques to analyze scientific literature both qualitatively and quantitatively, which is used to detect research status and hotspots in a specific area (Lin et al., 2022). Moreover, bibliometrics can use relevant parameters to present contributions from different countries, institutions, and authors to help with subsequent experimental strategies and funding decisions (Deng et al., 2022a). Bibliometric analysis has been broadly used in the medical area (Aksoy et al., 2021; Ma et al., 2021; Wu et al., 2021; Zhu et al., 2021; Shen et al., 2022). However, no targeted bibliometric analysis of scientific literature on APS has been carried out to date. Therefore, this study sought to present a bibliometric analysis of APS literature published during 2012–2021, thereby depicting the current research status, identifying the hotspots and development trends, and providing new references for future research directions of APS.

Materials and methods

Data collection

Web of Science (WoS) is a broad and reputable database channel for obtaining international academic sources, including more than 12,000 international academic periodicals (Wu et al., 2021). Publications regarding APS were extracted from the Science Citation Index Expanded (SCI-Expanded) of the WoS

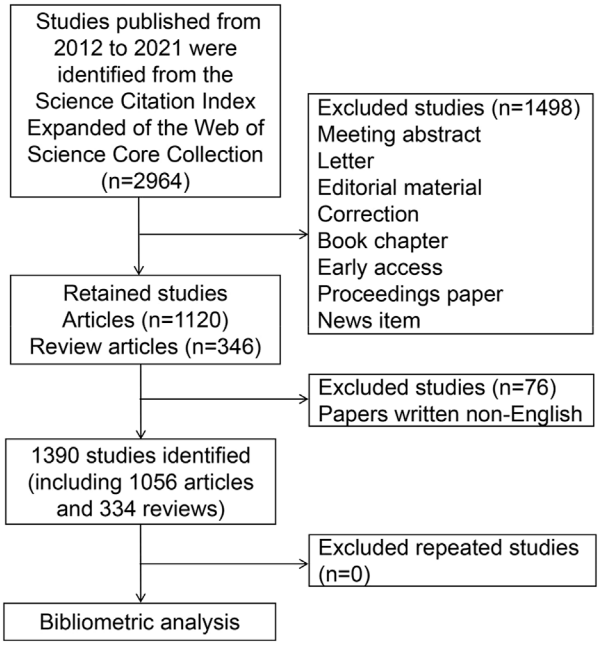

FIGURE 1
Flowchart of study retrieval and selection.

Core Collection (WoSCC) and downloaded within 1 day on 24 May 2022. To ensure high relevance of the content, terms referring to "antiphospholipid syndrome" were searched by title (TI).

The retrieval formula was as follows: TI = (antiphospholipid syndrome OR anti phospholipid syndrome OR anti-phospholipid syndrome OR antiphospholipid antibody syndrome OR anti phospholipid antibody syndrome OR anti-phospholipid antibody syndrome OR Hughes syndrome). The time range was limited from 2012 to 2021. Only English-language articles and reviews were included in this study. A total of 1,390 documents were ultimately acquired and stored as "plain text." Afterward, since CiteSpace can identify only files with a given name, these files were named "download_.txt". Finally, these publications were imported into CiteSpace software for de-duplication (Figure 1).

Data analysis

Microsoft Excel 2019 was used to evaluate the count of annual publications with APS research *via* a line chart. In addition, bibliometric analyses were carried out by three bibliometric tools. CiteSpace (version 5.8. R3), a Java-based tool, is a useful bibliometric software for analyzing scientific publications and presenting the knowledge framework *via* visualization results. Knowledge maps can intuitively capture the research hotspots and predict development trends within a specific area (Ma et al., 2021). In this work, the cooperation analysis of institutions and authors was conducted *via* CiteSpace. In addition, it was applied to implement a co-citation analysis of references and detect the burst references and keywords. In the network maps, each circle represents a research object, and the size of the circle is proportional to the publication or citation count. The line connecting the circles indicates the co-authorship or co-cited relationship, with thicker lines representing stronger collaboration or relevancy. The color of the line represents the time of the first co-authorship or occurrence, with a more yellow color meaning closer to 2021 and a more red color meaning closer to 2012. Centrality is an index used to evaluate the importance of elements in a map. The range of centrality is from 0 to 1. The higher centrality of an element means more frequent cooperation with other elements. Elements with a centrality value >0.1 usually imply significant influence, and the outermost ring of the element is displayed in purple. The following were CiteSpace's arguments: timespan: 2012–2021 (slice length = 1), selection criteria: g-index (k = 25), pruning: pathfinder, pruning sliced networks, pruning the merged network.

VOSviewer (version 1.6.17), another practical bibliometric tool created by Van Eck and Waltman, can construct and visualize bibliometric networks, allowing for a better comprehension of the framework and evolutionary trajectory of scientific research (van Eck and Waltman, 2010). Moreover, VOSviewer can offer three various types of maps: network, overlay, and density visualization maps. In the present research, this application was used to perform a co-occurrence analysis of the keywords and citation and co-citation relationships of journals.

Furthermore, the co-authorship of countries was conducted using a bibliometric online analysis platform (https://bibliometric.com/).

Results

Analysis of publication trends

After removing duplicates by CiteSpace software, a total of 1,390 publications of APS (1,056 articles and 334 reviews) were obtained from SCI-Expanded of WoSCC. Figure 2A exhibits the distribution of annual publications of APS literature, and research trends can be divided into two periods. From 2012 to 2016, the production of publications showed a gradual declining trend and reached a low point of 104 in 2016. From 2016 to 2021, the output of documents presented a rapid growth trend, with a peak in 2021. The quantity of papers published in 2021 was 1.68 times that in 2016.

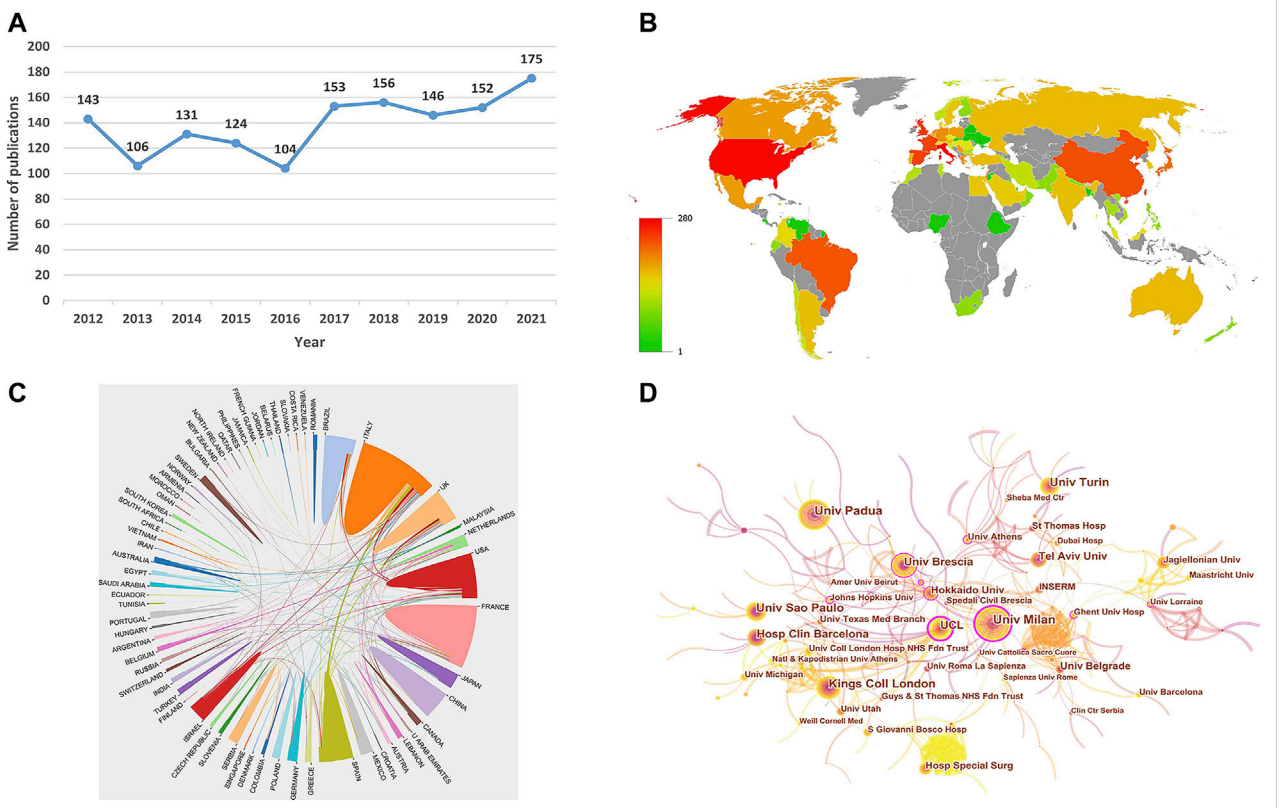

FIGURE 2
(A) Annual outputs of antiphospholipid syndrome (APS) from 2012 to 2021. **(B)** Map distribution on the sources of APS publications. **(C)** International cooperation analysis among countries by an online analysis platform. The curve between countries reflects the closeness of cooperation, thicker lines indicate closer cooperation. **(D)** International cooperation analysis among institutions by CiteSpace. Each circle represents an institution, and the size of the circle is proportional to the publication count. The circles with higher centrality (>0.1) are displayed with purple rings. The lines represent the strength of the co-authorship relationship, with thicker lines representing stronger collaboration. The color of the line represents the time of the first co-authorship, with a more yellow color meaning closer to 2021 and a more red color meaning closer to 2012.

TABLE 1 Top 10 countries with the highest productivity related to antiphospholipid syndrome (APS).

Rank	Country	Count (% of 1,390)	Number of papers per million people	Centrality
1	Italy	273 (19.64)	4.59	0.07
2	United States	258 (18.56)	0.78	0.09
3	United Kingdom	155 (11.15)	2.31	0.10
4	Spain	135 (9.71)	2.85	0.05
5	France	130 (9.35)	1.93	0.12
6	China	115 (8.27)	0.08	0.07
7	Brazil	104 (7.48)	0.49	0.02
8	Japan	78 (5.61)	0.62	0.02
9	Israel	67 (4.82)	7.27	0.06
10	Netherlands	55 (3.96)	3.15	0.04

Rank: based on the publication count. The demographic data were downloaded from the World Bank official website (https://data.worldbank.org.cn/).

TABLE 2 Top 10 most productive institutions related to APS.

Rank	Institution	Count (% of 1,390)	Centrality
1	Univ Padua (Italy)	69 (4.96)	0.03
2	Univ Milan (Italy)	62 (4.46)	0.27
3	Univ Sao Paulo (Brazil)	53 (3.81)	0.04
4	Univ Turin (Italy)	52 (3.74)	0.02
5	King's Coll London (United Kingdom)	50 (3.60)	0.05
6	UCL (United Kingdom)	49 (3.53)	0.27
7	Univ Brescia (Italy)	47 (3.38)	0.14
8	Hosp Clin Barcelona (Spain)	42 (3.02)	0.04
9	Tel Aviv Univ (Israel)	39 (2.81)	0.03
10	Univ Belgrade (Serbia)	28 (2.01)	0.09

Rank: based on the publication count.

A bibliometric online analysis platform for visualization of country collaboration

Figure 2B shows a world map revealing the production of each country. A total of 1,713 institutions from 73 countries were involved in 1,390 documents. The top 10 countries on APS are displayed in Table 1. The top three countries with the most publications were Italy (273, 19.64%), the United States (258, 18.56%), and the United Kingdom (155, 11.15%). In addition, considering the influence of the demographic profile of various countries on the quantity of papers published, a ratio indicator of the number of articles published per million population was adopted. After adjusting for population size, Israel ranked first with 7.27 articles per million people. In terms of centrality, the top three countries were France (0.12), the United Kingdom (0.10) and the United States (0.09). The cooperation relationships among the countries are shown in Figure 2C. The width of the line represents the frequency of collaboration between two countries, and thicker lines indicate closer cooperation. As illustrated in Figure 2C, Italy collaborates closely with the United Kingdom, Spain, and the United States.

CiteSpace for visualization of institution collaboration

Among the top 10 most productive institutions (Table 2), four were located in Italy, two in the United Kingdom, and one each in Brazil, Spain, Israel, and Serbia. Table 2 lists that the University of Padua contributes the largest number of documents (69, 4.96%), followed by the University of Milan (62, 4.46%), the University of Sao Paulo (53, 3.81%), the University of Turin (52, 3.74%), and King's College London (50, 3.60%). As exhibited in Figure 2D, each node denotes an institution, and the size of the node signifies the amount of papers produced by the institution. With respect to centrality, the University of Milan and the University College London ranked first with 0.27, followed by the University of Brescia (0.14) and the University of Belgrade (0.09). Figure 2D demonstrates that the research on APS in Italy and the United Kingdom is led by the University of Padua and King's College London, respectively.

CiteSpace for visualization of authors and co-cited authors

A total of 5,844 authors were responsible for these 1,390 publications. As presented in Table 3, Savino Sciascia is the most prolific author (44, 3.17%), followed by Ricard Cervera (39, 2.81%), Amelia Ruffatti (37, 2.66%), Pier Luigi Meroni (37, 2.66%), and Angela Tincani (36, 2.59%). Figure 3A illustrates that a certain degree of collaboration is observed among the various authors. The authors with the highest centrality were Laura Andreoli (0.34), Gerard Espinosa (0.31), and Angela Tincani (0.27), indicating their important bridging roles in APS research.

TABLE 3 Top 10 authors with the most publications involved in APS.

Rank	Author	Count (% of 1,390)	Centrality
1	Savino Sciascia	44 (3.17)	0.04
2	Ricard Cervera	39 (2.81)	0.00
3	Amelia Ruffatti	37 (2.66)	0.11
4	Pier Luigi Meroni	37 (2.66)	0.05
5	Angela Tincani	36 (2.59)	0.27
6	Laura Andreoli	28 (2.01)	0.34
7	Vittorio Pengo	27 (1.94)	0.01
8	Gerard Espinosa	27 (1.94)	0.31
9	Doruk Erkan	25 (1.80)	0.09
10	Maria Gerosa	23 (1.65)	0.17

Rank: based on the publication count.

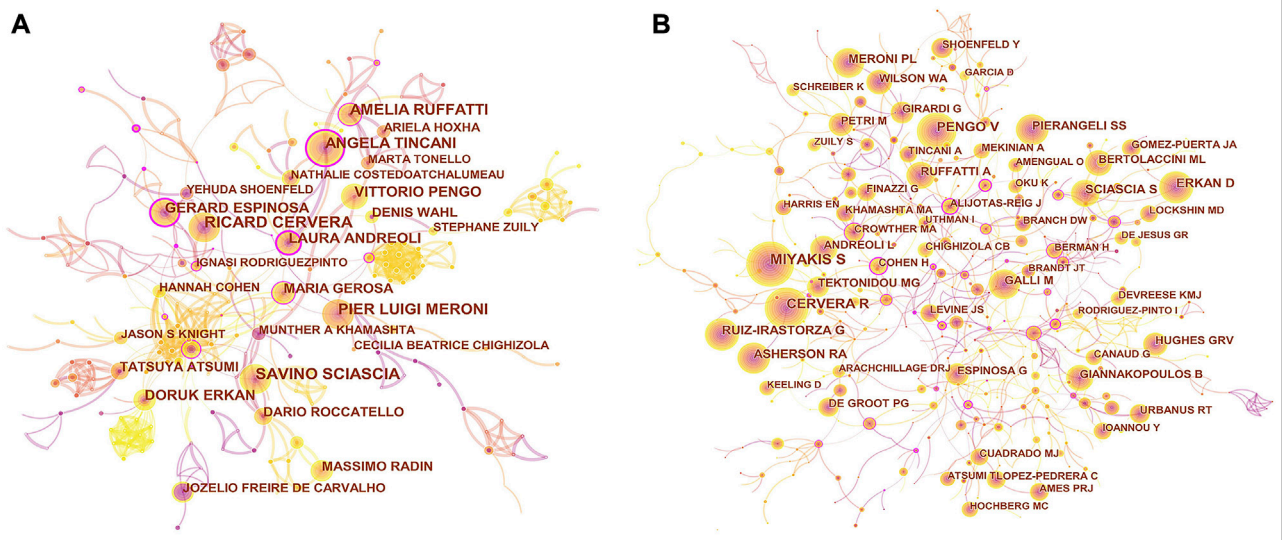

FIGURE 3
(A) CiteSpace visualization map of authors in APS research. **(B)** CiteSpace visualization map of co-cited authors involved in APS. Each circle represents an author, and the size of the circle is proportional to the publication count **(A)** or the number of citations **(B)**. The circles with higher centrality (>0.1) are displayed with purple rings. The lines represent the strength of the co-authorship **(A)** or co-cited relationship **(B)**, with thicker lines representing stronger collaboration **(A)** or relevancy **(B)**. The color of the line represents the time of the first co-authorship, with a more yellow color meaning closer to 2021 and a more red color meaning closer to 2012.

Co-cited authors are two authors cited by another literature at the same time. Table 4 displays that the top 10 co-cited authors are cited > 240 times. Miyakis S owned the most co-citations (962), followed by Cervera R (624), Pengo V (479), Erkan D (364), and Ruiz-Irastorza G (345). Regarding centrality, Sciascia S ranked first with 0.05. Figure 3B shows that Miyakis S is most frequently co-cited with Tektonidou MG, and the connection between the two authors is given in yellow. Notably, none of the top 10 co-cited authors have a purple outer ring (centrality > 0.1).

TABLE 4 Top 10 co-cited authors with the most citations involved in APS.

Rank	Co-cited author	Citation	Centrality
1	Miyakis S	962	0.00
2	Cervera R	624	0.01
3	Pengo V	479	0.00
4	Erkan D	364	0.01
5	Ruiz-Irastorza G	345	0.00
6	ASherson RA	327	0.00
7	Sciascia S	299	0.05
8	Meroni PL	286	0.00
9	Pierangeli SS	251	0.00
10	Galli M	242	0.02

Rank: based on the citation count.

VOSviewer for visualization of journals and co-cited journals

All documents related to APS research were distributed in 396 journals. Table 5 summarizes that the top 10 most active journals contribute 33.96% of the publications in this area (472). In detail, Lupus offered the highest volume of articles (168, 12.09%), followed by Autoimmunity Reviews (59, 4.24%), Clinical Rheumatology (39, 2.81%), Current Rheumatology Reports (38, 2.73%), and Rheumatology (37, 2.66%). The density map is used to exhibit the journals with publications ≥7 (Figure 4A). Of the top 10 periodicals, seven journals were Q1 in the Journal Citation Reports (JCR) 2021 standards, and Autoimmunity Reviews possessed the highest impact factor (IF; 17.390). In summary, these ten journals laid a firm foundation for future APS research.

Table 6 indicates that the most frequently co-cited journal is Lupus (4,012), followed by Arthritis and Rheumatology (3,330), Journal of Thrombosis and Haemostasis (3,247), Blood (2,694), and Autoimmunity Reviews (2,240). Of these top 10 co-cited periodicals, seven journals had citation times surpassing 1,600. A density map is employed to show the co-cited journals with citations ≥120 (Figure 4B). Of these periodicals, the New England Journal of Medicine (New Engl J Med) possessed the highest IF (176.079), followed by Annals of the Rheumatic Diseases (27.973) and Blood (25.476).

TABLE 5 Top 10 most productive journals related to APS.

Rank	Journal	Count (% of 1,390)	IF (2021)	JCR (2021)
1	Lupus	168 (12.09)	2.858	Q4
2	Autoimmunity Reviews	59 (4.24)	17.390	Q1
3	Clinical Rheumatology	39 (2.81)	3.650	Q3
4	Current Rheumatology Reports	38 (2.73)	4.686	Q2
5	Rheumatology	37 (2.66)	7.046	Q1
6	Thrombosis Research	35 (2.52)	10.407	Q1
7	Journal of Thrombosis and Haemostasis	29 (2.09)	16.036	Q1
8	Frontiers in Immunology	27 (1.94)	8.786	Q1
9	Journal of Autoimmunity	20 (1.44)	14.511	Q1
10	Seminars in Thrombosis and Hemostasis	20 (1.44)	6.398	Q1/Q2

Rank: based on the publication count. IF: impact factor. JCR: journal citation reports.

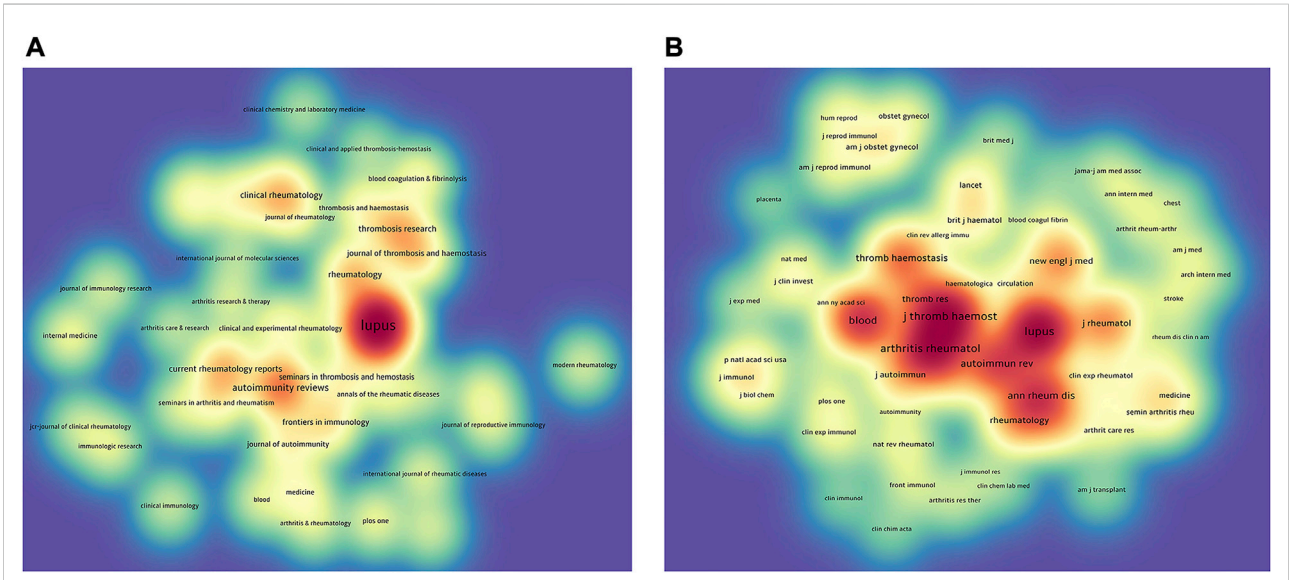

FIGURE 4
(A) VOSviewer density map of journals with publications ≥7 in APS research. The size of the title of the journal is proportional to the publication output. The more publications the journal produces, the closer the bottom background color is to red. **(B)** VOSviewer density map of co-cited journals with citations ≥120 involved in APS. The size of the title of the journal is proportional to the citation count. The more citations a journal receives, the closer the bottom background color is to red.

Analysis of highly-cited articles

Analyzing highly-cited papers helps to comprehend the basis of disciplinary research. Based on the number of citations, the top 10 most cited publications on APS are exhibited in Table 7. Of them, six belonged to original articles and four belonged to reviews. All these documents were published during 2012–2019, and all of them were cited more than 210 times. The highest number of citations was Giannakopoulos et al. (Giannakopoulos and Krilis, 2013) in the New Engl J Med (394), followed by Andreoli et al. (Andreoli et al., 2017) in Annals of the Rheumatic Diseases (334) and Cervera et al. (Cervera et al., 2015) in Annals of the Rheumatic Diseases (329). These studies were frequently regarded as a knowledge base in APS research.

CiteSpace for visualization of co-cited references and references burst

Reference co-citation analysis is a significant tool for exploring the evolution and discovering the developmental

TABLE 6 Top 10 co-cited journals with the most citations related to APS.

Rank	Co-cited journal	Citation	IF (2021)	JCR (2021)
1	Lupus	4,012	2.858	Q4
2	Arthritis and Rheumatology	3,330	15.483	Q1
3	Journal of Thrombosis and Haemostasis	3,247	16.036	Q1
4	Blood	2,694	25.476	Q1
5	Autoimmunity Reviews	2,240	17.390	Q1
6	Annals of the Rheumatic Diseases	2,234	27.973	Q1
7	Thrombosis and Haemostasis	1,643	6.681	Q1
8	Journal of Rheumatology	1,398	5.346	Q2
9	New England Journal of Medicine	1,384	176.079	Q1
10	Thrombosis Research	1,204	10.407	Q1

Rank: based on the citation count.

TABLE 7 Top 10 most cited publications on APS.

Rank	Title	First author	Journal	Year	Total citation
1	The pathogenesis of the antiphospholipid syndrome	Giannakopoulos, B	New England Journal of Medicine	2013	394
2	EULAR recommendations for women's health and the management of family planning, assisted reproduction, pregnancy and menopause in patients with systemic lupus erythematosus and/or antiphospholipid syndrome	Andreoli, L	Annals of the Rheumatic Diseases	2017	334
3	Morbidity and mortality in the antiphospholipid syndrome during a 10-year period: a multicentre prospective study of 1,000 patients	Cervera, R	Annals of the Rheumatic Diseases	2015	329
4	Rivaroxaban vs. warfarin in high-risk patients with antiphospholipid syndrome	Pengo, V	Blood	2018	311
5	EULAR recommendations for the management of antiphospholipid syndrome in adults	Tektonidou, MG	Annals of the Rheumatic Diseases	2019	297
6	Guidelines on the investigation and management of antiphospholipid syndrome	Keeling, D	British Journal of Haematology	2012	288
7	Diagnosis and management of the antiphospholipid syndrome	Garcia, D	New England Journal of Medicine	2018	270
8	The hyperferritinemic syndrome: macrophage activation syndrome, Still's disease, septic shock and catastrophic antiphospholipid syndrome	Rosario, C	BMC Medicine	2013	266
9	Rivaroxaban *versus* warfarin to treat patients with thrombotic antiphospholipid syndrome, with or without systemic lupus erythematosus (RAPS): a randomised, controlled, open-label, phase 2/3, non-inferiority trial	Cohen, H	Lancet Haematology	2016	225
10	Antiphospholipid syndrome	Schreiber, K	Nature Reviews Disease Primers	2018	215

Rank: based on the citation count.

frontiers in a given domain. CiteSpace is utilized for clustering analysis of co-cited references, and the whole network map is classified into 10 distinct clusters based on the log-likelihood ratio (LLR) algorithm (Figure 5A). In cluster analysis, the mean silhouette (S) value is an index of cluster homogeneity, and S >0.7 denotes the conviction of the clustering results. The modularity (Q) value is an index of the degree of grouping of nodes, and Q >0.3 denotes the significance of the clustering structure (Chen et al., 2012). The Q value was 0.5902 and the S value was 0.8428 in this work, indicating the reliability of the results. As described in Figure 5A, each cluster is represented by a different color, and the smaller the number label, the larger the cluster profile, meaning that the cluster contains more co-cited references (dots). Table 8 illustrates that "vitamin K antagonists (VKA)" is the largest cluster (#0), followed by "agapss" (#1), "CAPS" (#2), and "β2GPI" (#3). The timeline view in Figure 5B presents the evolution process of each cluster. The elements on the horizontal axis represent co-cited

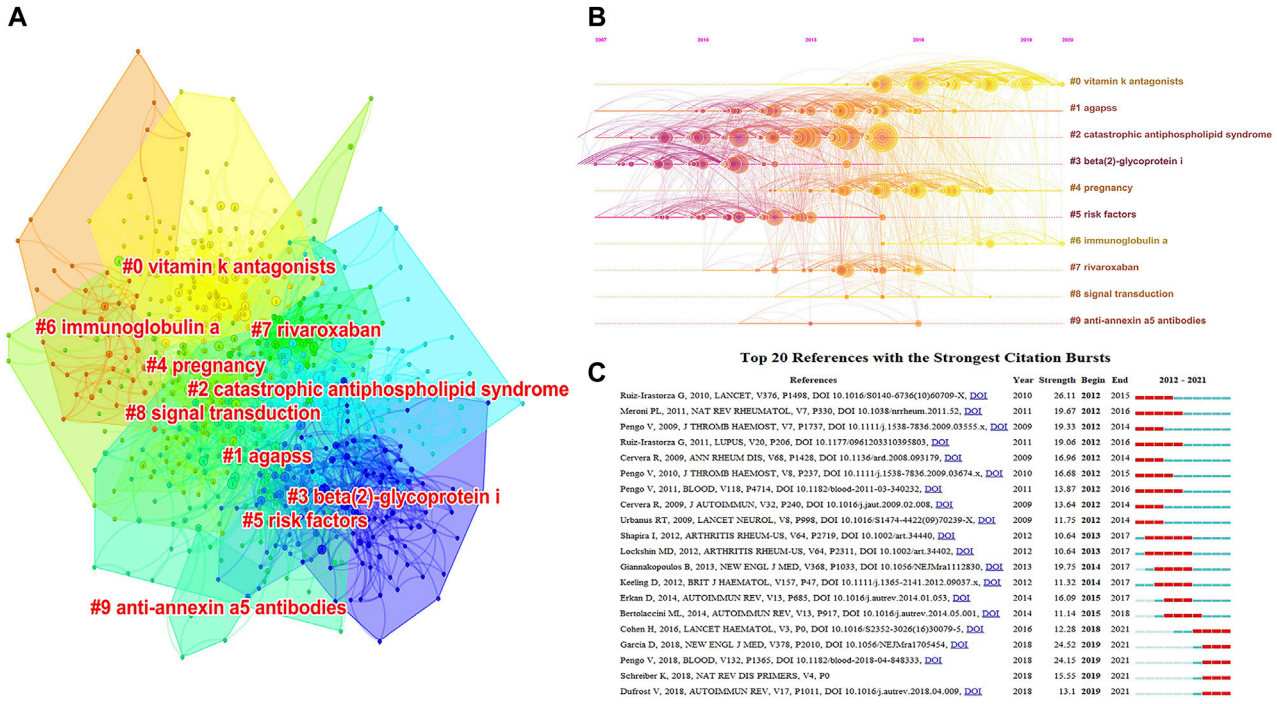

FIGURE 5
(A) Ten clustering maps of reference co-citation analysis based on CiteSpace. Each cluster is represented by a different color, and the smaller the number label, the larger the cluster profile and the more co-cited references (dots) it contains. **(B)** Timeline view map of reference co-citation analysis generated by CiteSpace. The elements on the horizontal axis represent co-cited references; the position of the element on the horizontal axis denotes the time of the first occurrence; and the line connecting the elements denotes the co-cited relationship. The size of the element is proportional to the citation count of the reference. A more yellow color indicates closer to the year 2021, and a more red color indicates closer to the year 2012. **(C)** Top 20 references with the strongest citation bursts by CiteSpace. Each rectangle represents a year. Green rectangles indicate the time interval, and red rectangles indicate the duration of the burst from the beginning to the end.

TABLE 8 The clusters information of co-cited references on APS.

Cluster ID	Size	Silhouette	Label (LLR)	Mean year
#0	116	0.865	Vitamin K antagonists	2017
#1	106	0.797	Agapss	2014
#2	100	0.831	Catastrophic antiphospholipid syndrome	2011
#3	87	0.842	Beta (2)-glycoprotein I	2009
#4	86	0.791	Pregnancy	2015
#5	55	0.835	Risk factors	2010
#6	39	0.903	Immunoglobulin A	2018
#7	36	0.912	Rivaroxaban	2014
#8	19	0.944	Signal transduction	2015
#9	6	0.994	Anti-annexin A5 antibodies	2013

Rank: based on the size. "Size" denotes the amount of co-cited references that a cluster contains. LLR: log-likelihood ratio.

references; the position of the element on the horizontal axis denotes the time of the first occurrence; and the line connecting the elements denotes the co-cited relationship. The size of the element is proportional to the citation count of the reference. A more yellow color indicates closer to the year 2021, and a more red color indicates closer to the year 2012. The research

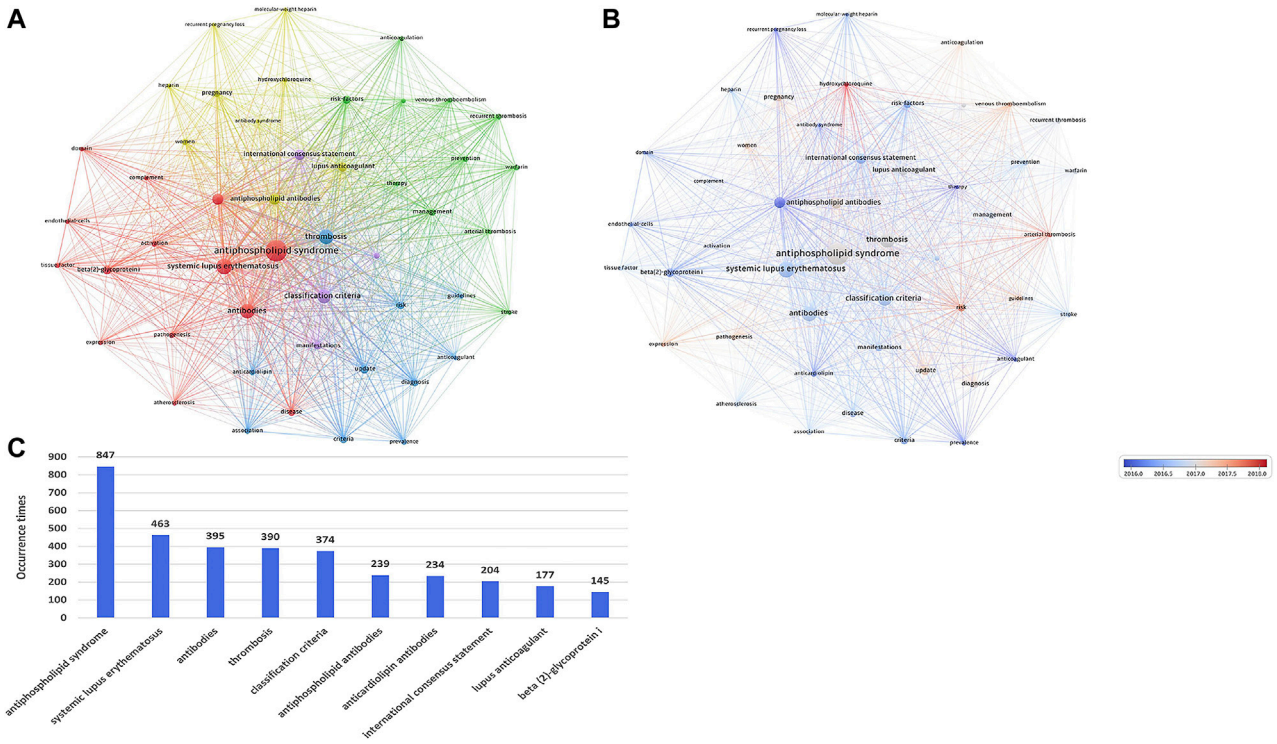

FIGURE 6
(A) Network clustering map of keywords' co-occurrence analysis based on VOSviewer. The minimum threshold for the number of keyword frequencies is 40. Keywords were divided into 5 clusters: cluster 1 (red), cluster 2 (green), cluster 3 (blue), cluster 4 (yellow), and cluster 5 (purple). Each keyword is represented as a node, and the node size is proportional to the frequency. The line between nodes denotes a co-occurrence relationship. The distance between nodes denotes the degree of relevancy, and the closer the distance, the higher the degree of relevancy. **(B)** Overlay map of keywords' co-occurrence analysis based on VOSviewer. The node color denotes the corresponding average occurrence time according to the color gradient in the lower right corner. Nodes displayed in blue represent keywords that appear relatively early, while nodes displayed in red represent keywords that appear later. **(C)** Top 20 keywords with the highest occurrence times.

hotspots have shifted from "β2GPI" (#3), "risk factors" (#5), and "CAPS" (#2) to "pregnancy" (#4), "VKA" (#0), and "immunoglobulin A (IgA)" (#6).

Burst detection is a useful approach for capturing rapid increases in the popularity of references or keywords, which denotes that the topic has received great attention over a set period. Figure 5C lists the top 20 references with the strongest citation bursts. The red segments in this picture signify the duration of the reference outbreak, and the green lines denote the time period. Of these co-cited references, Ruiz-Irastorza et al. (Ruiz-Irastorza et al., 2010) in Lancet had the strongest strength of burst (26.11), followed by Garcia et al. (Garcia and Erkan, 2018) in the New Engl J Med (24.52) and Pengo et al. (Pengo et al., 2018) in Blood (24.15). Notably, of these top 20 references, articles from Pengo V occupied one-fifth of the seats (4), demonstrating the profound influence of Pengo V in the APS field. While the outbreak of most references has ended, there are still five references in the citation burst, implying that these areas are research directions to focus on in the future.

VOSviewer for visualization of keywords co-occurrence and evolution and CiteSpace for visualization of keywords burst

The co-occurrence analysis of keywords reveals the dominating motifs within a specific field. In the clustering analysis, after merging keywords with the same content, 48 items were identified and classified into 5 clusters (minimum amount of frequencies of a keyword ≥40). As exhibited in Figure 6A, a keyword is represented as a node, and the size of the node reflects the frequency of the keyword.

The line between nodes denotes a co-occurrence relationship. The distance between nodes denotes the degree of relevancy, and the closer the distance, the higher the degree of relevancy. The five clusters, which were centered on APS, risk factors, thrombosis, aPL, and classification criteria, were each marked with a different color. Nodes with comparable characteristics were split into a color-marked cluster and displayed in red (cluster 1, studies on pathogenesis), green

(cluster 2, studies on risk factors and prevention), blue (cluster 3, studies on diagnosis), yellow (cluster 4, studies on treatment), and purple (cluster 5, studies on classification).

Figure 6B illustrates the dynamic evolution of keywords over time. Keywords that presented comparatively earlier are marked in blue, while keywords that emerged relatively later are marked in red. Keywords such as "therapy," "aCL antibodies," "recurrent pregnancy loss," "anticoagulant," and "antibody syndrome" were the major topics in the early stage. Keywords such as "venous thromboembolism (VTE)," "expression," "risk," "arterial thrombosis (AT)," and "hydroxychloroquine (HCQ)" evolved more recently, implying that these themes are attracting much attention at present.

As shown in Figure 6C, the top 10 keywords appear >140 times. The most commonly occurring keyword was APS (847), followed by SLE (463), antibodies (395), thrombosis (390), and classification criteria (374). Figure 7 presents the top 25 burst keywords. From 2017 to the present, the terms "neutrophil extracellular trap (NET)," "direct oral anticoagulant (DOAC)," "open label," "outcome," "HCQ," and "AT" are still in the outbreak period.

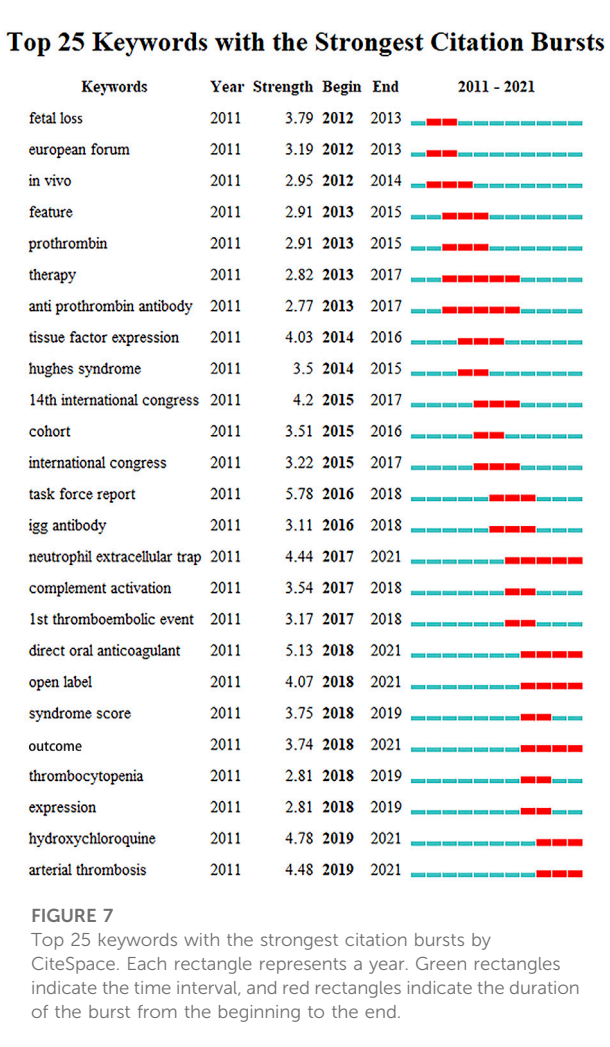

FIGURE 7
Top 25 keywords with the strongest citation bursts by CiteSpace. Each rectangle represents a year. Green rectangles indicate the time interval, and red rectangles indicate the duration of the burst from the beginning to the end.

Discussion

General information

Through bibliometrics, this study performs a systematic and visual analysis of the APS field and detects the research status and hotspots in the domain. The annual literature volume and trend can reveal the pace of development in a given field. According to qualitative and quantitative analysis using CiteSpace and VOSviewer software, scientific publications related to APS have been increasing continuously over the last 10 years. While the number of documents fluctuated and declined at certain time points, the overall trend gradually increased and reached its peak in 2021, with 175 papers, suggesting that the field of APS has attracted increasing attention from experts and scholars. In the work of Deng et al. and Liu et al., similar increasing trends were described (Deng et al., 2022b; Liu et al., 2022).

As shown in Table 1, Italy is the most productive country (273, 19.64%), followed by the United States (258, 18.56%) and the United Kingdom (155, 11.15%), which together account for 49.35% of the total. However, after adjusting for population size, Israel ranked on top, with 7.27 articles per million people. Of note, apart from Brazil and China, the rest of the top 10 countries all belong to developed countries. The main causes of differences in literature output between countries may be attributed to disparities in socioeconomic position, general research ability, and population size (Fan et al., 2017). In addition, only one of the top 10 countries had a centrality greater than 0.1, implying a lack of strong influence from these countries in the domain of APS. Regarding institutional contributions (Table 2), the University of Padua (Italy) had the greatest number of publications (69, 4.96%), whereas the University of Milan and the University College London ranked first in centrality value with 0.27, suggesting the high impact of these institutions in the field of APS. Among the top 10 institutions, eight were from developed countries, and there were four organizations from Italy, which helped reveal why Italy has been a leader in this field for the past decade. As shown in Figure 2C and Figure 2D, the close cooperation among countries and institutions is mainly focused on developed countries. To better foster the prosperity of this field, geographical barriers should be broken down, and exchanges and collaboration between developed and developing countries in this field should be further strengthened.

For authors (Table 3) and co-cited authors (Table 4), Savino Sciascia (44, 3.17%) contributes the most documents, while

Miyakis S owns the most co-citations (962). Five authors had a crucial bridging role among the top 10 authors (centrality >0.1). Notably, although Miyakis S had not published many papers, he topped the list of the top 10 co-cited authors. This phenomenon may be attributed to the high-impact article he has contributed (Miyakis et al., 2006). Professor Savino Sciascia is from San Giovanni Bosco Hospital in Italy. In 2013, Savino Sciascia and his colleagues developed and validated a risk score [global APS score (GAPSS)] in patients with SLE (Sciascia et al., 2013). The use of GAPSS significantly improved the prediction of the risk of thrombosis or pregnancy loss in SLE and transformed the concept of aPL as diagnostic antibodies to aPL as a risk factor for clinical events. In 2015, Savino Sciascia and his colleagues evaluated the clinical relevance of GAPSS in primary APS patients (Sciascia et al., 2015). They found higher GAPSS values in patients with only thrombosis compared to those with only pregnancy loss; individuals with recurrent thrombosis had higher GAPSS values than those without recurrence. They proposed that GAPSS values ≥11 were closely related to a higher risk of thrombotic recurrence. The study showed that the GAPSS was a useful method that significantly enhanced risk stratification regarding thrombosis in primary APS.

The analysis of journals indicated that Lupus is the most active periodical (168, 12.09%), followed by Autoimmunity Reviews (59, 4.24%) and Clinical Rheumatology (39, 2.81%) (Table 5). Scholars interested in APS research can pay more attention to these magazines because of their large volume of publications on APS. Regarding the sources of these top 10 magazines, nine are from countries in Western Europe and North America. None of the journals from Asian countries appeared on the list. Thus, Asian countries may enhance the construction of journals in the field of APS. For co-cited journals (Table 6), Lupus was the journal with the highest citations (4,012), followed by Arthritis and Rheumatology (3,330), Journal of Thrombosis and Haemostasis (3,247), Blood (2,694), and Autoimmunity Reviews (2,240). Of note, Lupus came out on top in both the most published and most co-cited journals, demonstrating its dominance in the area of APS. Of these top 10 co-cited journals, eight periodicals were located in the Q1 JCR region, implying that high-impact magazines had an interest in APS-related research. In addition, a 40% agreement rate is achieved between the top 10 periodicals and co-cited periodicals, implying a certain degree of focus on the simultaneous development of quantity and quality in these journals.

The impact of a publication can be reflected by the number of citations to some extent. Table 7 displays the top 10 most cited documents on APS. Specifically, Giannakopoulos et al. published "The pathogenesis of the antiphospholipid syndrome" in the New Engl J Med in 2013 (Giannakopoulos and Krilis, 2013), which was the most cited study (394). This essay provided a detailed overview of the thrombotic mechanisms of APS, including post-translational redox modifications of β2GPI, conformations of β2GPI, the "two hit" model, endothelial nitric oxide synthase, endothelial cells and monocytes, tissue factor, factor XI, platelets, annexin A5 anticoagulant shield and HCQ, complement and neutrophils, and disturbance of innate immunity, which were involved in the pathophysiology of APS. In summary, the top 10 publications with the highest citations were focused on the following topics: pathogenesis, the management of pregnancy, epidemiology, diagnosis and treatment.

The analysis of hotspots and frontiers

Reference co-citation analysis is a useful method to evaluate progress and identify hotspots within a specific field. As shown in Table 8, before 2014, the topics appearing more often are "β2GPI" (#3), "risk factors" (#5), "CAPS" (#2), and "anti-annexin A5 antibodies" (#9). After 2017, the more common subjects are "VKA" (#0) and "IgA" (#6), implying that the issues of the above clusters are the current research focuses in the domain. As the third most frequent cause of mortality worldwide (Bitsadze et al., 2022), VTE is considered to be the most common clinical feature of APS. VKA, primarily warfarin, is recognized as the gold standard for secondary prevention of thrombotic APS (Fujieda and Amengual, 2020). In individuals with APS and a first onset of VTE, a VKA with an international normalized ratio (INR) aim of two to three is recommended (Tektonidou et al., 2019). Proof from previous studies indicated that higher anticoagulation strength (INR 3.0–4.5) provided no additional benefit (Crowther et al., 2003; Finazzi et al., 2005). Due to the risk of bleeding with long-term VKA use, regular INR monitoring is essential. Prothrombin time-INR (PT-INR) is the standard way for monitoring the anticoagulant strength of VKA. However, in LA-positive APS patients, thromboplastin can be affected, resulting in PT-INR being raised (Cohen et al., 2021). Thus, more effective anticoagulation monitoring tools merit additional consideration. IgA isotypes of aPL are not presently included in standard diagnostic procedures for APS, and their value as a diagnostic marker is still up for debate (Sciascia et al., 2017). Several studies have demonstrated a link between the IgA isotypes and thrombosis (Pericleous et al., 2016; Tebo et al., 2016). Recently, Reshetnyak et al. reported that IgA aCL and IgA aβ2GPI had a high specificity (95% and 93%) but a low sensitivity (54% and 44%) in detecting APS (Reshetnyak et al., 2022). The diagnostic value of IgA aPL is worthy of further study in the future. The timeline view of co-cited references visually displays the evolutionary trajectory of each cluster (Figure 5B).

References with citation bursts mean the sudden rise of citations of specific publications in a given stage, which can serve as a practical technique to find emerging themes attracting great interest during some period of time. The top 20 references with the strongest citation bursts are presented in Figure 5C. It is

worth noting that while the bursts in most references were finished, five documents (20%) in the top 20 remain in a state of burst, focusing on the diagnosis and treatment of APS, suggesting that these research topics are the most up-to-date ones at the moment. Of these five papers, the essay with the highest burst strength (24.52) was published by Garcia et al. (Garcia and Erkan, 2018) in the New Engl J Med in 2018. This paper expounded on evidence-based recommendations for the recognition and diagnosis of APS, as well as therapy recommendations for patients with persistently positive aPL. Pengo et al. (Pengo et al., 2018) offered the second-ranked essay (24.15), which compared the efficacy and safety of rivaroxaban *versus* warfarin in high-risk triple-positive (presence of LA, aβ2GP1, and aCL) APS patients. The results showed 11 (19%) events in the rivaroxaban arm and 2 (3%) in the warfarin arm. Finally, the trial was terminated early due to an excess of events in patients in the rivaroxaban group. Schreiber et al. (Schreiber et al., 2018) offered the third-ranked essay (15.55), which described the pathogenesis, diagnosis, and treatment of APS in detail and provided an outlook on future research subjects. Dufrost et al. (Dufrost et al., 2018) offered the fourth-ranked essay (13.1), which evaluated the prevalence of recurrent thrombosis in patients with APS receiving DOACs treatment and detected risk factors related to recurrent thrombosis. The results indicated a higher thrombotic risk in a portion of individuals with APS treated with DOACs. Cohen et al. (Cohen et al., 2016) offered the fifth-ranked essay (12.28), which compared the efficacy and safety of rivaroxaban and warfarin in patients with thrombotic APS for the first time. The results showed that compared to the use of warfarin, the use of rivaroxaban resulted in a 100% increase in endogenous thrombin potential and a 40% reduction in peak thrombin generation, with a similar incidence of adverse effects.

In addition to references, keywords also serve as a representation of the main motifs and core contents in a given field. Based on the co-occurrence analysis of high-frequency keywords (Figure 6A), five current research directions for APS were recognized, which are as follows: "the pathogenesis of APS," "the prevention and risk factors for APS," "the research on the diagnosis of APS," "the treatment of APS" and "the classification for APS." Moreover, we conduct the evolution process of high-frequency keywords to better understand the mutative course of the APS research themes (Figure 6B). Keywords are marked with various colors depending on the average occurrence time of the items, that is, red keywords arise on average later than blue keywords. In the early period, the majority of blue keywords appeared in cluster 3 (blue, focusing on diagnosis) and cluster 5 (purple, focusing on classification). In recent years, most of the red keywords appear in cluster 1 (red, focusing on pathogenesis), cluster 2 (green, focusing on risk factors and prevention) and cluster 4 (yellow, focusing on treatment), suggesting that after 2018, more studies will be concentrated on the pathogenesis, risk factors and prevention and treatment of APS.

Furthermore, we identified the top 25 burst keywords between 2012 and 2021. Of these, we primarily focus on those topics that continue to explode into 2021, which indicates that these subjects are potential research frontiers for the future. As shown in Figure 7, these keywords are mainly associated with clinical studies on the treatment and mechanism research of APS, including NET, DOAC, open label, outcome, HCQ, and AT. Neutrophils contribute to thrombosis by releasing substances into the extracellular space, primarily DNA and histones, defined as NETs (Meng et al., 2017). A cross-sectional study found that NETs contribute to activated protein C resistance, which plays a role in the hypercoagulable condition of APS individuals (Foret et al., 2022). Mazetto et al. (Mazetto et al., 2022) recently reported that patients with thrombotic APS have elevated levels of gene expression associated with neutrophil activity and the release of NETs, which may be involved in thrombus formation.

In terms of treatment, as mentioned earlier, warfarin is considered the gold standard for secondary prevention of thrombotic APS (Fujieda and Amengual, 2020). However, the presence of APS patients on warfarin requiring frequent blood draws to monitor INR and warfarin intolerance has led to considerable interest in the use of DOACs instead of VKA (Ghembaza and Saadoun, 2020). The major benefits of DOACs *versus* VKA are their standardized dose regimen, faster and more predictable anticoagulant reaction, absence of frequent laboratory monitoring, and less major bleeding (Wiggins et al., 2020). Several recent randomized controlled and open-label studies have compared the efficacy of DOACs *versus* VKA in patients with APS (Cohen et al., 2016; Pengo et al., 2018; Ordi-Ros et al., 2019), and their outcomes appear to be contradictory, but it is worth noting that these trials were conducted in diverse APS populations. The results of Pengo et al.'s study (all of 120 patients were triple aPL positive) indicated that 12% of those using rivaroxaban experienced thromboembolic events, compared to 0% of those taking warfarin (Pengo et al., 2018). The outcomes of Cohen et al.'s study (28% of 116 patients were triple aPL positive) showed that neither the VKA nor rivaroxaban groups had experienced recurrent thrombosis after 210 days of follow-up (Cohen et al., 2016). The results of Ordi-Ros et al.'s study (61% of 190 patients were triple aPL positive) suggested that the VKA and rivaroxaban groups had no statistical difference in the rate of recurrent thrombosis, although higher AT occurred in the rivaroxaban group (Ordi-Ros et al., 2019). A more recent meta analysis of seven randomised controlled studies demonstrated that VKA is a more effective treatment option for individuals with APS than DOACs, especially rivaroxaban, given that DOACs use is linked to a 69% higher risk of thromboembolic events (Koval et al., 2021). To demonstrate the effectiveness of DOACs in different subgroups of APS

patients and different types of DOACs for the treatment of APS, further high-quality studies are warranted.

Moreover, additional treatment with HCQ, an antimalarial agent, has been frequently mentioned in the literature in recent years. HCQ has already been demonstrated to reduce the risk of thrombosis in individuals with SLE (Petri, 2011). Kravvariti et al. (Kravvariti et al., 2020) reported the results of a randomized controlled study, which suggested that HCQ could also reduce the incidence of thrombosis and lower APL titers in APS patients. A similar result was reported in a retrospective study by Nuri et al. (Nuri et al., 2017), which found that HCQ could also decrease APL levels and significantly diminish the recurrence rate of AT in individuals with APS. Several studies have attributed the thromboprophylaxis effects of HCQ to its anti-inflammatory, immunoregulatory, metabolic and antithrombotic properties (Ben-Zvi et al., 2012; Andrade and Tektonidou, 2016).

Stroke and transient ischaemic attack (TIA) are the two symptoms of APS that occur most frequently in the arterial circulation. It was reported that the prevalence of stroke and TIA in individuals with APS was 13.1% and 7.0%, respectively (Cervera et al., 2002). An analysis of five cohort studies suggested that low-dose aspirin reduced the risk of first AT in patients with aPL (Arnaud et al., 2015). A greater probability of recurrence exists in APS patients with AT compared to those with VTE. However, the optimal anticoagulant target after AT is still up for debate. For individuals with AT, the Galveston guidelines suggested either high-intensity warfarin at an INR >3.0 or combined treatment with low-dose aspirin plus warfarin (INR 2.0–3.0) (Ruiz-Irastorza et al., 2011). Adequate and robust antithrombotic treatment strategies after AT is an urgent issue that needs to be addressed.

Limitations

The study had several limitations. First, although limiting the search to titles can improve the accuracy and relevance of our search results, it is inevitable that articles related to the topic may be missed, and using a combination of titles and logical operators may further improve the accuracy of the search results (Cheng et al., 2022a; 2022b). Second, the bibliometric information included only data from the WoSCC database, leaving out data from other major databases, which might result in the exclusion of a few relevant studies. Third, we looked only at documents published in English and excluded those published in other languages, which indicated that non-English speaking countries' contributions may be neglected to some extent. Last, the data extraction took place on 24 May 2022, which might slightly influence the analysis results by the time.

Conclusions

This is the first bibliometric analysis of the quantity and quality of APS-related publications, which suggests that global research on APS has increased continuously over the past 10 years. Globally, Italy and the United States are the leading countries in the field. The University of Padua, the University of Milan, and the University of Sao Paulo are the top three most prolific institutions. Lupus ranks first in both the most published and most co-cited journals. Savino Sciascia and Spiros Miyakis are the most productive and most co-cited authors, respectively. Notably, "VKA" and "IgA" are current research foci in the domain of APS. The clinical studies on the treatment and mechanism research of APS are recognized as promising research frontiers. In conclusion, this bibliometric study presents the current research status and frontiers in the APS field, which can explore potential collaboration opportunities and provide a reference for subsequent investment decisions and research directions.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

TW, WH, and ZY designed the study. TW and WH wrote the original draft. JQ, YL, and YZ collected the data. HJ, JW, and JZ verified the data. TW, ZJ, and LC performed software analyses. ZY supervised the research and revised the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Aksoy, U., Kucuk, M., Versiani, M. A., and Orhan, K. (2021). Publication trends in micro-CT endodontic research: A bibliometric analysis over a 25-year period. *Int. Endod. J.* 54 (3), 343–353. doi:10.1111/iej.13433

Andrade, D., and Tektonidou, M. (2016). Emerging therapies in antiphospholipid syndrome. *Curr. Rheumatol. Rep.* 18 (4), 22. doi:10.1007/s11926-016-0566-z

Andreoli, L., Bertsias, G. K., Agmon-Levin, N., Brown, S., Cervera, R., Costedoat-Chalumeau, N., et al. (2017). EULAR recommendations for women's health and the management of family planning, assisted reproduction, pregnancy and menopause in patients with systemic lupus erythematosus and/or antiphospholipid syndrome. *Ann. Rheum. Dis.* 76 (3), 476–485. doi:10.1136/annrheumdis-2016-209770

Arnaud, L., Mathian, A., Devilliers, H., Ruffatti, A., Tektonidou, M., Forastiero, R., et al. (2015). Patient-level analysis of five international cohorts further confirms the efficacy of aspirin for the primary prevention of thrombosis in patients with antiphospholipid antibodies. *Autoimmun. Rev.* 14 (3), 192–200. doi:10.1016/j.autrev.2014.10.019

Ben-Zvi, I., Kivity, S., Langevitz, P., and Shoenfeld, Y. (2012). Hydroxychloroquine: From malaria to autoimmunity. *Clin. Rev. Allergy Immunol.* 42 (2), 145–153. doi:10.1007/s12016-010-8243-x

Bitsadze, V., Khizroeva, J., Alexander, M., and Elalamy, I. (2022). Venous thrombosis risk factors in pregnant women. *J. Perinat. Med.* 50 (5), 505–518. doi:10.1515/jpm-2022-0008

Borghi, M. O., Beltagy, A., Garrafa, E., Curreli, D., Cecchini, G., Bodio, C., et al. (2020). Anti-phospholipid antibodies in COVID-19 are different from those detectable in the anti-phospholipid syndrome. *Front. Immunol.* 11, 584241. doi:10.3389/fimmu.2020.584241

Cervera, R. (2017). Antiphospholipid syndrome. *Thromb. Res.* 151 (1), S43–S47. doi:10.1016/S0049-3848(17)30066-X

Cervera, R., Piette, J. C., Font, J., Khamashta, M. A., Shoenfeld, Y., Camps, M. T., et al. (2002). Antiphospholipid syndrome: Clinical and immunologic manifestations and patterns of disease expression in a cohort of 1, 000 patients. *Arthritis Rheum.* 46 (4), 1019–1027. doi:10.1002/art.10187

Cervera, R., Rodriguez-Pinto, I., and Espinosa, G. (2018). The diagnosis and clinical management of the catastrophic antiphospholipid syndrome: A comprehensive review. *J. Autoimmun.* 92, 1–11. doi:10.1016/j.jaut.2018.05.007

Cervera, R., Serrano, R., Pons-Estel, G. J., Ceberio-Hualde, L., Shoenfeld, Y., de Ramon, E., et al. (2015). Morbidity and mortality in the antiphospholipid syndrome during a 10-year period: A multicentre prospective study of 1000 patients. *Ann. Rheum. Dis.* 74 (6), 1011–1018. doi:10.1136/annrheumdis-2013-204838

Chen, C., Hu, Z., Liu, S., and Tseng, H. (2012). Emerging trends in regenerative medicine: A scientometric analysis in CiteSpace. *Expert Opin. Biol. Ther.* 12 (5), 593–608. doi:10.1517/14712598.2012.674507

Cheng, K., Guo, Q., Shen, Z., Yang, W., Wang, Y., Sun, Z., et al. (2022a). Bibliometric analysis of global research on cancer photodynamic therapy: Focus on nano-related research. *Front. Pharmacol.* 13, 927219. doi:10.3389/fphar.2022.927219

Cheng, K., Guo, Q., Yang, W., Wang, Y., Sun, Z., and Wu, H. (2022b). Mapping knowledge landscapes and emerging trends of the links between bone metabolism and diabetes mellitus: A bibliometric analysis from 2000 to 2021. *Front. Public Health* 10, 918483. doi:10.3389/fpubh.2022.918483

Cohen, H., Efthymiou, M., and Devreese, K. M. J. (2021). Monitoring of anticoagulation in thrombotic antiphospholipid syndrome. *J. Thromb. Haemost.* 19 (4), 892–908. doi:10.1111/jth.15217

Cohen, H., Hunt, B. J., Efthymiou, M., Arachchillage, D. R. J., Mackie, I. J., Clawson, S., et al. (2016). Rivaroxaban versus warfarin to treat patients with thrombotic antiphospholipid syndrome, with or without systemic lupus erythematosus (raps): A randomised, controlled, open-label, phase 2/3, non-inferiority trial. *Lancet. Haematol.* 3 (9), e426–e436. doi:10.1016/S2352-3026(16)30079-5

Cohen, H., and Isenberg, D. A. (2021). How I treat anticoagulant-refractory thrombotic antiphospholipid syndrome. *Blood* 137 (3), 299–309. doi:10.1182/blood.2020004942

Crowther, M. A., Ginsberg, J. S., Julian, J., Denburg, J., Hirsh, J., Douketis, J., et al. (2003). A comparison of two intensities of warfarin for the prevention of recurrent thrombosis in patients with the antiphospholipid antibody syndrome. *N. Engl. J. Med.* 349 (12), 1133–1138. doi:10.1056/NEJMoa035241

Deng, P., Shi, H., Pan, X., Liang, H., Wang, S., Wu, J., et al. (2022a). Worldwide research trends on diabetic foot ulcers (2004-2020): Suggestions for researchers. *J. Diabetes Res.* 2022, 7991031. doi:10.1155/2022/7991031

Deng, P., Wang, S., Sun, X., Qi, Y., Ma, Z., Pan, X., et al. (2022b). Global trends in research of gouty arthritis over past decade: A bibliometric analysis. *Front. Immunol.* 13, 910400. doi:10.3389/fimmu.2022.910400

Duarte-Garcia, A., Pham, M. M., Crowson, C. S., Amin, S., Moder, K. G., Pruthi, R. K., et al. (2019). The epidemiology of antiphospholipid syndrome: A population-based study. *Arthritis Rheumatol.* 71 (9), 1545–1552. doi:10.1002/art.40901

Dufrost, V., Risse, J., Reshetnyak, T., Satybaldyeva, M., Du, Y., Yan, X. X., et al. (2018). Increased risk of thrombosis in antiphospholipid syndrome patients treated with direct oral anticoagulants. Results from an international patient-level data meta-analysis. *Autoimmun. Rev.* 17 (10), 1011–1021. doi:10.1016/j.autrev.2018.04.009

Fan, G., Han, R., Zhang, H., He, S., and Chen, Z. (2017). Worldwide research productivity in the field of minimally invasive spine surgery: A 20-year survey of publication activities. *Spine* 42 (22), 1717–1722. doi:10.1097/BRS.0000000000001393

Finazzi, G., Marchioli, R., Brancaccio, V., Schinco, P., Wisloff, F., Musial, J., et al. (2005). A randomized clinical trial of high-intensity warfarin vs. Conventional antithrombotic therapy for the prevention of recurrent thrombosis in patients with the antiphospholipid syndrome (WAPS). *J. Thromb. Haemost.* 3 (5), 848–853. doi:10.1111/j.1538-7836.2005.01340.x

Foret, T., Dufrost, V., Salomon du Mont, L., Costa, P., Lakomy, C., Lagrange, J., et al. (2022). A new pro-thrombotic mechanism of neutrophil extracellular traps in antiphospholipid syndrome: Impact on activated protein C resistance. *Rheumatology* 61 (7), 2993–2998. doi:10.1093/rheumatology/keab853

Foret, T., Dufrost, V., Salomon Du Mont, L., Costa, P., Lefevre, B., Lacolley, P., et al. (2021). Systematic review of antiphospholipid antibodies in COVID-19 patients: Culprits or bystanders? *Curr. Rheumatol. Rep.* 23 (8), 65. doi:10.1007/s11926-021-01029-3

Fujieda, Y., and Amengual, O. (2020). New insights into the pathogenic mechanisms and treatment of arterial thrombosis in antiphospholipid syndrome. *Eur. J. Rheumatol.* 8 (2), 93–99. doi:10.5152/eurjrheum.2020.20058

Garcia, D., and Erkan, D. (2018). Diagnosis and management of the antiphospholipid syndrome. *N. Engl. J. Med.* 378 (21), 2010–2021. doi:10.1056/NEJMra1705454

Ghembaza, A., and Saadoun, D. (2020). Management of antiphospholipid syndrome. *Biomedicines* 8 (11), E508. doi:10.3390/biomedicines8110508

Giannakopoulos, B., and Krilis, S. A. (2013). The pathogenesis of the antiphospholipid syndrome. *N. Engl. J. Med.* 368 (11), 1033–1044. doi:10.1056/NEJMra1112830

Hughes, G. R. (1983). Thrombosis, abortion, cerebral disease, and the lupus anticoagulant. *Br. Med. J.* 287 (6399), 1088–1089. doi:10.1136/bmj.287.6399.1088

Karahan, S., Erol, K., Yuksel, R. C., Artan, C., and Celik, I. (2022). Antiphospholipid antibodies in COVID-19-associated pneumonia patients in intensive care unit. *Mod. Rheumatol.* 32 (1), 163–168. doi:10.1080/14397595.2021.1892257

Koval, N., Alves, M., Plácido, R., Almeida, A. G., Fonseca, J. E., Ferreira, J. J., et al. (2021). Direct oral anticoagulants versus vitamin K antagonists in patients with antiphospholipid syndrome: Systematic review and meta-analysis. *RMD Open* 7 (2), e001678. doi:10.1136/rmdopen-2021-001678

Kravvariti, E., Koutsogianni, A., Samoli, E., Sfikakis, P. P., and Tektonidou, M. G. (2020). The effect of hydroxychloroquine on thrombosis prevention and antiphospholipid antibody levels in primary antiphospholipid syndrome: A pilot open label randomized prospective study. *Autoimmun. Rev.* 19 (4), 102491. doi:10.1016/j.autrev.2020.102491

Lin, W., Luo, Y., Liu, F., Li, H., Wang, Q., Dong, Z., et al. (2022). Status and trends of the association between diabetic nephropathy and diabetic retinopathy from 2000 to 2021: Bibliometric and visual analysis. *Front. Pharmacol.* 13, 937759. doi:10.3389/fphar.2022.937759

Liu, Y., Zheng, B., Hong, J., and Liu, Y. (2022). Mapping theme trends and knowledge structure of sjögren's syndrome (SS), A bibliometric analysis from 2010 to 2021. *Clin. Rheumatol.* 41 (9), 2779–2789. doi:10.1007/s10067-022-06196-x

Luigi Meroni, P., Toubi, E., and Shoenfeld, Y. (2019). Are anti-phospholipid syndrome and systemic lupus erythematosus two different diseases? A 10-year late remake. *Isr. Med. Assoc. J.* 21 (7), 491–493.

Ma, D., Yang, B., Guan, B., Song, L., Liu, Q., Fan, Y., et al. (2021). A bibliometric analysis of pyroptosis from 2001 to 2021. *Front. Immunol.* 12, 731933. doi:10.3389/fimmu.2021.731933

Mazetto, B. de M., Hounkpe, B. W., da Silva Saraiva, S., Vieira-Damiani, G., Dos Santos, A. P. R., Jacinto, B. C., et al. (2022). Association between neutrophil

extracellular traps (NETs) and thrombosis in antiphospholipid syndrome. *Thromb. Res.* 214, 132–137. doi:10.1016/j.thromres.2022.05.001

Meng, H., Yalavarthi, S., Kanthi, Y., Mazza, L. F., Elfline, M. A., Luke, C. E., et al. (2017). *In vivo* role of neutrophil extracellular traps in antiphospholipid antibody-mediated venous thrombosis. *Arthritis Rheumatol.* 69 (3), 655–667. doi:10.1002/art.39938

Miyakis, S., Lockshin, M. D., Atsumi, T., Branch, D. W., Brey, R. L., Cervera, R., et al. (2006). International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J. Thromb. Haemost.* 4 (2), 295–306. doi:10.1111/j.1538-7836.2006.01753.x

Nuri, E., Taraborelli, M., Andreoli, L., Tonello, M., Gerosa, M., Calligaro, A., et al. (2017). Long-term use of hydroxychloroquine reduces antiphospholipid antibodies levels in patients with primary antiphospholipid syndrome. *Immunol. Res.* 65 (1), 17–24. doi:10.1007/s12026-016-8812-z

Ordi-Ros, J., Saez-Comet, L., Perez-Conesa, M., Vidal, X., Riera-Mestre, A., Castro-Salomo, A., et al. (2019). Rivaroxaban versus vitamin K antagonist in antiphospholipid syndrome: A randomized noninferiority trial. *Ann. Intern. Med.* 171 (10), 685–694. doi:10.7326/M19-0291

Pengo, V., Denas, G., Zoppellaro, G., Jose, S. P., Hoxha, A., Ruffatti, A., et al. (2018). Rivaroxaban vs warfarin in high-risk patients with antiphospholipid syndrome. *Blood* 132 (13), 1365–1371. doi:10.1182/blood-2018-04-848333

Pericleous, C., Ferreira, I., Borghi, O., Pregnolato, F., McDonnell, T., Garza-Garcia, A., et al. (2016). Measuring IgA anti-β2-glycoprotein I and IgG/IgA anti-domain I antibodies adds value to current serological assays for the antiphospholipid syndrome. *PLoS One* 11 (6), e0156407. doi:10.1371/journal.pone.0156407

Petri, M. (2020). Antiphospholipid syndrome. *Transl. Res.* 225, 70–81. doi:10.1016/j.trsl.2020.04.006

Petri, M. (2011). Use of hydroxychloroquine to prevent thrombosis in systemic lupus erythematosus and in antiphospholipid antibody-positive patients. *Curr. Rheumatol. Rep.* 13 (1), 77–80. doi:10.1007/s11926-010-0141-y

Reshetnyak, T., Cheldieva, F., Cherkasova, M., Lila, A., and Nasonov, E. (2022). IgA antiphospholipid antibodies in antiphospholipid syndrome and systemic lupus erythematosus. *Int. J. Mol. Sci.* 23 (16), 9432. doi:10.3390/ijms23169432

Rodriguez-Pinto, I., Espinosa, G., Erkan, D., Shoenfeld, Y., and Cervera, R.CAPS Registry Project Group (2018). The effect of triple therapy on the mortality of catastrophic anti-phospholipid syndrome patients. *Rheumatology* 57 (7), 1264–1270. doi:10.1093/rheumatology/key082

Rodriguez-Pinto, I., Moitinho, M., Santacreu, I., Shoenfeld, Y., Erkan, D., Espinosa, G., et al. (2016). Catastrophic antiphospholipid syndrome (CAPS): Descriptive analysis of 500 patients from the international CAPS registry. *Autoimmun. Rev.* 15 (12), 1120–1124. doi:10.1016/j.autrev.2016.09.010

Ruiz-Irastorza, G., Crowther, M., Branch, W., and Khamashta, M. A. (2010). Antiphospholipid syndrome. *Lancet* 376 (9751), 1498–1509. doi:10.1016/S0140-6736(10)60709-X

Ruiz-Irastorza, G., Cuadrado, M. J., Ruiz-Arruza, I., Brey, R., Crowther, M., Derksen, R., et al. (2011). Evidence-based recommendations for the prevention and long-term management of thrombosis in antiphospholipid antibody-positive patients: Report of a task force at the 13th international congress on antiphospholipid antibodies. *Lupus* 20 (2), 206–218. doi:10.1177/0961203310395803

Schreiber, K., Sciascia, S., de Groot, P. G., Devreese, K., Jacobsen, S., Ruiz-Irastorza, G., et al. (2018). Antiphospholipid syndrome. *Nat. Rev. Dis. Prim.* 4, 17103. doi:10.1038/nrdp.2017.103

Sciascia, S., Amigo, M. C., Roccatello, D., and Khamashta, M. (2017). Diagnosing antiphospholipid syndrome: 'Extra-Criteria' manifestations and technical advances. *Nat. Rev. Rheumatol.* 13 (9), 548–560. doi:10.1038/nrrheum.2017.124

Sciascia, S., Sanna, G., Murru, V., Roccatello, D., Khamashta, M. A., and Bertolaccini, M. L. (2013). Gapss: The global anti-phospholipid syndrome score. *Rheumatology* 52 (8), 1397–1403. doi:10.1093/rheumatology/kes388

Sciascia, S., Sanna, G., Murru, V., Roccatello, D., Khamashta, M. A., and Bertolaccini, M. L. (2015). The global anti-phospholipid syndrome score in primary APS. *Rheumatology* 54 (1), 134–138. doi:10.1093/rheumatology/keu307

Shen, J., Shen, H., Ke, L., Chen, J., Dang, X., Liu, B., et al. (2022). Knowledge mapping of immunotherapy for hepatocellular carcinoma: A bibliometric study. *Front. Immunol.* 13, 815575. doi:10.3389/fimmu.2022.815575

Tebo, A. E., Willis, R., Jaskowski, T. D., Guerra, M., Pierangeli, S. S., Salmon, J., et al. (2016). Clinical significance and correlations between anti-β2 glycoprotein I IgA assays in antiphospholipid syndrome and/or systemic lupus erythematosus. *Clin. Chim. Acta.* 460, 107–113. doi:10.1016/j.cca.2016.06.025

Tektonidou, M. G., Andreoli, L., Limper, M., Amoura, Z., Cervera, R., Costedoat-Chalumeau, N., et al. (2019). EULAR recommendations for the management of antiphospholipid syndrome in adults. *Ann. Rheum. Dis.* 78 (10), 1296–1304. doi:10.1136/annrheumdis-2019-215213

Trahtemberg, U., Rottapel, R., Dos Santos, C. C., Slutsky, A. S., Baker, A., and Fritzler, M. J. (2021). Anticardiolipin and other antiphospholipid antibodies in critically ill COVID-19 positive and negative patients. *Ann. Rheum. Dis.* 80 (9), 1236–1240. doi:10.1136/annrheumdis-2021-220206

van Eck, N. J., and Waltman, L. (2010). Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics* 84 (2), 523–538. doi:10.1007/s11192-009-0146-3

Wiggins, B. S., Dixon, D. L., Neyens, R. R., Page, R. L., and Gluckman, T. J. (2020). Select drug-drug interactions with direct oral anticoagulants: JACC review topic of the week. *J. Am. Coll. Cardiol.* 75 (11), 1341–1350. doi:10.1016/j.jacc.2019.12.068

Wilson, W. A., Gharavi, A. E., Koike, T., Lockshin, M. D., Branch, D. W., Piette, J. C., et al. (1999). International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: Report of an international workshop. *Arthritis Rheum.* 42 (7), 1309–1311. doi:10.1002/1529-0131(199907)42:7<1309::AID-ANR1>3.0.CO;2-F

Wu, H., Li, Y., Tong, L., Wang, Y., and Sun, Z. (2021). Worldwide research tendency and hotspots on hip fracture: A 20-year bibliometric analysis. *Arch. Osteoporos.* 16 (1), 73. doi:10.1007/s11657-021-00929-2

Zhang, Y., Xiao, M., Zhang, S., Xia, P., Cao, W., Jiang, W., et al. (2020). Coagulopathy and antiphospholipid antibodies in patients with COVID-19. *N. Engl. J. Med.* 382 (17), e38. doi:10.1056/NEJMc2007575

Zhu, S., Liu, Y., Gu, Z., and Zhao, Y. (2021). A bibliometric analysis of advanced healthcare materials: Research trends of biomaterials in healthcare application. *Adv. Healthc. Mat.* 10 (10), e2002222. doi:10.1002/adhm.202002222

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Assessment by ABPM verified the presence of hypertension in patients with self-reported hypertension, pregnant women, as well as differences between ethnicities in women aged 38-39 years in the Ribeirão Preto cohort

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Introduction: Arterial hypertension is a global health problem and one of the main risk factors for cardiovascular diseases (CVD), and therefore for morbidity and mortality among adult men and women. Factors related to obstetric history, family history, sociodemographic characteristics, and lifestyle habits are known determinants of arterial hypertension.

Methods: Case-control study of women belonging to the 1978/79 birth cohort conducted in the city of Ribeirão Preto/SP. Sociodemographic data, presence of comorbidities, maternal comorbidities, paternal comorbidities, comorbidities during pregnancy, and biometric and biophysical markers associated with blood pressure measured by 24-h ambulatory blood pressure monitoring (ABPM) were assessed in women aged 38–39 years. We want to study which variables of the previous sentence are related to the presence of hypertension measured by ABPM.

Results: Data from 281 women were analyzed. Our results showed that ethnicity, a history of hypertension, and gestational hypertension reported by the women were significantly associated with the presence of hypertension measured by ABPM. Other factors such as marital status, educational level, comorbidities of the woman, paternal or maternal comorbidities, anthropometric measurements or serum levels of cardiovascular markers were not associated with the presence of hypertension measured by ABPM.

Conclusion: We conclude that ethnicity, self-reported hypertension, and gestational hypertension are associated with arterial hypertension measured by ABPM.

KEYWORDS

ambulatory blood pressure monitoring, comorbidities, hypertension during pregnancy, blood pressure, hypertension

Introduction

Birth cohort studies have been high priority on the research agenda of developed countries for scientific and technological advancement (Brandstetter et al., 2019). Arterial hypertension is a chronic disease and a public health problem in Brazil and in the world. In addition, it is one of the main risk factors for cardiovascular diseases (CVD) and a leading cause of death worldwide (Brant et al., 2022) (Ribeiro et al., 2016) Arterial hypertension is characterized by persistently raised blood pressure, i.e., systolic blood pressure (SBP) ≥ 130 mmHg and/or diastolic blood pressure (DBP) ≥ 80 mmHg, measured with the correct technique on three different occasions in the absence of antihypertensive medication (Whelton et al., 2018).

After pregnancy, women with hypertensive disorders of pregnancy such as gestational hypertension and preeclampsia are at increased risk of developing CVD (Honigberg and Natarajan, 2020). Preeclampsia is a specific pregnancy syndrome that affects about 5%–8% of pregnancies and is a leading cause of maternal, fetal and neonatal mortality. The condition is characterized by raised blood pressure after the 20th week of gestation (Rana et al., 2019).

Metabolic syndrome is defined by the health word organization (WHO) as a cluster of conditions that include arterial hypertension, abdominal obesity, dyslipidemia, and altered glucose metabolism (Fahed et al., 2022). It is estimated that about one quarter of the world's population has metabolic syndrome, corresponding to more than one billion people. In Brazil, a prevalence of metabolic syndrome in the adult population of 29.6% has been reported, which can reach more than 40% in age groups over 60 years (Oliveira et al., 2020; de Siqueira Valadares et al., 2022).

Studies of blood pressure measurements are uncommon in the Brazilian population and most existing ones are restricted to certain locations and using different information, a fact impairing comparison of the data (Jardim et al., 2020). In addition, different diagnostic criteria exist for estimating the population prevalence of arterial hypertension, such as cut-off point and the use or not of associated medications (Zhou et al., 2021)

Arterial hypertension is a chronic disease whose incidence is increasing worldwide and that is reported to be one of the main causes of CVD, with a consequent impact on mortality, morbidity, and quality of life (Mills et al., 2020). Knowledge of the most important and prevalent risk factors related to the pathophysiology of this disease in women will permit to prevent this condition by proposing early interventions and more appropriate treatments (Carey et al., 2018). Our hypothesis was that with the technique measured by ambulatory blood pressure monitoring the study would be able to identify hypertension in the woman's gestational phase, in self-reported hypertension and other variables in women aged close to 40 years.

Materials and methods

Study design

Analytical, retrospective, case-control study that included female patients of the Ribeirão Preto Cohort 1978/1979. The 1978/79 cohort, as the group of participants is called, is the first National Cohort in Brazil and has 6827 live births in the city of Ribeirão Preto. In addition to the assessment at birth, these individuals were also assessed at school age, at military enlistment, at 23–25 years, and in their 5th assessment (2016/2017), in adulthood (38–39 years), in which 1775 were evaluated. Individuals being 929 women. Reinforcing the data of the participants of this study were carried out on women who were aged between 38 and 39 years. These women were invited to attend spontaneously interviews and examinations at the University of São Paulo, at the Hospital das Clínicas of the Faculty of Medicine of Ribeirão Preto. Of the 929 patients who attended, 281 were accepted to have the ambulatorial blood pressure monitoring (ABPM) placed. The placement of the ABPM device was an option for the participants and not a criterion of the study. Measurement blood pressure was performed for 24 h from ABPM placement. ABPM analysis measures data on mean 24-h systolic and diastolic blood pressure, and mean systolic and diastolic blood pressure during wakefulness and sleep. After these data, the study´s cardiology physicians lauded the participants´ABPM and classified them as hypertensive or normotensive (de Souza et al., 2022). The inclusion criteria were female participants, not pregnant at the time of the interview and who accepted the measurement by ABPM. And the exclusion criterion was male and women who refused to be measured by ABPM.

Evaluation of anthropometric measurements

The evaluation of anthropometric measurements was made through measurements of weight, height, calculation of Body

Mass Index (BMI), waist circumference (WC), and neck circumference (NC).

Arterial stiffness assessment

Pulse wave velocity (PWV) analysis is a simple, non-invasive, and reliable diagnostic method for the assessment of arterial stiffness. (47) The computerized system Sphygmocor® Software Version 9.0, AtCor Medical Pty Ltd., was used to record and analyze PWV. The measurement of PWV was performed with the participant in the supine position after a 5-min rest and an acclimatized room (22–24°C). An inelastic measuring tape graduated in centimeters, was used to measure the distances between the carotid wrist and the sternal notch and between the sternum notch and the femoral wrist. External transducers were placed directly on the skin in the right carotid artery and the right femoral artery. Capture the recording of pulse waves for a minimum time interval of 10 s. The PWV measurement is automatically calculated by the ratio of the carotid-femoral distance and the time interval between the two pulses.

Blood and urine collection

The participants had blood collection performed at the Gynecology Laboratory of the Hospital das Clínicas of the Faculty of Medicine of the University of São Paulo in the city of Ribeirão Preto. Blood collection was not performed on an empty stomach. From each subject, 20 ml of whole blood was collected and stored in conical plastic tubes (BD-Becton Dickinson, Plymouth, United Kingdom). The women did not use any medication that could alter the results of laboratory tests. Blood samples were processed within a maximum of 2 h after collection. The serum was stored at −80°C for measurement of all serum variables at the same time.

Serum concentrations of creatinine, total cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides, C-reactive protein, homocysteine, basal insulin, and glucose were quantified in an automated biochemistry analyzer (Weiner, Rosario, Argentina). The glomerular filtration rate was estimated from creatinine and cystatin C using the CKD-EPI 2009 equation. (15) Glycated hemoglobin (HbA1c) was determined by HPLC (Bio-Rad D-10, Hercules, CA).

Questionnaire applied to women

The questionnaire asked about demographic variables such as skin color, marital status, social benefit, and educational level (Years of schooling). After the questionnaire, ABPM was measured, and for each demographic variable mentioned above, evaluated the presence of hypertension.

In the questionnaire, the patient was asked about the presence of comorbidities. Among the questions on the questionnaire, the patient was asked if she was aware of the presence of the following comorbidities: high blood glucose/diabetes, hypercholesterolemia, hypertension, obesity, thrombosis, arrhythmia, angina, infarction, stroke, kidney disease. After this questionnaire, the participants placed the ABPM, and the presence of hypertension measured by the ABPM was evaluated. With this, we obtained two groups about to with concerning these variables of the presence of comorbidities, the hypertensive related to these comorbidities and the normotensive related to these comorbidities.

In the questionnaire, the women were asked about the following paternal and maternal comorbidities: knowledge of the father's disease, paternal high blood glucose/diabetes, hypercholesterolemia reported by the father, cardiovascular disease described by the father, paternal hypertension, paternal obesity, paternal thrombosis, paternal arrhythmia, paternal angina, paternal infarction, paternal stroke, paternal kidney disease, knowledge of the mother's disease, maternal high blood glucose/diabetes, hypercholesterolemia reported by the mother, cardiovascular disease reported by the mother, maternal hypertension, maternal obesity, maternal thrombosis, maternal arrhythmia, maternal angina, maternal infarction, maternal stroke, and maternal kidney disease. And for each variable of paternal or maternal comorbidity, evaluated the presence or absence of hypertension.

Another evaluation performed was the presence of hypertension measured by MAPA in pregnant women with the following questions for women if they were aware of the following comorbidities during pregnancy: self-reported preeclampsia and gestational hypertension.

Blood pressure measurement by ambulatory blood pressure monitoring

In this study, blood pressure was measured by ABPM. The installation of the device (A&D Company Limited, Kitamoto-shi, Saitama-ken, Japan) was offered to all women on the day of the interview but the decision to use the device was voluntary.

The patient was classified as hypertensive based on ABPM if the mean SBP ≥130 mmHg or mean DBP ≥80 mmHg (Whelton et al., 2018; Flynn et al., 2022). The obstetric history on the presence of hypertensive syndromes and current hypertension was obtained with the questionnaire during the interview and not from the medical record (Barbieri et al., 2006; Malta et al., 2018; Hurrell et al., 2022).

Statistical analysis

Continuous and semi-continuous variables were compared using a Gauss curve. Data were found to be nonparametric by the

TABLE 1 Description of ABPM results (n = 281).

	Median	25%	75%
ABPM mean 24-h SBP	113.0	108.0	120.0
ABPM mean 24-h DBP	72.0	68.0	79.0
ABPM mean awake SBP	117.0	111.0	122.0
ABPM mean awake DBP	76.0	72.0	84.0
ABPM mean sleep SBP	106.0	100.0	113.0
ABPM mean sleep DBP	65.0	60.0	71.0

Data expressed as median and percentiles. ABPM, ambulatory blood pressure monitoring; SBP, systolic blood pressure; DBP, diastolic blood pressure.

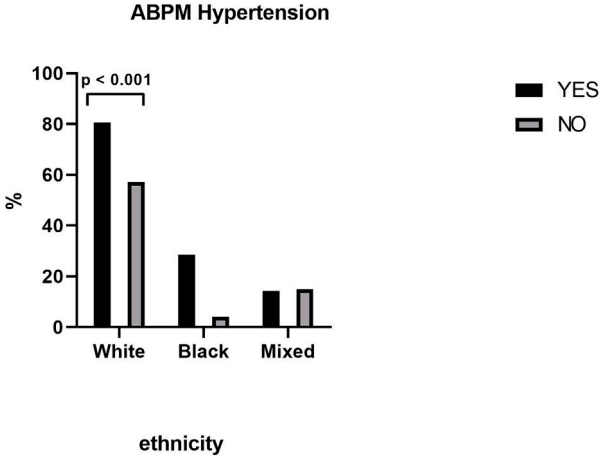

FIGURE 1
ABPM Hypertension between ethnicities.

Kolmogorov-Smirnov and Shapiro-Wilk tests and were therefore reported as median and percentiles (25–75). The geometric mean was used when the sample showed a high frequency of modal values (>20%). Two independent groups were compared by the Mann-Whitney test with Bonferroni correction. Categorical data were expressed as absolute (n) and relative (%) frequency. The contingency matrices were analyzed by Pearson's chi-squared test and complex matrices with $p \leq 0.05$.

Results

Of the 929 patients of the cohort, only 281 underwent ABPM. Twenty-four-hour SBP and DBP were recorded, as well as sleeping and awake SBP and DBP, and are expressed as median and percentiles (Table 1). The median SBP was 113 mmHg and the median DBP was 72 mmHg. Blood pressures differed between the sleep and wake periods, with a median SBP and DBP of 117 and 76 mmHg during the wake period, respectively, while SBP was 106 mmHg and DBP was 65 mmHg during the sleep period.

Figure 1 shows the demographic variable of the e. A significant difference (p = 0.001) was only observed for ethnicity, with a higher prevalence of hypertensive women among white patients. In Table 2 there was no difference in marital status, i.e., if the women were married or lived with a partner, if they were separated or divorced, or if they were single or widowed, concluding that marital status does not influence the presence or absence of hypertension measured by ABPM. Receiving social benefit or educational level also did not affect the presence or absence of hypertension measured by ABPM.

Table 3 and Figure 2 shows that there was a statistically significant difference between patients with self-reported hypertension and with hypertension measured by ABPM when asked about the presence of current hypertension. In Table 3 no significant difference was observed between women with hypertension and normotensive measured by ABPM when asked about the presence of high blood glucose or diabetes, or the presence of hypercholesterolemia. ABPM also did not detect hypertension in women with obesity (Figure 2). There was also no difference in the presence of other self-reported comorbidities such as thrombosis, arrhythmia, angina, infarction, stroke, or chronic kidney disease between hypertensive and normotensive women.

As can be seen in Table 4, there was no difference between patients with hypertension and without hypertension measured by ABPM when asked about the presence of paternal comorbidities. The women were asked whether they had knowledge of the father's disease, whether their father had high blood glucose or diabetes, and whether they had knowledge of hypercholesterolemia or CVD reported by the father. The women undergoing ABPM were also asked about other paternal comorbidities such as hypertension, obesity, thrombosis, arrhythmia, angina, infarction, stroke, and kidney disease, and no association of the presence of hypertension with these variables was found.

Table 5 shows the analysis of the presence of maternal comorbidities reported by the participants according to the presence of hypertension measured by ABPM. Knowledge of maternal diseases such as high blood glucose, diabetes, hypercholesterolemia reported by the mother, CVD reported by the mother, hypertension, obesity, thrombosis, arrhythmia, angina, infarction, stroke or kidney disease did not differ between hypertensive and non-hypertensive women.

When we evaluated the presence of hypertensive disorders of pregnancy, we found a significant difference in patients classified as hypertensive by ABPM when asked about gestational hypertension (p < 0.0001) and we found no presence of hypertension measured by ABPM when the patient was asked about having preeclampsia (Figure 3).

TABLE 2 Evaluation of the presence of hypertension (ABPM) according to demographic variables.

ABPM hypertension		No		Yes		Pearson X^2
		n	%	n	%	Sig
Marital status	With a partner	172	64.4%	10	71.4%	0.932
	Separated/Divorced	28	10.5%	1	7.1%	
	Single	64	24.0%	3	21.4%	
	Widowed	3	1.1%	0	0.0%	
Social benefit	No	181	70.2%	9	64.3%	0.641
	Yes	77	29.8%	5	35.7%	
Educational level (years of schooling)	12 or more	114	42.9%	4	28.6%	0.559
	5 to 8	28	10.5%	1	7.1%	
	9 to 11	120	45.1%	9	64.3%	
	≤4	4	1.5%	0	0.0%	

Data expressed as absolute (n) and relative (%) frequency. Pearson's chi-squared test.

TABLE 3 Evaluation of the presence of hypertension (ABPM) according to the patient's self-reported comorbidities.

ABPM hypertension		No		Yes		Pearson X
		n	%	n	%	Sig
High blood glucose/diabetes	No	232	86.9%	13	92.9%	0.515
	Yes	35	13.1%	1	7.1%	
Hypercholesterolemia	No	209	78.6%	11	78.6%	1.000
	Yes	57	21.4%	3	21.4%	
Hypertension	No	213	79.8%	4	28.6%	<0.0001
	Yes	54	20.2%	10	71.4%	
Obesity	No	147	55.1%	6	42.9%	0.372
	Yes	120	44.9%	8	57.1%	
Thrombosis	No	260	97.4%	14	100.0%	0.540
	Yes	7	2.6%	0	0.0%	
Arrhythmia	No	243	92.0%	11	78.6%	0.080
	Yes	21	8.0%	3	21.4%	
Angina	No	246	92.8%	13	92.9%	0.997
	Yes	19	7.2%	1	7.1%	
Infarction	No	265	99.3%	14	100.0%	0.745
	Yes	2	0.7%	0	0.0%	
Stroke	No	266	99.6%	14	100.0%	0.819
	Yes	1	0.4%	0	0.0%	
Kidney disease	No	238	89.1%	11	78.6%	0.225
	Yes	29	10.9%	3	21.4%	

Data expressed as absolute (n) and relative (%) frequency. Pearson's chi-squared test.

Table 6 shows the evaluation of the presence of hypertension measured by ABPM according to anthropometric parameters (waist circumference, neck circumference, weight, height, BMI, weight-to-height ratio and no significant difference was observed between hypertensive and non-hypertensive women. As can be seen in Table 7, there was no significant difference in markers of CVD (serum triglycerides, cholesterol, LDL, HDL, creatinine, glucose, C-reactive protein, homocysteine, basal insulin, and

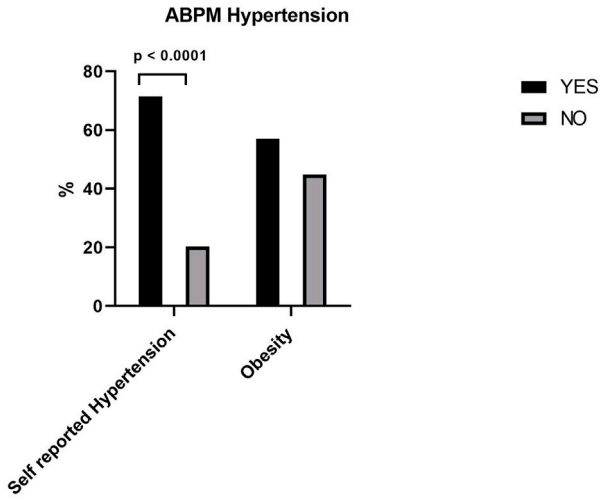

FIGURE 2
ABPM Hypertension between self reported hypertension and obese women.

glycated hemoglobin) between hypertensive and non-hypertensive women based on ABPM. Also in Table 7, there was no difference in pulse wave velocity between hypertensive and non-hypertensive women.

Discussion

The main findings of the present study were: that white women had hypertension detected by the ABPM; women who reported that they were aware of being hypertensive in the interview when they were measured by the ABPM had their hypertension demonstrated by blood pressure data; gestational hypertension measured by the ABPM presented hypertensive.

Two approaches were used in this study to assess the presence of arterial hypertension: one was through a questionnaire, the women answered if they knew if they were hypertensive and answered positively and the other was through quantitative measures such as measured by ABPM confirming hypertension. Hypertension was reported during the interview

TABLE 4 Evaluation of the presence of hypertension (ABPM) according to paternal comorbidities.

ABPM hypertension		No		Yes		Pearson X^2
		n	%	n	%	Sig
Knowledge of the father's disease	No	43	16.4%	2	14.3%	0.834
	Yes	219	83.6%	12	85.7%	
Paternal high blood glucose/diabetes	No	151	70.9%	9	75.0%	0.760
	Yes	62	29.1%	3	25.0%	
Hypercholesterolemia reported by the father	No	141	68.4%	7	58.3%	0.466
	Yes	65	31.6%	5	41.7%	
Cardiovascular disease reported by the father	No	165	93.8%	10	90.9%	0.709
	Yes	11	6.3%	1	9.1%	
Paternal hypertension	No	106	50.7%	5	41.7%	0.542
	Yes	103	49.3%	7	58.3%	
Paternal obesity	No	155	72.1%	11	91.7%	0.137
	Yes	60	27.9%	1	8.3%	
Paternal thrombosis	No	196	94.7%	11	91.7%	0.655
	Yes	11	5.3%	1	8.3%	
Paternal arrhythmia	No	182	87.9%	10	83.3%	0.638
	Yes	25	12.1%	2	16.7%	
Paternal angina	No	171	82.2%	8	72.7%	0.428
	Yes	37	17.8%	3	27.3%	
Paternal infarction	No	177	82.7%	9	75.0%	0.496
	Yes	37	17.3%	3	25.0%	
Paternal stroke	No	197	91.2%	11	91.7%	0.956
	Yes	19	8.8%	1	8.3%	
Paternal kidney disease	No	186	88.6%	10	83.3%	0.583
	Yes	24	11.4%	2	16.7%	

Data expressed as absolute (n) and relative (%) frequency. Pearson's chi-squared test.

TABLE 5 Evaluation of the presence of hypertension (ABPM) according to maternal comorbidities.

ABPM hypertension		No		Yes		Pearson X
		n	%	n	%	Sig
Knowledge of the mother's disease	No	6	2.3%	0	0.0%	0.570
	Yes	260	97.7%	14	100.0%	
Maternal high blood glucose/diabetes	No	173	67.1%	8	57.1%	0.444
	Yes	85	32.9%	6	42.9%	
Hypercholesterolemia reported by the mother	No	136	54.0%	10	71.4%	0.201
	Yes	116	46.0%	4	28.6%	
Cardiovascular disease reported by the mother	No	80	44.9%	8	72.7%	0.073
	Yes	98	55.1%	3	27.3%	
Maternal hypertension	No	102	39.5%	4	28.6%	0.413
	Yes	156	60.5%	10	71.4%	
Maternal obesity	No	148	57.1%	10	71.4%	0.292
	Yes	111	42.9%	4	28.6%	
Maternal thrombosis	No	239	94.1%	14	100.0%	0.349
	Yes	15	5.9%	0	0.0%	
Maternal arrhythmia	No	201	79.1%	13	92.9%	0.213
	Yes	53	20.9%	1	7.1%	
Maternal angina	No	199	77.7%	11	84.6%	0.559
	Yes	57	22.3%	2	15.4%	
Maternal infarction	No	229	89.1%	13	92.9%	0.658
	Yes	28	10.9%	1	7.1%	
Maternal stroke	No	243	94.2%	12	85.7%	0.202
	Yes	15	5.8%	2	14.3%	
Maternal kidney disease	No	229	90.2%	14	100.0%	0.218
	Yes	25	9.8%	0	0.0%	

Data expressed as absolute (n) and relative (%) frequency. Pearson's chi-squared test.

by 19.1% of the women, and the prevalence of hypertensive among women measure by ABPM was 4.98% In addition, 4.95% of the participants reported preeclampsia during a pregnancy.

In our study, we demonstrated a statistically significant difference in white skin color in which women were detected as having hypertension by ABPM while other ethnicities such as black and mixed women did not have this difference. The studies by Mendes et al. (2018) and Paula et al. (2020) discuss the differences between hypertensive and normotensive people when the topic is ethnicity or race. Other demographic characteristics such as marital status and educational level did not show a statistical difference showing the presence or absence of hypertension when measured by the ABPM.

Regarding cardiovascular comorbidities, our study found no significant associations between hypertension measured by ABPM and diabetes, obesity, infarction, stroke, chronic kidney disease, or other comorbidities. Comparing these data with the literature, it is known that hypertension is associated with several risk factors for cardiovascular diseases (CVD), including lipid disorders such as high levels of cholesterol and triglycerides, obesity, and tobacco and alcohol consumption. It is also known that the presence of more than one risk factor for CVD such as heart attack, chronic kidney disease and stroke are increased in patients with hypertension (Levine et al., 2018; Unger et al., 2020). In our study, due to the small number of women, we did not find this association. Regarding paternal and maternal comorbidities, our study did not find hypertension to be associated with these comorbidities It is also known that paternal and maternal comorbidities influence the presence of hypertension, another result different from the literature in our study (Levine et al., 2018; Unger et al., 2020).

Our study also assessed the presence of hypertension measured by ABPM according to with median measures of anthropometric parameters, including abdominal circumference, neck circumference, weight, height, BMI, waist-to-height ratio. There was no significant difference in these parameters, i.e., the anthropometric measurements, always considering that the anthropometric parameters are with median values, of the participants are not related to the presence of hypertension. In the literature we find that we

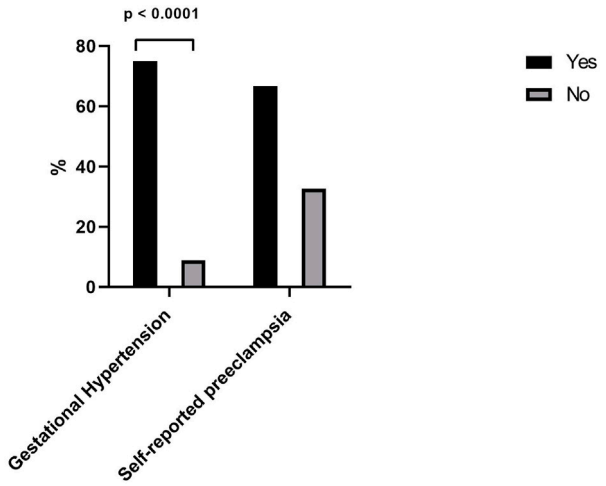

FIGURE 3
ABPM Hypertension between gestational hypertension and self reported hypertension.

found that in the study by Cassani et al. (2009) only waist circumference was associated with hypertension detected by conventional blood pressure measurement, while other anthropometric parameters were not associated with hypertension, as observed in our study with median measurements. Schommer et al. (2014) also reported an association of waist circumference with hypertension in 10- to 18-year-old adolescents (Cassani et al., 2009; Schommer et al., 2014).

When hypertensive patients based on ABPM were evaluated in our study regarding the presence of hypertensive disorders of pregnancy, there was a significant difference when these patients were asked about the presence of gestational hypertension, women who reported gestational hypertension were hypertensive based on ABPM. However, there was no relationship between the presence of self-reported preeclampsia and hypertension measured by ABPM. Metoki et al. (2022) recommend ABPM in hypertensive and overweight pregnant women. In a review article, Bello et al. (2018) recommend the use of ABPM in pregnant women as a tool to guide the diagnosis and management of hypertensive disorders of pregnancy (Bello et al., 2018; Metoki et al., 2022).

We also observed no significant differences in serum cardiovascular markers such as LDL, HDL, C-reactive protein, triglycerides or cholesterol between women with hypertension measured by ABPM and non-hypertensive women, also in this case the evaluation of cardiovascular markers was with average values (Babkowski et al., 2021). Kidambi et al. (2020) reported that, in African Americans, 24-h ABPM is a limited predictor of CVD.

The pulse wave velocity measure is a measure of arterial stiffness and there was no relationship with the detections of the presence of hypertension in women, probably due to the low mean age of women.

Our study has several strengths such as the assessment of many different variables–from demographic variables such as participants' comorbidities and paternal and maternal comorbidities of the participants to anthropometric and serum dosage variables–to determine the presence of hypertension measured by the ABPM. This study also serves to reinforce the use of the ABPM to measure hypertension in women aged close to 40 years to encourage future research and cohorts to investigate hypertension in this age group in women. However, among the limitations of the study are that the women who underwent evaluation by ABPM were relatively few (n = 281), that is, the acceptance of the placement of the ABPM voluntarily by women was low, with only 281 accepting to do this specific evaluation. Of the total of 929 women who participated in the cohort. Another limitation is that these women were evaluated at a relatively young age (38–39 years) for the development of

TABLE 6 Evaluation of the presence of hypertension (ABPM) according to anthropometric measurements.

ABPM hypertension	No			Yes			Mann-whitney
	Median	25%	75%	Median	25%	75%	Sig
Waist circumference	87.0	79.0	98.0	93.0	85.0	107.0	0.113
Neck circumference	35.0	33.0	37.0	35.8	34.0	38.0	0.094
Weight	71.8	62.4	87.2	77.1	65.5	97.3	0.361
Height	162.0	158.0	166.0	160.8	157.0	166.0	0.756
BMI	27.6	24.0	33.2	29.1	26.0	33.3	0.340
Waist-height ratio	0.54	0.49	0.61	0.54	0.53	0.69	0.112

Data expressed as median and percentile (25%–75%). Mann-Whitney test.

TABLE 7 Evaluation of the presence of hypertension (ABPM) according to serum levels of cardiovascular disease markers.

ABPM hypertension	No			Yes			Mann-whitney
	Median	25%	75%	Median	25%	75%	Sig
Triglycerides	108.0	76.0	153.0	150.5	98.0	197.0	0.100
Cholesterol	174.5	152.0	197.0	195.5	161.0	212.0	0.221
LDL	100.0	82.0	123.5	103.5	85.0	133.0	0.710
HDL	46.9	40.2	55.1	46.4	38.2	61.1	0.752
Creatinine	0.80	0.71	0.88	0.84	0.69	0.93	0.632
Glucose	88.0	79.0	97.0	85.4	77.0	100.1	0.649
C-reactive protein	0.31	0.12	0.66	0.40	0.21	0.55	0.389
Homocysteine	7.7	6.8	9.3	7.7	6.5	9.3	0.806
Basal insulin	27.2	14.7	53.0	33.6	29.0	43.0	0.519
Glycated hemoglobin	5.3	5.0	5.6	5.3	5.1	5.4	0.935
PWV [(a)]	7.0	6.2	7.8	7.9	6.6	8.5	0.130

(a) Pulse wave velocity; Data expressed as median and percentile (25%–75%). Mann-Whitney test.

cardiovascular diseases, it is consolidated in the literature that cardiovascular diseases, including hypertension, appear more frequently in older age groups, more specifically over 50 and over 60 years.

Conclusion

We conclude that ethnicity, self-reported hypertension, and the presence of hypertension during pregnancy are associated with arterial hypertension measured by ABPM. We did not find this association for the other parameters analyzed in part because the women analyzed in the study were aged 39–39 years. However, the identification of risk factors in women by ABPM is important for preventing future CVD, reducing morbidity and mortality and improving quality of life and longevity.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by The Ethics Committee of the University Hospital of the Ribeirão Preto Medical School approved this study under protocol number 45485915.7.0000.5440. The patients/participants provided their written informed consent to participate in this study.

Author contributions

TB–Project administration, data curation, formal analysis, writing—original draft, writing—review and editing. EV–project administration, data curation, formal analysis, writing—original draft, writing—review and editing. RL–writing—original draft, writing—review and editing. ED–writing—original draft, writing—review and editing. VS–writing—original draft, writing—review and editing. RC–project administration, data curation, formal analysis, writing—original draft, writing—review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Barbieri, M. A., Bettiol, H., Silva, A. A. M., Cardoso, V. C., Simões, V. M. F., Gutierrez, M. R. P., et al. (2006). Health in early adulthood: The contribution of the 1978/79 Ribeirão Preto birth cohort. *Braz. J. Med. Biol. Res.* 39, 1041–1055. doi:10.1590/S0100-879X2006000800007

Bello, N. A., Miller, E., Cleary, K., Wapner, R., Shimbo, D., and Tita, A. T. (2018). Out of office blood pressure measurement in pregnancy and the postpartum period. *Curr. Hypertens. Rep.* 20, 101. doi:10.1007/s11906-018-0901-z

Brandstetter, S., Toncheva, A. A., Niggel, J., Wolff, C., Gran, S., Seelbach-Göbel, B., et al. (2019). KUNO-kids birth cohort study: Rationale, design, and cohort description. *Mol. Cell. Pediatr.* 6, 1. doi:10.1186/s40348-018-0088-z

Brant, L. C. C., Nascimento, B. R., Veloso, G. A., Gomes, C. S., Polanczyk, C., de Oliveira, G. M. M., et al. (2022). Burden of cardiovascular diseases attributable to risk factors in Brazil: Data from the "global burden of disease 2019" study. *Rev. Soc. Bras. Med. Trop.* 55, e0263. doi:10.1590/0037-8682-0263-2021

Camafort-Babkowski, M., Adeseye, A., Coca, A., Damasceno, A., de Simone, G., Dorobantu, M., et al. (2021). The global ambulatory blood pressure monitoring (ABPM) in heart failure with preserved ejection fraction (HFpEF) registry. Rationale, design and objectives. *J. Hum. Hypertens.* 35, 1029–1037. doi:10.1038/s41371-020-00446-8

Carey, R. M., Muntner, P., Bosworth, H. B., and Whelton, P. K. (2018). Prevention and control of hypertension: JACC health promotion series. *J. Am. Coll. Cardiol.* 72, 1278–1293. doi:10.1016/j.jacc.2018.07.008

Cassani, R. S. L., Nobre, F., Pazin-Filho, A., and Schmidt, A. (2009). Relationship between blood pressure and anthropometry in a cohort of brazilian men: A cross-sectional study. *Am. J. Hypertens.* 22, 980–984. doi:10.1038/ajh.2009.104

de Siqueira Valadares, L. T., de Souza, L. S. B., Salgado Júnior, V. A., de Freitas Bonomo, L., de Macedo, L. R., and Silva, M. (2022). Prevalence of metabolic syndrome in Brazilian adults in the last 10 years: A systematic review and meta-analysis. *BMC Public Health* 22, 327. doi:10.1186/s12889-022-12753-5

de Souza, M. P., Lopes, P. C., Bazo, G., Rocha, P. R. H., Lorencini, D. A., Bettiol, H., et al. (2022). Hypertension defined by the 2017 ACC/AHA guideline is more accurate than 2018 ESC/ESH for detecting early vascular aging in young adults. *Med. (United States)* 101, E28841. doi:10.1097/MD.0000000000028841

Fahed, G., Aoun, L., Zerdan, M. B., Allam, S., Zerdan, M. B., Bouferraa, Y., et al. (2022). Metabolic syndrome: Updates on pathophysiology and management in 2021. *Int. J. Mol. Sci.* 23, 786. doi:10.3390/ijms23020786

Flynn, J. T., Urbina, E. M., Brady, T. M., Baker-Smith, C., Daniels, S. R., Hayman, L. L., et al. (2022). Ambulatory blood pressure monitoring in children and adolescents: 2022 update: A scientific statement from the American heart association. *Hypertension* 79, e114–e124. doi:10.1161/HYP.0000000000000215

Honigberg, M. C., and Natarajan, P. (2020). Women's cardiovascular health after hypertensive pregnancy: The long view from labor and delivery becomes clearer. *J. Am. Coll. Cardiol.* 75, 2335–2337. doi:10.1016/j.jacc.2020.01.064

Hurrell, A., Webster, L., Chappell, L. C., and Shennan, A. H. (2022). The assessment of blood pressure in pregnant women: Pitfalls and novel approaches. *Am. J. Obstet. Gynecol.* 226, S804–S818. doi:10.1016/j.ajog.2020.10.026

Jardim, T. V., Rosner, B., Bloch, K. V., Kuschnir, M. C. C., Szklo, M., and Jardim, P. C. V. (2020). Blood pressure reference values for Brazilian adolescents: Data from the study of cardiovascular risk in adolescents (ERICA study). *J. Pediatr.* 96, 168–176. doi:10.1016/j.jped.2018.09.003

Jiang, Z., Sun, T., He, Y., Gou, W., Zuo, L., Shi, Y, et al. (2020). Dietary fruit and vegetable intake, gut microbiota, and type 2 diabetes: Results from two large human cohort studies. *BMC Med.* 18, 371. doi:10.1186/s12916-020-01842-0

Kidambi, S., Wang, T., Chelius, T., Nunuk, I., Agarwal, P., Laud, P., et al. (2020). Twenty-four-hour versus clinic blood pressure levels as predictors of long-term cardiovascular and renal disease outcomes among African Americans. *Sci. Rep.* 10, 11685. doi:10.1038/s41598-020-68466-5

Levine, G. N., Al-Khatib, S. M., Beckman, J. A., Birtcher, K. K., Bozkurt, B., Brindis, R. G., et al. (2018). 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: A report of the American college of cardiology/American heart association task force on clinical practice guidelines. *Hypertension* 71, 13–115. doi:10.1161/HYP.0000000000000065

Malta, D. C., Gonçalves, R. P. F., Machado, Í. E., Freitas, M. I. de F., Azeredo, C., and Szwarcwald, C. L. (2018). Prevalence of arterial hypertension according to different diagnostic criteria, National Health Survey. *Rev. Bras. Epidemiol.* 21, e180021. doi:10.1590/1980-549720180021.supl.1

Marques, A. P., Szwarcwald, C. L., de Souza-Junior, P. R. B., Malta, D. C., and Montilla, D. E. R. (2019). Prevalence of arterial hypertension in Brazilian adults and its associated factors and activity limitations: A cross-sectional study. *Sao Paulo Med. J.* 137, 312–321. doi:10.1590/1516-3180.2018.0251220719

Mendes, P. M., Nobre, A. A., Griep, R. H., Guimarães, J. M. N., Juvanhol, L. L., Barreto, S. M., et al. (2018). Association between perceived racial discrimination and hypertension: Findings from the ELSA-Brasil study. *Cad. Saude Publica* 34, e00050317. doi:10.1590/0102-311X00050317

Metoki, H., Iwama, N., Hamada, H., Satoh, M., Murakami, T., Ishikuro, M., et al. (2022). Hypertensive disorders of pregnancy: Definition, management, and out-of-office blood pressure measurement. *Hypertens. Res.* 45, 1298–1309. doi:10.1038/s41440-022-00965-6

Mills, K. T., Stefanescu, A., and He, J. (2020). The global epidemiology of hypertension. *Nat. Rev. Nephrol.* 16, 223–237. doi:10.1038/s41581-019-0244-2

Oliveira, L. V. A., dos Santos, B. N. S., Machado, Í. E., Malta, D. C., Velasquez-Melendez, G., and Felisbino-Mendes, M. S. (2020). Prevalence of the metabolic syndrome and its components in the Brazilian adult population. *Cien. Saude Colet.* 25, 4269–4280. doi:10.1590/1413-812320202511.31202020

Paula, D. P., Lopes, L. J., Mill, J. G., Fonseca, M. J. M., and Griep, R. H. (2020). Identifying patterns of diurnal blood pressure variation among ELSA-Brasil participants. *J. Clin. Hypertens.* 22, 2315–2324. doi:10.1111/jch.14066

Rana, S., Lemoine, E., Granger, J., and Karumanchi, S. A. (2019). Preeclampsia: Pathophysiology, challenges, and perspectives. *Circ. Res.* 124, 1094–1112. doi:10.1161/CIRCRESAHA.118.313276

Ribeiro, A. L. P., Duncan, B. B., Brant, L. C. C., Lotufo, P. A., Mill, J. G., and Barreto, S. M. (2016). Cardiovascular health in Brazil trends and perspectives. *Circulation* 133, 422–433. doi:10.1161/CIRCULATIONAHA.114.008727

Schommer, V. A., Barbiero, S. M., Cesa, C. C., Oliveira, R., Silva, A. D., and Pellanda, L. C. (2014). Excess weight, anthropometric variables and blood pressure in schoolchildren aged 10 to 18 years. *Arq. Bras. Cardiol.* 102, 312–318. doi:10.5935/abc.20140038

Unger, T., Borghi, C., Charchar, F., Khan, N. A., Poulter, N. R., Prabhakaran, D., et al. (2020). 2020 international society of hypertension global hypertension practice guidelines. *Hypertension* 75, 1334–1357. doi:10.1161/HYPERTENSIONAHA.120.15026

Whelton, P. K., Carey, R. M., Aronow, W. S., Carey, D. E., Jr, Collins, K. J., Himmelfarb, C. D., et al. (2018). 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: A report of the American college of cardiology/American heart association task force on clinical practice guidelines. *J. Am. Coll. Cardiol.* 71, e127–e248. doi:10.1016/j.jacc.2017.11.006

Zhou, B., Carrillo-Larco, R. M., Danaei, G., Riley, L. M., Paciorek, C. J., Stevens, G. A., et al. (2021). Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: A pooled analysis of 1201 population-representative studies with 104 million participants. *Lancet* 398, 957–980. doi:10.1016/S0140-6736(21)01330-1

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The safety and efficacy of esketamine in comparison to dexmedetomidine during drug-induced sleep endoscopy in children with obstructive sleep apnea hypopnea syndrome: A randomized, controlled and prospective clinical trial

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Background and Purpose: Data and high-quality studies of anesthetic methods for children with obstructive sleep apnea hypopnea syndrome (OSAHS) who undergo drug-induced sleep endoscopy (DISE) are limited. Research on pediatric DISE using esketamine has never been reported before. To test the safety and efficacy of esketamine during DISE in children with OSAHS, we compare esketamine (Group K) with dexmedetomidine (Group D) in this study.

Methods: 100 children with ASA I~II grade, prepared for an elective adenotonsillectomy under general anesthesia, were enrolled in this study and randomized into two groups. Midazolam 0.1 mg/kg was administered intravenously for both groups. In Group D a 1 μg/kg bolus of dexmedetomidine was given over 10 min followed by the infusion rate 1 μg/kg/hr to the end of DISE. Group K received a 1.0 mg/kg IV bolus of esketamine over 10 s followed by the infusion rate 1 mg/kg/hr to the end of DISE.

Abbreviations: OSAHS, obstructive sleep apnea hypopnea syndrome; PSG, polysomnography; BMI, Body Mass Index; RDI, respiratory disturbance index; AHI, Apnea-hypopnea Index; ASA, American Society of Anesthesiologists; DISE, drug-induced sleep endoscopy; UMSS, University of Michigan Sedation Scale; ABJ score, Awakening and behavior judgment score for newborns and children; PONV, postoperative nausea and vomiting; BIS, bispectral index; ECG, electrocardiogram; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; RR, respiratory rate; SpO2, pulse oxygen saturation; AEs, adverse effects; SD, standard deviation; ANOVA, one-way analysis of variance.

Results: Group K had a higher percentage of success than Group D (p = 0.008). The onset time of Group K was shorter than that of Group D (p = 0.000). The University of Michigan Sedation Scale (UMSS) score of Group K was higher than that of Group D (p = 0.005). The risk of adverse effects (AEs) was lower in Group K (p = 0.000). In Group D, systolic and diastolic blood pressure (SBP and DBP) and heart rate (HR) all decreased, while in Group K, SBP, DBP, and HR hardly changed.

Conclusion: Esketamine in comparison to dexmedetomidine provides more effective and safer depth of anesthesia for OSAHS pediatric DISE by ensuring short onset time, deep sedation, and few AEs.

Clinical Trial Registration: ClincalTrials.gov, identifier NCT04877639

KEYWORDS

dexmedetomidine, esketamine, drug-induced sleep endoscopy (DISE), obstructive sleep apnea hypoapnea syndrome, pediatrics

Introduction

Obstructive sleep apnea hypopnea syndrome (OSAHS) is generally believed to be a common health problem (Li et al., 2010). 2%–4% of children with OSAHS are associated with a substantial morbidity (Park et al., 2011), including failure of growth, impaired neurocognitive and neurobehavioral abnormalities (Miano et al., 2011), systemic hypertension, pulmonary hypertension (Goldbart et al., 2010), cor pulmonale, etc (Kelly et al., 2010). A great number of children with OSAHS are cured after adenotonsillectomy. However, 10%–20% of children have continuing symptoms after surgery (Bhattacharjee et al., 2010). One of the reasons for uncured OSAHS is failure to identify all sites of the upper airway obstruction (Marcus et al., 2012).

There has been increased interest in using drug-induced sleep endoscopy (DISE), an emerging endoscopic technology, to assess upper airway obstruction in patients with OSAHS in a sleep-like state induced and maintained by anesthetic drugs. Using a flexible nasal endoscope, DISE locates the sites and patterns of airway collapse accurately, predicts the benefit of surgery, and customizes a targeted surgical approach for each patient (Capasso et al., 2016).

To mimic physiological sleep with decreased oxygen saturation levels, the ideal anesthetic administration during DISE should involve the use of titrable pharmacological agents with short biological half-life and minor influence on muscle tone and respiratory drive (Liu et al., 2020). Dynamic evaluation of DISE in children with OSAHS is often achieved by using sedatives and anesthetics including benzodiazepines, pentobarbital, remifentanil, propofol, ketamine, dexmedetomidine, and their combination (Cho et al., 2015; Liu et al., 2020). Propofol acts through the inhibitory neurotransmitter GABA to diminish behavioral responsiveness as if in a state of non-rapid eye movement (NREM) sleep (Murphy et al., 2011). It may compromise the airway due to muscular flaccidity and respiratory drive suppression. As a selective alpha-2 adrenergic agonist, dexmedetomidine is a highly recommended agent for DISE owing to its analgesic, amnesic and anxiolytic characteristics. Ehsan reviewed and concluded that drugs such as dexmedetomidine have the least impact on respiratory control and may be most effective in DISE (Ehsan et al., 2016). Though dexmedetomidine successfully induces sedation for non-invasive procedures, it does not provide sufficient depth of anesthesia when used as a sedative/anesthetic alone for invasive procedures (Mahmoud and Mason, 2015). Based on his review, Liu thought that the optimal scheme might be made by combining ketamine with dexmedetomidine (Liu et al., 2020). As a non-competitive n-methyl-d- aspartic acid (NMDA) receptor antagonist, ketamine offers good analgesia and amnesia with natural respiratory pattern, however, its role as a sedative has been restricted by the occurrence of vomiting and psychomimetic side effects (Sruthi et al., 2018; Wang et al., 2019). The substance ketamine is a racemate comprising two enantiomers—mirror-like configurated molecules S (+)- and R (−)- ketamine. Compared to both the racemic and R (−)-ketamine, S (+)-enantiomer demonstrated the greater efficacy with lower dosage in experimental studies on animals (Schmidt et al., 2005).

Children with OSAHS are vulnerable to upper airway obstruction during sedation and anesthesia because they are more sensitive to the respiratory inhibitive effects of hypnotics and sedatives. We need to avoid using airway intervention to improve airway patency for them. Thus, it poses a challenge to obtain perfect dynamic airway assessment during DISE for these patients. It is urgent and critical to find good anesthetic drugs for their DISE. Data and high-quality studies of anesthetic methods for OSAHS pediatric DISE are limited (Liu et al., 2020). Research on pediatric DISE using esketamine has never been reported before. To test the safety and efficacy of esketamine during DISE in children with OSAHS, we compare esketamine (Group K) with dexmedetomidine (Group D) in this study.

Materials and methods

Ethics approval

This study, approved by the Ethical Board for Clinical/Scientific Research Project of Zhongnan Hospital of Wuhan University (Approval Number: 2021071), was conducted in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki. Trial Registration: ClincalTrials.gov. Identifier: NCT04877639. Chinese Clinical Trial Registry: http://www.chictr.org. Number: ChiCTR2100045914.

Study design, setting and population

100 children with ASA Ⅰ~Ⅱ grade who are prepared for an elective adenotonsillectomy under general anesthesia were enrolled between 17 May 2021 and 22 November 2021 at Department of Otorhinolaryngology-Head and Neck Surgery of Zhongnan Hospital of Wuhan University in the study. Inclusion criteria included 1) 3–12 years old, and 2) informed consent from subjects' legal guardian. Exclusion criteria were 1) ASA physical status>Ⅲ, 2)a baseline oxygen saturation<95%, 3) Body Mass index (BMI) > 30 kg/m^2, 4)Mallampati score iv, 5) chronic heart/lung/liver/kidney diseases, iind-iiird degree a-v block, psychiatric illness, (6 drug abuse or history of chronic analgesic use, and 7) allergy against the study medications (dexmedetomidine or esketamine). The subjects were numbered according to their treatment order and randomized into two groups (Group D and Group K). Randomization was achieved by computer generated random numbers hidden in a sealed opaque bag. A nurse, who was not involved in the study, read the numbers and assigned two groups.

In the inpatient ward, an intravenous (IV) catheter was inserted for all children in the two groups. Midazolam 0.1 mg/kg was administered intravenously for both groups. In Group D a 1 μg/kg bolus of dexmedetomidine (U2102003, Hu Nan Ke Lun Pharmaceutical Co. Ltd., China) was given over 10 min followed by the infusion rate 1 μg/kg/hr to the end of DISE. Group K received a 1.0 mg/kg IV bolus of esketamine (210126BL, Jiang Su Heng Rui Pharmaceuticals, China) over 10 s followed by the infusion rate 1 mg/kg/hr to the end of DISE. The dexmedetomidine and esketamine were each diluted in a 50 ml syringe separately and labeled as infusion A and B respectively, and administered using syringe pumps (WZS-50F6 Double channel micro-infusion pump, Smiths medical, China) by an anesthesiologist, who covered all syringes and infusion sets as well as the screen of the syringe pumps by aluminum foil paper to assure blindness of the study.

With their heads remaining neutral, the patients in both groups were supine and could breathe spontaneously. Throughout the procedure the patients received continuous oxygen, and their pulse oximetry, electrocardiogram and blood pressure were monitored. The level of sedation was monitored with an A-2000 BIS monitor (BIS LoC 2 channel, BIS Complete Monitoring System, 2011 Covidien 11c, Singapore). After UMSS>3 and BIS 65–75, the nostrils, the nasopharynx, the oral cavity and the hypopharynx were checked by a flexible fibrous laryngoscope to ascertain airway obstructions. The base of tongue and supraglottic structures were also checked. The patients in both groups were given propofol 0.5 mg/kg when they moved during DISE. After DISE, intubation was employed and then mechanical ventilation was achieved by IPPV. An elective adenotonsillectomy under general anesthesia was performed at last.

Observational index

Demographic and PSG data: Demographic and polysomnography (PSG) data, including age, gender, height, weight, American Society of Anesthesiologists (ASA) physical status, Body Mass Index (BMI), respiratory disturbance index (RDI), apnea hypopnea index (AHI), severity of OSAHS and Mallampati Score (Smith et al., 2020), were gathered.

Vital signs: Heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), electrocardiogram (ECG), respiratory rate (RR) and pulse oxygen saturation (%, SpO_2) were collected before medication and DISE (T0), 5 min after medication and before DISE (T1), 1 min after start of DISE (T2), 1 min after completion of DISE (T3), 1 min after tracheal intubation (T4), 1 min after extubation (T5) and 30 min after extubation (T6).

Time for each procedure: Onset time, DISE time, operation duration, recovery time and residence time were recorded.

Percentage of success: The ratio of completed DISE cases and total cases was calculated.

UMSS score, BIS and ABJ score: Depth of sedation was evaluated by the University of Michigan Sedation Scale (UMSS) (Malviya et al., 2002; Haberland et al., 2011) at T1 and the bispectral index (BIS) (Ibrahim et al., 2001) at T0, T1, T2, T3, T4, T5, and T6. Awakening and behavior judgment score for newborns and children (ABJ score) (Pees et al., 2003) was recorded at T6.

Adverse effects (AEs) and corresponding treatments: AEs such as hypoxemia (SpO_2<90%, Apnea>20s), laryngospasm, patient movement, abortion of examination, PONV, delirium and propofol rescue were observed during and after DISE. Hypoxemia was relieved by oxygen therapy. Laryngospasm was treated by the positive pressure ventilation and/or administration of propofol. Propofol 1.0 mg/kg was used for delirium or uncontrolled movements. 0.15 mg/kg of Ondansetron and/or 0.25 mg/kg of dexamethasone (below 10 mg) were given for postoperative nausea and vomiting (PONV) (Martin et al., 2019). Other AEs were minor and transient, which needed no special treatment.

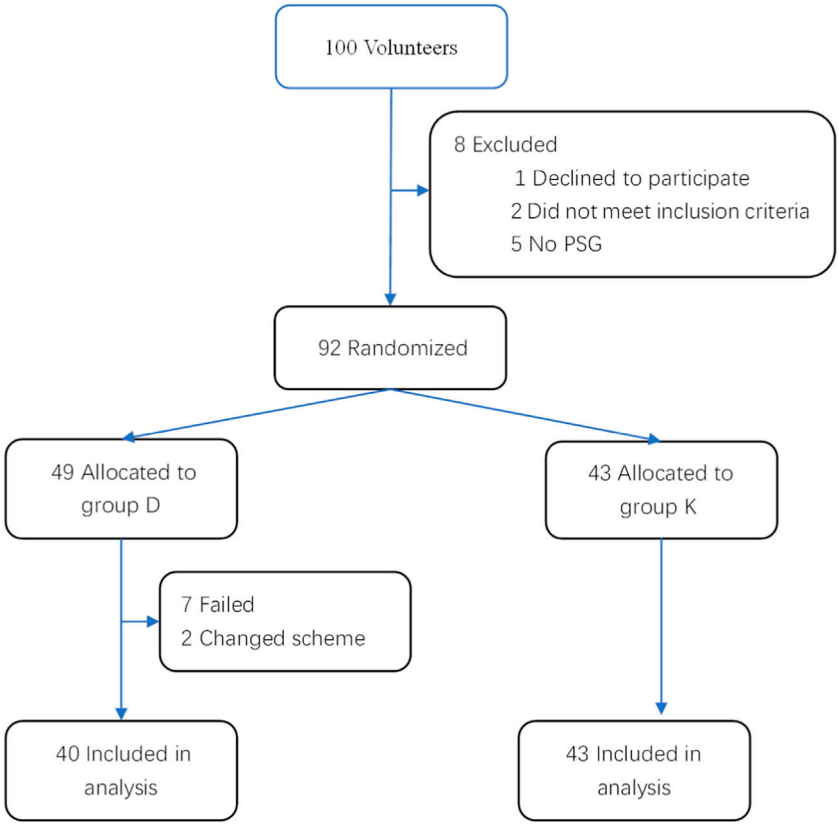

FIGURE 1
Flow diagram illustrating patients' enrollment throughout the study.

Statistical analysis

Power analysis performed by using the nQuery Advisor with an inter-group difference of 20.4 and a standard deviation of 23, $\alpha = 0.05$, $\beta = 0.2$ (power = 80%), assuming a dropout rate of 10%, indicated that at least 22 subjects would be needed for each group. The sample size of each treatment group was estimated based on the differences and variations observed in a previous study by Evans et al. (2003).

Continuous variables with normal distribution (Height, Recovery time, HR, SBP, DBP, BIS) were presented as mean ± standard deviation, and continuous variables with non-normal distribution (Age, Weight, BMI, RDI, AHI, Onset time, DISE time, Operation duration, Residence time, RR and SpO_2) were represented by median (interquartile range). Frequency (%) was used for categorical variables (Gender, UMSS score, ABJ score, severity of OSAHS, ASA physical status, Mallampati score, Percentage of success and AEs).

Continuous variables with normal distribution were compared between the two groups by independent sample *t* test, and these variables at different time points within each group were compared by repeated measure ANOVA. Continuous variables with non-normal distribution were compared between the two groups by Wilcoxon rank sum test. Non-rank categorical variables (Percentage of success, AEs) were tested by Chi-square test, and rank categorical variables (UMSS score, ABJ score, severity of OSAHS, ASA physical status, Mallampati score) were tested by Wilcoxon rank sum test. All statistical analysis was conducted with SPSS26 software. $P < 0.05$ was considered statistically significant.

Results

Demographic and polysomnography data

100 children were enrolled in the study. Of them, 2 did not meet the inclusion criteria, 1 declined to participate, 5 had no PSG, 2 changed scheme, and 7 failed to undergo the complete DISE, and thus we dropped these 17 children from the study. Their data were not used in analysis, but the 7 children who had early termination of DISE were included in the calculation of percentage of success (Figure 1).

Demographic and PSG data had no difference between the two groups ($p > 0.05$). PSG monitoring demonstrated some degree of

TABLE 1 Demographic and PSG data.

Characteristic	Total (n = 83)	Group D (n = 40)	Group K (n = 43)	p-value
Gender (n%)				0.600
Male	42 (50.6%)	19 (47.5%)	23 (53.5%)	
Female	41 (49.4%)	21 (52.5%)	20 (46.5%)	
Age(m)	79 (60.50–99)	70.50 (56.25–99)	83.50 (69.75–100.25)	0.433
Height (cm)	122.90 ± 15.37	119.94 ± 16.39	125.71 ± 13.94	0.100
Weight (kg)	24 (18–30)	21.00 (17.13–26.38)	25.50 (18.00–32.63)	0.050
BMI (kg.m-2)	15.53 (14.50–18.01)	15.38 (14.44–16.89)	16.08 (14.67–18.68)	0.183
RDI	1.4 (1.00–3.45)	1.25 (1.00–3.03)	1.65 (0.95–3.65)	0.600
AHI	1.4 (0.98–3.38)	1.25 (1.00–3.03)	1.65 (0.95–3.65)	0.498
Severity of OSAHS				0.426
No	15 (18.1%)	9 (22.5%)	6 (14.0%)	
I	57 (68.7%)	24 (60.0%)	33 (76.7%)	
II	6 (7.2%)	4 (10.0%)	2 (4.7%)	
III	5 (6.0%)	3 (7.5%)	2 (4.7%)	
ASA physical status				0.916
I	17 (20.5%)	8 (20.0%)	9 (20.9%)	
II	66 (79.52%)	32 (80.0%)	34 (79.1%)	
Mallampati Score				0.905
I	64 (77.11%)	30 (75.0%)	34 (79.1%)	
II	17 (20.48%)	9 (22.5%)	8 (18.6%)	
III	2 (2.41%)	1 (2.5%)	1 (2.33%)	

PSG, polysomnography; BMI, body mass index; RDI, respiratory disturbance index; AHI, Apnea-hypopnea Index. OSA, obstructive sleep apnea; ASA, American Society of Anesthesiologist. Data presented as mean ± SD, median (interquartile range) or frequency (%). p presented the comparison between the two groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

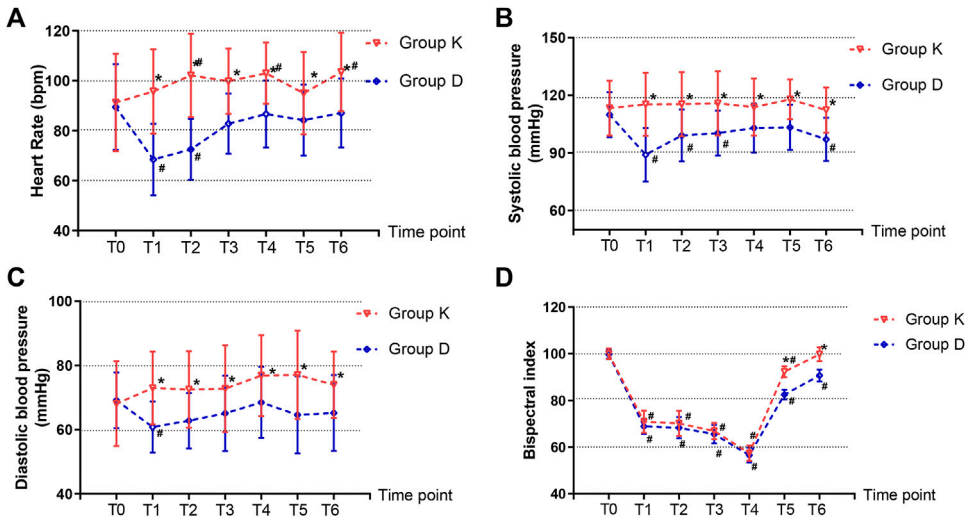

FIGURE 2
Hemodynamic changes and the bispectral index (BIS) records. **(A)** Heart rate (HR). **(B)** Systolic blood pressure (SBP). **(C)** Diastolic blood pressure (DBP). **(D)** The bispectral index (BIS). Data are expressed as mean ± SD, Compare between two groups *$p < 0.05$. Compare within time points of the same group $^{\#}p < 0.05$.

TABLE 2 Time for each procedure.

Time (min)	Total	Group D	Group K	p-value
Onset (min)	3 (2–12)	12 (10.25–14)	2 (2–3)	0.000*
DISE(min)	12 (12–12)	12 (12–12)	12 (12–12)	0.851
Operation (min)	50 (35–60)	50 (40–60)	45 (35–60)	0.204
Recovery (min)	47.55 ± 16.639	54.18 ± 18.812	50.40 ± 17.389	0.344
Residence (min)	30 (30–37)	30 (30–35)	30 (30–40)	0.649

DISE, drug-induced sleep endoscopy. Data presented as mean ± SD, or median (interquartile range). Compare between two groups *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

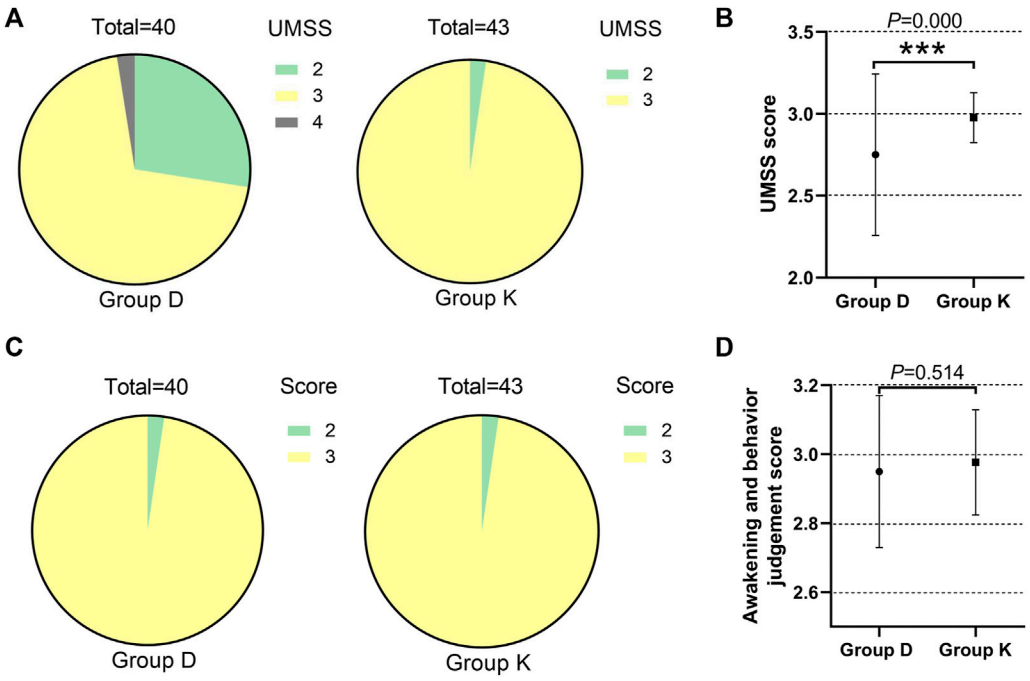

FIGURE 3
Different effects of two groups during drug-induced sleep endoscopy in children with obstructive sleep apnea hypopnea syndrome. **(A)**The number of subjects with each UMSS score in two groups. **(B)** UMSS score. **(C)**The number of subjects with each ABJ score in two groups. **(D)**ABJ score. Data are expressed as median (interquartile range) or frequency (%), Compare between two groups *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

OSAHS on 68 subjects, of whom 57 had mild OSAHS, 6 had moderate OSAHS, and 5 had severe OSAHS (Table 1).

Vital signs

Baseline HR, SBP and DBP did not differ between the two groups. Compared with T0, SBP and DBP at T1 T2 T3 T4 T5 T6, and HR at T1 T3 T5 in Group K were not significantly different. Compared with T0, HR, SBP and DBP all decreased at T1 in Group D. Compared with Group D, children receiving esketamine had higher HR, SBP and DBP at T1,T2,T3,T4, T5, and T6 (Figures 2A,B,C,).

The SpO_2 values recorded were 100% (95% CI, 99%–100%) for the esketamine group and 100% (95% CI, 98%–100%) for the dexmedetomidine group respectively. SpO_2 values did not differ between the two groups ($p = 0.135$).

No abnormal ECG and RR were observed in both groups.

Time for each procedure

Onset time, from starting of dexmedetomidine or esketamine infusion to DISE beginning, was 12 (95% CI, 10.25–14) min in Group D, and onset time of Group K was 2 (95% CI, 2–3) min.

TABLE 3 UMSS score and Awakening and behavior judgment (ABJ) score.

Score	Total (83)	Group D (40)	Group K (43)	p-value
Awakening and behavior judgment				0.514
2	3 (3.61%)	2 (5.00%)	1 (2.33%)	
3	80 (96.39%)	38 (95.00%)	42 (97.6%)	
UMSS				0.005**
2	12 (14.46)	11 (27.50%)	1 (2.33%)	
3	70 (84.34)	28 (70.00%)	42 (97.67%)	
4	1 (1.20%)	1 (2.50%)	0 (0.00%)	

UMSS: University of Michigan Sedation Scale. Data presented as frequency (%). Group D vs. Group K *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

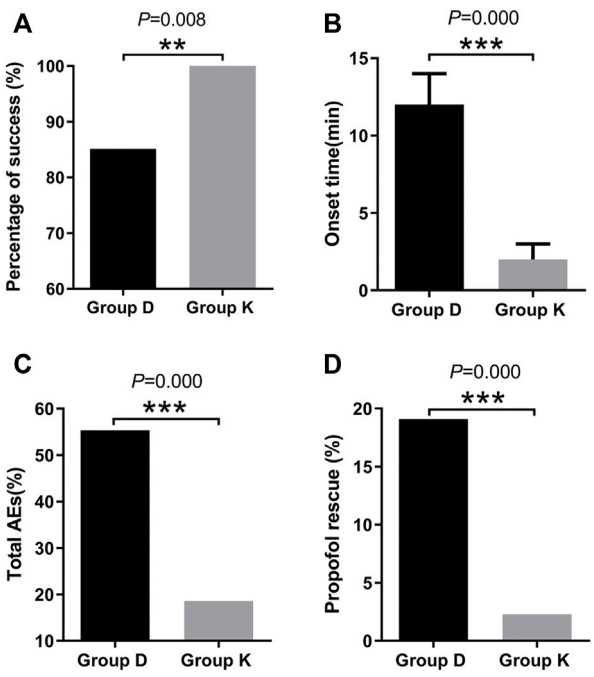

FIGURE 4
Different effects of two groups during drug-induced sleep endoscopy in children with obstructive sleep apnea hypopnea syndrome. **(A)**Percentage of success. **(B)**Onset time. **(C)**Total AEs. **(D)**Propofol rescue. Data are expressed as median (interquartile range) or frequency (%), Compare between two groups *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

The onset time of Group K was shorter than that of Group D (p = 0.000) (Table 2) (Figure 4B).

DISE time, from the beginning to the end of endoscopy, was 12.0 (95% CI, 12.0–12.0) min in both groups. DISE time had no significant difference between the two groups (Table 2) (p = 0.851).

The mean operation duration of the two groups, from the beginning to the end of the operation, was 50 (95% CI, 35–60) min. The operation duration had no significant difference between the two groups (Table 2) (p = 0.204).

Recovery time, from discontinuing of dexmedetomidine or esketamine infusion to eye opening on verbal contact, was 54.18 ± 18.81 min in Group D, and recovery time of Group K was 50.40 ± 17.39 min. The recovery time had no significant difference between the two groups (Table 2) (p = 0.344).

The mean residence time at PACU, from entering PACU to leaving PACU, was 30 (95%CI, 30–37) min. The residence-time at PACU had no significant difference between the two groups (Table 2) (p = 0.649).

University of michigan sedation scale score, bispectral index and awakening and behavior judgment score

UMSS score: Depth of sedation was evaluated by UMSS after completion of bolus dose administration. Most subjects had a UMSS score of 2 or 3 at the time of evaluation. The number of subjects with each score varied between the two groups. For the subjects receiving dexmedetomidine, 11 had a score of 2 and 28 had a score of 3. For the subjects receiving esketamine, 1 had a score of 2, and 42 had a score of 3. Group K had a higher UMSS score than Group D (p = 0.005) (Table 3) (Figures 3A,B).

BIS: BIS at T0, T1, T2, T3 and T4 had no significant difference between the two groups. Group K had higher BIS at T5 and T6 than Group D (Figure 2D).

ABJ score: All subjects had an ABJ score of 2 or 3. The number of subjects with each score varied between the two groups. Of the subjects receiving dexmedetomidine, 2 had a score of 2 and 38 had a score of 3. And of the subjects receiving esketamine, 1 had a score of 2 and 42 had a score of 3. The ABJ scores didn't vary between the two groups (p = 0.514) (Table 3) (Figures 3C,D).

Adverse effects, propofol rescue and percentage of success

AEs: In Group D, 26 of 47 patients experienced AEs, and 8 of 43 patients experienced AEs in Group K. The number of

TABLE 4 Adverse effects, Propofol rescue and Percentage of success.

Symptoms	D group (n = 47)	K group (n = 43)	p-value
Patient movement	14 (29.8%)	0 (0.0%)	
Cry	2 (4.3%)	1 (2.3%)	
Sleepy	3 (6.4%)	0 (0.0%)	
Laryngospasm	1 (2.1%)	0 (0.0%)	
Hypoxemia	4 (8.5%)	6 (14.0%)	
Allergy	1 (2.1%)	0 (0.0%)	
PONV	0 (0.0%)	0 (0.0%)	
Sore throat	0 (0.0%)	0 (0.0%)	
Nystagmus	0 (0.0%)	0 (0.0%)	
Hypertension	0 (0.0%)	0 (0.0%)	
Delirium	0 (0.0%)	0 (0.0%)	
Coughing	1 (2.1%)	0 (0.0%)	
Salivation	0 (0.0%)	1 (2.3%)	
Overnight respiratory events	0 (0.0%)	0 (0.0%)	
Related to drugs			
Certainly	23 (48.9%)	6 (14.0%)	
Probably	3 (6.4%)	2 (4.7%)	
Total	26 (55.3%)	8 (18.6%)	0.000***
propofol rescue	9 (19.1%)	1 (2.3%)	0.000***
Percentage of success	40 (85.11%)	43 (100%)	0.008**

PONV, postoperative nausea and vomiting. Data presented as frequency (%). Group D vs. Group K *p < 0.05, **p < 0.01, ***p < 0.001.

AEs varied greatly between the two groups (p = 0.000). The risk of AEs and propofol rescue was higher in Group D than in Group K (p = 0.000) (Table 4) (Figures 4C,D).

Percentage of success: The ratios of completed DISE cases and total cases, were 85.11% (40/47) in Group D and 100% (43/43) in Group K respectively. Overall, DISE was successfully completed in 92.22% (83/90) of cases. In Group D, DISE was not completed in 7 patients. Group K had a higher percentage of success than Group D (p = 0.008) (Table 4) (Figure 4A).

Discussion

In this prospective, randomized and blinded clinical trial, esketamine iv can provide higher percentage of success, shorter onset time, deeper sedation and fewer AEs, therefore it is superior to dexmedetomidine iv.

It can be seen from this study that the respective percentage of success was 85.11% (40/47) in Group D and 100% (43/43) in Group K. Since 7 patients failed to undergo the complete DISE in Group D, Group K had a higher percentage of success than Group D (p = 0.008). The reasons for increased risk of DISE failure of Group D were probably delayed onset and slighter sedation.

Our findings revealed that esketamine had quicker onset than dexmedetomidine. The respective onset time was 12 (95% CI, 10.25–14) min in Group D and 2 (95% CI, 2–3) min in Group K, which was consistent with Pees' (Pees et al., 2003) and Tekeli's research (Tekeli et al., 2020). Our difference from Pee's research (Pees et al., 2003) was the subjects were adults in Pee's but children in ours. Our study showed no significant difference in recovery time between the two groups (p = 0.344). It was probably because drugs including dexmedetomidine and esketamine had been eliminated during the whole period of DISE followed by adenotonsillectomy.

The UMSS score of Group K was higher than that of Group D, which proved that esketamine had preferred sedation. This result was similar to Pees' (Pees et al., 2003). Contrary to the previous studies made by Lo YL and Haberland CM (Haberland et al., 2011; Lo et al., 2015), difference in BIS between the two groups at T0, T1, T2, T3, and T4 were not significant. The BIS was one of the most accurate and sensitive indicators of accessing conscious state by a single numeric value, scaled from 0 to 100 (Lo et al., 2015; Jones et al., 2021). However, according to Ibrahim AE (Ibrahim et al., 2001), BIS scores associated with depth of anesthesia were dependent upon the anesthetic agent being used. They were relatively ineffective during sedation with ketamine, nitrous oxide, or dexmedetomidine, and could be unpredictable in the presence of opioids. Further research was needed.

AEs occurred in 8 of 43 patients in Group K and 26 of 47 patients in Group D. The risk of AEs was proved to be lower in Group K (p = 0.000).

No abnormal ECG and RR were observed in both groups. SpO_2 between the two groups had no significant difference ($p = 0.135$). It was shown that neither dexmedetomidine nor esketamine had airway intervention or significant oxygen desaturation. This gave further support to earlier experimental results (Mahmoud et al., 2009; Ehsan et al., 2016). Compared with T0, HR, SBP and DBP all decreased at T1 in Group D. In accordance with Nelson's research (Nelson et al., 2003), dexmedetomidine might cause marked hemodynamic instability, especially bradycardia and hypotension.

Compared with T0, SBP and DBP at T1 T2 T3 T4 T5 T6, and HR at T1 T3 T5 in Group K were not significantly different. Our research revealed for the first time that esketamine, administered along with midazolam, had little influence on the circulatory and respiratory system during OSAHS pediatric DISE.

The pharmacokinetic parameters of esketamine and S-norketamine are both similar in the pure isomer and the racemate. Also, there were no sex differences in the pharmacokinetics of esketamine and S-norketamine in the pure isomer. However, compared with racemate ketamine, esketamine had a shorter recovery time and orientation recovery time, which present potential clinical advantages (Wang et al., 2019). Esketamine possesses a higher efficiency and mainly acts on N-methyl-D-aspartate (NMDA) receptor and integrates sedation, analgesia, and the anesthesia effect (Smits et al., 2017; Van de Bunt et al., 2017; Kalmoe et al., 2020; Harder et al., 2022; Li et al., 2022). Its analgesic effect is twice that of ketamine; therefore, lower clinical doses of esketamine are demanded, and side effects (such as nightmare, delirium, and agitation) are decreased (Zhang et al., 2022). It has been adopted in some European countries for decades and has been used in Chinese hospitals in recent years. Besides treating depression, esketamine is applied for clinical sedation and analgesia associated with same-day bidirectional endoscopy (Long et al., 2022), pediatric dental surgery (Xin et al., 2021) and mechanical ventilation in ICU patients (Song et al., 2022) When it is used for bronchoscopy, esketamine relaxes bronchiolar muscles and inhibits bronchial constriction (Huang et al., 2022).

The following are several limitations of our research:

First, the DISE technique doesn't reliably induce REM sleep, which is closely connected with upper airway obstruction (Capasso et al., 2016);

Second, the depth of sleep and wakefulness cannot be assessed effectively in an accurate and consistent way. Based on previous research, adequate depth of sedation and anesthesia for DISE in OSAHS children was UMSS score of 3 or BIS 65–75 in this study (Stierer and Ishman, 2015; Lo et al., 2015; Shields et al., 2005; Malviya et al., 2002). The scores for pediatric awakening depth were very few. A reliable and valid one was ABJ score for newborns and children (Pees et al., 2003). So UMSS score, BIS and ABJ score were adopted in our study.

Third, this study, carried out at a clinical DISE center specializing in the care of OSAHS children, is only a reflection of the experience of a single center.

Fourth, in the absence of dose-response studies, we cannot determine the effect of using larger or smaller doses in our study.

Fifth, in our study DISE was not done alone but performed before adenotonsillectomy.

Last but not least, the majority of pediatric patients in our study have mild OSAHS or even suspected ones.

Further research on dosage related effects of esketamine for pediatric DISE needs to be done at multiple centers. The relationship between BIS scores and depth of anesthesia under esketamine for OSAHS pediatric DISE should be explored.

Conclusion

Esketamine in comparison to dexmedetomidine provides more effective and safer depth of anesthesia for OSAHS pediatric DISE by ensuring short onset time, deep sedation, and few AEs.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethical Board for Clinical/Scientific Research Project of Zhongnan Hospital of Wuhan University (Approval Number: 2021071). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

ZY, XQ, SM, and LX helped analyze the data and write the manuscript. ZY, SM, and ZT helped collect the data. ZY, CX, and SX helped design the study, critically revise the manuscript and finally approve the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Bhattacharjee, R., Kheirandish-Gozal, L., Spruyt, K., Mitchell, R. B., and Promchiarak, J. N., (2010). Adenotonsillectomy outcomes in treatment of obstructive sleep apnea in children: A multicenter retrospective study. *Am. J. Respir. Crit. Care Med.* 182 (5), 676–683. doi:10.1164/rccm.200912-1930OC

Capasso, R., Rosa, T., Tsou, D. Y., Nekhendzy, D., Drover, J., Collins, S., et al. (2016). Variable findings for drug-induced sleep endoscopy in obstructive sleep apnea with propofol versus dexmedetomidine. *Otolaryngol. Head. Neck Surg.* 154 (4), 765–770. doi:10.1177/0194599815625972

Cho, J. S., Soh, S., Kim, E. J., Cho, H. J., Shin, S., Kim, H. J., et al. (2015). Comparison of three sedation regimens for drug-induced sleep endoscopy. *Sleep. Breath.* 19 (2), 711–717. doi:10.1007/s11325-015-1127-9

Ehsan, Z., Mahmoud, M., Shott, S. R., Amin, R. S., and Ishman, S. L. (2016). The effects of anesthesia and opioids on the upper airway: A systematic review. *Laryngoscope* 126 (1), 270–284. doi:10.1002/lary.25399

Evans, R. G., Crawford, M. W., Noseworthy, M. D., and Yoo, S. J. (2003). Effect of increasing depth of propofol anesthesia on upper airway configuration in children. *Anesthesiology* 99 (3), 596–602. doi:10.1097/00000542-200309000-00014

Goldbart, A. D., Levitas, A., Greenberg-Dotan, S., Ben Shimol, A., Broides, M., Puterman, M., et al. (2010). B-type natriuretic peptide and cardiovascular function in young children with obstructive sleep apnea. *Chest* 138 (3), 528–535. doi:10.1378/chest.10-0150

Haberland, C. M., Baker, S., and Liu, H. (2011). Bispectral index monitoring of sedation depth in pediatric dental patients. *Anesth. Prog.* 58 (2), 66–72. doi:10.2344/0003-3006-58.2.66

Harder, M., Fiegl-Lechner, A., Oberacher, H., Horvath, U. E. I., Schlager, A., Jeske, M., et al. (2022). Stability evaluation of morphine, hydromorphone, metamizole and esketamine containing analgesic mixtures applied for patient-controlled analgesia in hospice and palliative care. *Biomed. Chromatogr.* 36 (4), e5340. doi:10.1002/bmc.5340

Huang, X., Ai, P., Wei, C., Sun, Y., and Wu, A. (2022). Comparison of the effects of esketamine/propofol and sufentanil/propofol on the incidence of intraoperative hypoxemia during bronchoscopy: Protocol for a randomized, prospective, parallel-group trial. *J. Clin. Med.* 11 (15), 4587. doi:10.3390/jcm11154587

Ibrahim, A. E., Taraday, J. K., and Kharasch, E. D. (2001). Bispectral index monitoring during sedation with sevoflurane, midazolam, and propofol. *Anesthesiology* 95 (5), 1151–1159. doi:10.1097/00000542-200111000-00019

Jones, J. H., Nittur, V. R., Fleming, N., and Applegate, R. L. (2021). Simultaneous comparison of depth of sedation performance between SedLine and BIS during general anesthesia using custom passive interface hardware: Study protocol for a prospective, non-blinded, non-randomized trial. *BMC Anesthesiol.* 21 (1), 105. doi:10.1186/s12871-021-01326-5

Kalmoe, M. C., Janski, A. M., Zorumski, C. F., Nagele, P., Palanca, B. J., and Conway, C. R. (2020). Ketamine and nitrous oxide: The evolution of NMDA receptor antagonists as antidepressant agents. *J. Neurol. Sci.* 412, 116778. doi:10.1016/j.jns.2020.116778

Kelly, A., Dougherty, S., Cucchiara, A., Marcus, C. L., and Brooks, L. J. (2010). Catecholamines, adiponectin, and insulin resistance as measured by HOMA in children with obstructive sleep apnea. *Sleep* 33 (9), 1185–1191. doi:10.1093/sleep/33.9.1185

Li, A. M., So, H. K., Au, C. T., Ho, C., Lau, J., Ng, S. K., et al. (2010). Epidemiology of obstructive sleep apnoea syndrome in Chinese children: A two-phase community study. *Thorax* 65 (11), 991–997. doi:10.1136/thx.2010.134858

Li, X., Xiang, P., Liang, J., Deng, Y., and Du, J. (2022). Global trends and hotspots in esketamine research: A bibliom etric analysis of past and estimation of future trends. *Drug Des. devel. Ther.* 16, 1131–1142. doi:10.2147/DDDT.S356284

Liu, K. A., Liu, C. C., Alex, G., Szmuk, P., and Mitchell, R, B. (2020). Anesthetic management of children undergoing drug-induced sleep endoscopy: A retrospective review. *Int. J. Pediatr. Otorhinolaryngol.* 139 (12), 110440. doi:10.1016/j.ijporl.2020.110440

Lo, Y. L., Ni, Y. L., Wang, T. Y., Lin, H. Y., Li, D. P., White, J. R., et al. (2015). Bispectral index in evaluating effects of sedation depth on drug-induced sleep endoscopy. *J. Clin. Sleep. Med.* 11 (9), 1011–1020. doi:10.5664/jcsm.5016

Long, Y. Q., Feng, C. D., Ding, Y. Y., Feng, X. M., Liu, H., Ji, F. H., et al. (2022). Esketamine as an adjuvant to ciprofol or propofol sedation for same-day bidirectional endoscopy: Protocol for a randomized, double-blind, controlled trial with factorial design. *Front. Pharmacol.* 3 (13), 821691. doi:10.3389/fphar.2022.821691

Mahmoud, M., Gunter, J., Donnelly, L. F., Wang, Y., Nick, T. G., and Sadhasivam, S. (2009). A comparison of dexmedetomidine with propofol for magnetic resonance imaging sleep studies in children. *Anesth. Analg.* 109 (3), 745–753. doi:10.1213/ane.0b013e3181adc506

Mahmoud, M., and Mason, K. P. (2015). Dexmedetomidine: Review, update, and future considerations of paediatric perioperative and periprocedural applications and limitations. *Br. J. Anaesth.* 115 (2), 171–182. doi:10.1093/bja/aev226

Malviya, S., Voepel-Lewis, T., Tait, A. R., Merkel, S., Tremper, K., and Naughton, N. (2002). Depth of sedation in children undergoing computed tomography: Validity and reliability of the university of Michigan sedation Scale (UMSS). *Br. J. Anaesth.* 88 (2), 241–245. doi:10.1093/bja/88.2.241

Marcus, C. L., Brooks, L. J., Draper, K. A., Gozal, D., Halbower, A. C., Jones, J., et al. (2012). Diagnosis and management of childhood obstructive sleep apnea syndrome. *Pediatrics* 130 (3), e714–e755. doi:10.1542/peds.2012-1672

Martin, C. S., Deverman, S. E., Norvell, D. C., Cusick, J. C., Kendrick, A., and Koh, J. (2019). Randomized trial of acupuncture with antiemetics for reducing postoperative nausea in children. *Acta Anaesthesiol. Scand.* 63 (3), 292–297. doi:10.1111/aas.13288

Miano, S., Paolino, M. C., Urbano, A., Parisi, P., Massolo, A. C., Castaldo, R., et al. (2011). Neurocognitive assessment and sleep analysis in children with sleep-disordered breathing. *Clin. Neurophysiol.* 122 (2), 311–319. doi:10.1016/j.clinph.2010.06.019

Murphy, M., Bruno, M. A., Riedner, B. A., Boveroux, P., Noirhomme, Q., Landsness, E. C., et al. (2011). Propofol anesthesia and sleep: a high-density EEG study. *Sleep* 34 (3), 283–91A. doi:10.1093/sleep/34.3.283

Nelson, L. E., Lu, J., Guo, T., Saper, C. B., Franks, N. P., and Maze, M. (2003). The alpha2-adrenoceptor agonist dexmedetomidine converges on an endogenous sleep-promoting pathway to exert its sedative effects. *Anesthesiology* 98 (2), 428–436. doi:10.1097/00000542-200302000-00024

Park, J. G., Ramar, K., and Olson, E, J. (2011). Updates on definition, consequences, and management of obstructive sleep apnea. *Mayo Clin. Proc.* 86 (6), 549–554. quiz 554-555. doi:10.4065/mcp.2010.0810

Pees, C., Haas, N. A., Ewert, P., Berger, F., and Lange, P. E. (2003). Comparison of analgesic/sedative effect of racemic ketamine and S(+)-ketamine during cardiac catheterization in newborns and children. *Pediatr. Cardiol.* 24 (5), 424–429. doi:10.1007/s00246-002-0356-4

Schmidt, A., Oye, I., and Akeson, J. (2005). Cerebral physiological responses to bolus injection of racemic, S(+)- or R(-)-ketamine in the pig. *Acta Anaesthesiol. Scand.* 49 (10), 1436–1442. doi:10.1111/j.1399-6576.2005.00838.x

Shields, C. H., Styadi-Park, G., McCown, M. Y., and Creamer, K. M. (2005). Clinical utility of the bispectral index score when compared to the University of Michigan Sedation Scale in assessing the depth of outpatient pediatric sedation. *Clin. Pediatr.* 44 (3), 229–236. doi:10.1177/000992280504400306

Smith, D. F., He, S., Peddireddy, N, S., Vairavan Manickam, P. C., Heubi, H., Shott, S. R., et al. (2020). Effectiveness of pediatric drug-induced sleep endoscopy for REM-predominant obstructive sleep apnea. *Sleep. Breath.* 24 (4), 1705–1713. doi:10.1007/s11325-020-02056-7

Smits, G. J., Kuypers, M. I., Mignot, L. A., Reijners, E. P., Oskam, E., Doom, K. V., et al. (2017). Procedural sedation in the emergency department by Dutch emergency physicians: A prospective multicentre observational study of 1711 adults. *Emerg. Med. J.* 34 (4), 237–242. doi:10.1136/emermed-2016-205767

Song, X., Wang, F., Dong, R., Zhu, K., and Wang, C. (2022). Efficacy and safety of remimazolam tosilate combined with esketamine for analgesic sedation in mechanically ventilated ICU patients: A single-arm clinical study protocol. *Front. Med.* 9, 832105. doi:10.3389/fmed.2022.832105

Sruthi, S., Mandal, B., Rohit, M. K., and Puri, G. D. (2018). Dexmedetomidine versus ketofol sedation for outpatient diagnostic transesophageal echocardiography: A randomized controlled study. *Ann. Card. Anaesth.* 21 (2), 143–150. doi:10.4103/aca.ACA_171_17

Stierer, T. L., and Ishman, S. L. (2015). Bispectral index in evaluating effects of sedation depth on drug-induced sleep endoscopy: DISE or No dice. *J. Clin. Sleep. Med.* 11 (9), 965–966. doi:10.5664/jcsm.5002

Tekeli, A. E., Oguz, A. K., Tuncdemir, Y. E., and Almali, N. (2020). Comparison of dexmedetomidine-propofol and ketamine-propofol administration during sedation-guided upper gastrointestinal system endoscopy. *Med. Baltim.* 99 (49), e23317. doi:10.1097/MD.0000000000023317

van de Bunt, J. A., Veldhoen, E. S., Nievelstein, R. A. J., Hulsker, C. C. C., Schouten, A. N. J., and van Herwaarden, M. Y. A. (2017). Effects of esketamine sedation compared to morphine analgesia on hydrostatic reduction of intussusception: A case-cohort comparison study. *Paediatr. Anaesth.* 27 (11), 1091–1097. doi:10.1111/pan.13226

Wang, J., Huang, J., Yang, S., Cui, C., Ye, L., Wang, S. Y., et al. (2019). Pharmacokinetics and safety of esketamine in Chinese patients undergoing painless gastroscopy in comparison with ketamine: A randomized, open-label clinical study. *Drug Des. devel. Ther.* 13, 4135–4144. doi:10.2147/DDDT.S224553

Xin, N., Xu, H., and Yue, C. (2021). Comparison between dexmedetomidine and esketamine in pediatric dentistry surgery. *Transl. Pediatr.* 10 (12), 3159–3165. doi:10.21037/tp-21-435

Zhang, C., He, J., Shi, Q., Bao, F., and Xu, J. (2022). Subanaesthetic dose of esketamine during induction delays anaesthesia recovery a randomized, double-blind clinical trial. *BMC Anesthesiol.* 22 (1), 138. doi:10.1186/s12871-022-01662-0

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Precision caffeine therapy for apnea of prematurity and circadian rhythms: New possibilities open up

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Caffeine is the globally consumed psychoactive substance and the drug of choice for the treatment of apnea of prematurity (AOP), but its therapeutic effects are highly variable among preterm infants. Many of the molecular underpinnings of the marked individual response have remained elusive yet. Interestingly, the significant association between *Clock* gene polymorphisms and the response to caffeine therapy offers an opportunity to advance our understanding of potential mechanistic pathways. In this review, we delineate the functions and mechanisms of human circadian rhythms. An up-to-date advance of the formation and ontogeny of human circadian rhythms during the perinatal period are concisely discussed. Specially, we summarize and discuss the characteristics of circadian rhythms in preterm infants. Second, we discuss the role of caffeine consumption on the circadian rhythms in animal models and human, especially in neonates and preterm infants. Finally, we postulate how circadian-based therapeutic initiatives could open new possibilities to promote precision caffeine therapy for the AOP management in preterm infants.

KEYWORDS

apnea of prematurity, caffeine, chronopharmacology, circadian rhythms, preterm infants

1 Introduction

Caffeine, one of the bioactive methylxanthines that exist in a variety of natural and processed foods and beverages, is the most frequently consumed psychoactive substance in the world (Gonzalez de Mejia and Ramirez-Mares, 2014; van Dam et al., 2020; Rodak et al., 2021). Studies have confirmed that ingested caffeine has profound effects on the function and health of various systems in the human body through the combination of several molecular mechanisms including the antagonism of adenosine receptors, inhibition of phosphodiesterase, and mobilization of intracellular calcium (Nehlig et al., 1992; Cappelletti et al., 2015; Rodak et al., 2021; Yang et al., 2021). Among these effects of caffeine, the most well-known are those on the central nervous system,

such as the regulation of sleep-wake states, learning-memory functions, cognitive-behavioral performances, attention-alertness functions, and mood-consciousness states (Nehlig et al., 1992; Snel and Lorist, 2011; Spaeth et al., 2014; Urry and Landolt, 2015). Therefore, it is no surprise that many people are accustomed to taking caffeinated beverages or foods to combat sleep deprivation induced fatigue and circadian rhythm sleep disorder caused by shift work or rapid transmeridian travel (Landolt, 2015; Clark and Landolt, 2017; Arendt, 2018), while some other people intentionally avoid caffeine in their daily life so as not to interfere with regular sleep habits (Snel and Lorist, 2011).

On the other hand, the therapeutic use of caffeine is very common in clinical practice. Caffeine acts as a potent analgesic adjuvant and is often added to a variety of over-the-counter and prescription analgesics due to its anti-inflammatory and vasoconstrictive effects (Cappelletti et al., 2015; van Dam et al., 2020; Rodak et al., 2021). More strikingly, caffeine is the drug of choice for the treatment of apnea of prematurity (AOP) (Eichenwald et al., 2016; Kumar and Lipshultz, 2019; Moschino et al., 2020; Long et al., 2021) and becomes one of the most commonly prescribed medications in the neonatal intensive care unit (NICU) (Hsieh et al., 2014; Krzyżaniak et al., 2016), evidenced by its short-term and long-term efficacy and safety in reducing apnea, facilitating extubation, preventing bronchopulmonary dysplasia, ameliorating retinopathy of prematurity, reducing patent ductus arteriosus, and improving neurodevelopmental outcome that have been demonstrated in the Caffeine for Apnea of Prematurity (CAP) trial (Schmidt et al., 2006; Schmidt et al., 2007). Assuredly, compared with other methylxanthines, caffeine has higher therapeutic index, longer half-life, and better tolerability (Henderson-Smart and De Paoli, 2010; Henderson-Smart and Steer, 2010; Abdel-Hady et al., 2015). Inspiringly, caffeine has been clinically applied in the treatment of AOP for nearly 50 years, which has created a typical successful story in pediatrics (Kreutzer and Bassler, 2014; Dobson and Hunt, 2018; Williamson et al., 2021).

Recently, the association between caffeine and circadian rhythms has attracted widespread attentions (Landolt, 2015). Many intriguing phenomena occurred, and the underlying mechanisms have been tentatively investigated by several studies conducted in adults and animals (Spaeth et al., 2014), but we still know very little about the truth. Fortunately, however, our previous study revealed that the circadian rhythms in premature infants might play a sophisticated role in determining the efficacy of caffeine therapy (Guo et al., 2022). Therefore, it will be very interesting to summarize the current relevant studies to know about the progress of this research field.

To the best of our knowledge, there is no comprehensive summary of the most recent advances in the circadian rhythms in preterm infants and caffeine therapy. Thus, to fill this knowledge gap, in this review, we begin by introducing the coexistence of tough challenges and new insights in the current caffeine therapy for AOP. Then, our novel findings (Guo et al., 2022) push us to delineate the functions and mechanisms of human circadian rhythms first for better understanding the deep theoretical logic underlying those clinical phenotypes. As a key part of circadian development, an up-to-date knowledge of the formation and ontogeny of human circadian rhythms during the perinatal period are also concisely discussed. Undoubtedly, what attracts our attention the most is the research progress on the effects of caffeine on human circadian rhythms, especially for premature infants, and the progress on the sophisticated roles of circadian rhythms in the response to caffeine therapy for those babies with AOP. Therefore, based on the increasing evidence, a new possibility opens up in this area of research in light of the circadian rhythms.

2 Tough challenges and new findings in current caffeine therapy for AOP

To be honest, the tough challenges are always there for the current AOP therapy with caffeine. The optimal dose regimen, timing and duration of therapy, necessity of therapeutic drug monitoring, and variable clinical outcomes of caffeine in preterm infants remain controversial (Gentle et al., 2018; Davis, 2020; Saroha and Patel, 2020). Impressively, however, those problems related to the clinical use of caffeine in preterm infants have been widely concerned and discussed as the continuous deepening of research, especially as the application of several innovative research technologies, such as artificial intelligence, predictive modeling, and machine learning (Koch et al., 2017; Shirwaikar, 2018; Faramarzi et al., 2021; Dai et al., 2022). Interestingly, several novel findings in those studies provide valuable references for determining the optimal initial dose, tailoring the maintenance dose, enhancing clinical decision making, and then for promoting the achievement of consensus on those tough challenges (Abdel-Hady et al., 2015; Eichenwald, 2020; Moschino et al., 2020).

The clinical response bears the brunt. The most tough and urgent problem is the significant interindividual variability in response to caffeine therapy (He et al., 2021). It remains unclear why some preterm infants have well-controlled outcomes while others have not. To make matters worse, the frequent episodes of apnea among those lacking efficacy cannot be well controlled by solely increasing the dose of caffeine (Saroha and Patel, 2020).

Tentatively to explore the underlying factors that determine the interindividual response to caffeine therapy, a single-center and retrospective study was conducted by our team (He et al., 2021; Guo et al., 2022). In line with previous study (Saroha and Patel, 2020), the plasma concentration of caffeine could not explain the variable efficacy for preterm infants yet (He et al., 2021). Arguably, such highly variable response could not be explained either by the genetic polymorphisms of various genes encoding the metabolic enzymes and transporters (Guo et al.,

2022). However, genetic polymorphisms involved in caffeine's target receptors, directly and indirectly, and quite unexpectedly, in regulation of circadian rhythms were significantly associated with the variable response to caffeine therapy (Guo et al., 2022). Such novel finding bears good clinical significance and is inspirational for future studies to delve into the biological mechanisms.

3 The functions and mechanisms of human circadian rhythms

Due to the rotation of Earth, almost all life forms on the planet have evolved a biological timer to adapt to the daily changes in the environment (Du Pre et al., 2014; Dong et al., 2020; Jha et al., 2021). The endogenous biological clock is commonly called as the circadian (from Latin, meaning "about a day") rhythms (Dong et al., 2020; Ruan et al., 2021). It is proven that the inherent period of the human pacemaker clock is close to 25 h in most people (Ohdo et al., 2019; Dong et al., 2020). However, because of the entrainment by environmental time signals, or so-called zeitgebers (from German, meaning "time givers") (Bicker et al., 2020; Ruan et al., 2021), the inherited circadian pacemaker manifests itself in a 24-h pattern (Ohdo et al., 2019; Dong et al., 2020).

3.1 The functions of human circadian rhythms

Circadian rhythms regulate various behavioral, physiological, psychological, and endocrine functions in humans (Froy, 2007; Ribas-Latre and Eckel-Mahan, 2016; Allada and Bass, 2021; Kinouchi et al., 2021; Thosar and Shea, 2021; Zhang and Jain, 2021). One can imagine that circadian dysfunction would cause multiple negative impacts, both short term and long term, which lead to the increased susceptibility to many diseases, decreased quality of life, and even reduced life expectancy (Froy and Miskin, 2007; Jagannath et al., 2013; Roenneberg and Merrow, 2016; Valenzuela et al., 2016; Logan and McClung, 2019; Xu and Lu, 2019; Allada and Bass, 2021). Interestingly, the onsets and symptoms of many diseases, such as stroke, asthma, and depression, also display clear circadian characteristics (Jagannath et al., 2013; Hsieh et al., 2018; Cederroth et al., 2019; Dobrek, 2021; Ruan et al., 2021), which are called as the circadian pathology signs (Cederroth et al., 2019). Speaking of pharmacology, circadian rhythms affect the absorption, distribution, metabolism, and excretion (ADME) or called the pharmacokinetic processes as well as the efficacy and adverse effects of many drugs, which is well known as the chronopharmacology or chronotherapy (Dallmann et al., 2016; Ohdo et al., 2019; Dong et al., 2020; Dobrek, 2021; Nahmias and Androulakis, 2021). Given the importance of circadian rhythms, three researchers who discovered the basic of biological clock in studies of *Drosophila* were awarded the Nobel Prize in 2017 (Dobrek, 2021; Ruan et al., 2021).

3.2 The mechanisms of human circadian rhythms

Back in the 1990s, the discovery of several circadian clock genes, such as *Clock*, *Bmal1*, *Per*, and *Cry* (Takahashi, 2004), proved that almost all human cells express these genes and have the capacity to generate circadian oscillations (Du Pre et al., 2014; Takahashi, 2017), which thwarted the previous neuro-centric view that the master clock is located only in the brain (Takahashi, 2017). As is generally believed and well understood, at the systemic level, the human circadian system consists of the inputs, circadian oscillators, and outputs (Figure 1) (Takahashi, 2017; Cederroth et al., 2019; Huang et al., 2020; Ruan et al., 2021), while at the cellular level, it consists of several cell-autonomous molecular oscillators that composed of three transcriptional-translational feedback loops that are widespread throughout the body (Figure 2) (Du Pre et al., 2014; Takahashi, 2017; Logan and McClung, 2019; Huang et al., 2020; Sumova and Cecmanova, 2020; Ruan et al., 2021).

3.2.1 Physiological basis

The regulation and maintenance of human circadian rhythms depend on the synergy of the input pathways, central and peripheral clocks, and output pathways (Figure 1) (Huang et al., 2020). The input pathway senses and transmits the environmental rhythm signals to the central circadian clocks (Ruan et al., 2021), which act as the biological rhythm pacemaker to transmit the generated rhythm signals to the periphery through the output pathway (Du Pre et al., 2014; Jha et al., 2021), and then cooperate with the endogenous clock systems of peripheral tissues and organs to regulate the gene expression, cellular function, physiological activity, and metabolism of the body (Huang et al., 2020).

Light, the major input signal in the suprachiasmatic nuclei (SCN) of the circadian system, is perceived by the intrinsically photosensitive retinal ganglion cells (ipRGCs) (Zele et al., 2011), which express the photopigment melanopsin and are modulated by the rods and cones in the retina (Van Cruchten et al., 2017). Then, the ipRGCs generated and transmitted electric rhythm signals to the central clock system that located in the SCN of the hypothalamus through a neural pathway called the retinohypothalamic tract (RHT) (Logan and McClung, 2019; Dong et al., 2020; Jha et al., 2021).

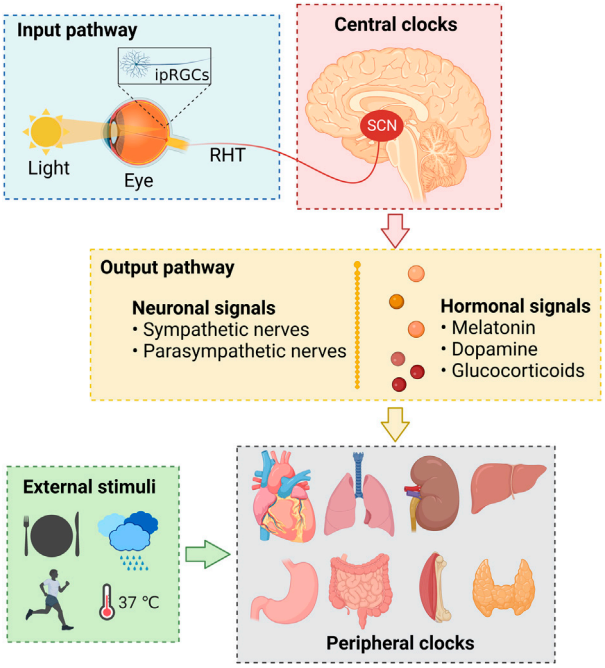

FIGURE 1
The physiological basis of human circadian rhythms. ipRGCs, intrinsically photosensitive retinal ganglion cells; RHT, retinohypothalamic tract; SCN, suprachiasmatic nuclei.

The SCN is comprised of neurons that express the neuropeptide arginine vasopressin (AVP) and vasoactive intestinal polypeptide (VIP), which are essential for the circadian light transduction (Ono et al., 2021). The AVP and VIP neurons in the SCN master pacemaker are also regulated by the neurotransmitters released by the ipRGCs, such as excitatory glutamate and pituitary adenylate cyclase-activating polypeptide (PACAP) (Dong et al., 2020; Jha et al., 2021; Ruan et al., 2021). Subsequently, the SCN transmits such perceived rhythm information *via* neuronal and hormonal signals (Logan and McClung, 2019), and coordinates other oscillators in extra-SCN brain regions and peripheral tissues and organs, such as heart, lung, liver, and kidney (Takahashi, 2017; Huang et al., 2020).

It is worth mentioning that in addition to be regulated by the SCN master pacemaker, the peripheral clocks could also directly and SCN-independently receive external stimuli, such as food intake, exercise, temperature, and humidity (Figure 1) (Du Pre et al., 2014; Xu and Lu, 2019; Huang et al., 2020).

3.2.2 Molecular mechanism

Three interlocked transcriptional feedback loops constitute the complex molecular clock networks at the cellular level (Figure 2) (Takahashi, 2017; Ruan et al., 2021). The core loop regulates human circadian rhythms with a period of approximately 24-h through a negative feedback mechanism (Huang et al., 2020; Allada and Bass, 2021). The circadian locomotor output cycles kaput (CLOCK) or neuronal PAS domain-containing protein 2 (NPAS2) forms heterodimers with the brain and muscle ARNT-like 1 (BMAL1) *via* binding to the E-box elements to regulate the transcription of clock-controlled genes (CCGs), including those encoding the period (PER) and cryptochrome (CRY) proteins (Takahashi, 2017; Logan and McClung, 2019; Dong et al., 2020). PER and CRY proteins accumulate in the cytoplasm in the morning (Ruan et al., 2021), then heterodimerize and translocate into the nucleus as negative regulators directly interact with CLOCK-BMAL1 to suppress their transcriptional activity in the late afternoon or evening (Takahashi, 2017; Xu and Lu, 2019). As the suppression progresses, PER and CRY proteins are gradually degraded *via* the ubiquitination through specific E3 ligase complexes and *via* the proteasome (Takahashi, 2017). At the same time, the transcription activity of CLOCK-BMAL1 is restored, and a new cycle will restart over the next morning (Ruan et al., 2021).

Besides, another two families of nuclear receptors, REV-ERBs and retinoic acid receptor-related orphan receptors (RORs), are also the direct targets of CLOCK-BMAL1 that stabilize the core loop, regulate the transcription in a distinct phase, and thus form the secondary or called the stabilization loop (Xu and Lu, 2019). The REV-ERBs inhibit the transcription

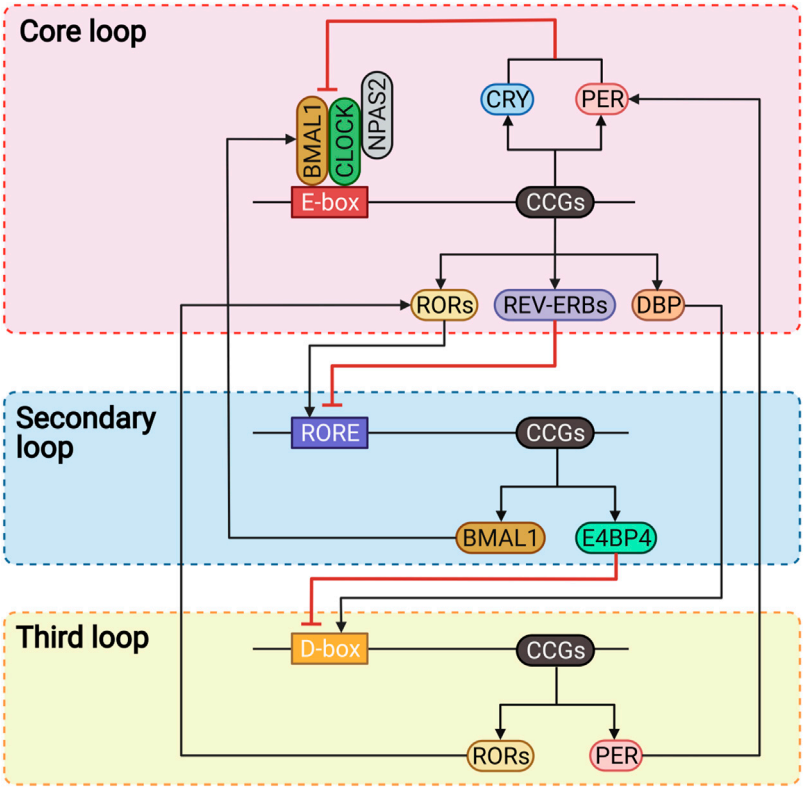

FIGURE 2
The molecular mechanism of human circadian rhythms. BMAL1, brain and muscle ARNT-like 1; CCGs, clock-controlled genes; CLOCK, circadian locomotor output cycles kaput; CRY, cryptochrome; DBP, D-box binding protein; E4BP4, E4 promoter-binding protein 4; NPAS2, neuronal PAS domain-containing protein 2; PER, period; RORE, ROR/REV-ERB response elements; RORs, retinoic acid receptor-related orphan receptors.

of BMAL1 by competitively binding to the ROR/REV-ERB response elements (RORE) (Hsieh et al., 2018; Ruan et al., 2021). Conversely, the RORs are the positive regulators that bind to the RORE to promote the transcription of BMAL1 (Logan and McClung, 2019; Huang et al., 2020).

The third loop involves the proline- and acidic amino acid-rich basic leucine zipper (PAR-bZIP) factors, such as the D-box binding protein (DBP) and the repressor E4 promoter-binding protein 4 (E4BP4), which competitively bind to the D-box elements, and are driven by the core loop and stabilization loop, respectively (Takahashi, 2017; Ruan et al., 2021). DBP and E4BP4 contribute to circadian robustness by synergistically regulating the expression of RORs and PER proteins (Takahashi, 2017; Dong et al., 2020; Ruan et al., 2021).

Collectively, these three interactive feedback loops regulate the transcription and translation of CCGs by binding to the *cis*-elements, including E-box, RORE, and D-box, in their gene promoter and enhancer element regions (Dong et al., 2020; Ruan et al., 2021). In addition to these three transcriptional-translational feedback loops, several post-transcriptional and post-translational mechanisms, such as phosphorylation, acetylation, and ubiquitination of circadian proteins, also play important roles in regulating the circadian rhythms (Figure 2) (Takahashi, 2017; Xu and Lu, 2019; Huang et al., 2020).

4 The formation and development of human circadian rhythms

The physiological and molecular mechanisms of human circadian rhythms have been well described, but the formation and development during ontogenesis remain poorly understood (Astiz and Oster, 2020; Sumova and Cecmanova, 2020). Moreover, most studies were performed in rodents and non-human primates, which hinders our understanding of the

developmental circadian physiology for humans (Rivkees, 2003; Sumova and Cecmanova, 2020). Nevertheless, the existing evidence reveals that the formation and development of circadian rhythms are the continuously morphological, structural, and functional maturation processes of tissues and organs with ontogenesis (Rivkees, 2003; Seron-Ferre et al., 2012).

4.1 The formation of circadian rhythms: Does fetus have circadian rhythms?

As early as 1975, a rat study (Deguchi, 1975) found, for the first time, that the mammalian fetal clock oscillators could be detected already at or before birth and be entrained by the mother. Subsequent studies have revealed that the fetus of rat, hamster, sheep, baboon, and other mammalians exhibited the circadian rhythms of metabolic activity (Reppert, 1992; Serón-Ferré et al., 1993; Mirmiran and Lunshof, 1996; Seron-Ferre et al., 2012) and the expressions of canonical clock genes (Seron-Ferre et al., 2007; Du Pre et al., 2014; Sumova and Cecmanova, 2020).

In human fetus, circadian rhythms in several physiological and endocrine functions, including heart rate (Lunshof et al., 1998), breathing patterns (Patrick and Challis, 1980), limb movements (Einspieler et al., 2021), sleep-wake states (Peirano et al., 2003; Bennet et al., 2018), and hormone levels (Serón-Ferré et al., 2001a) have been detected at different stages of pregnancy (Seron-Ferre et al., 2007; Du Pre et al., 2014; Wong et al., 2022). Impressively, Frigato et al. (2009), first observed the rhythmic expression of clock genes such as *Per2* in the HTR-8/SVneo cells derived from human extravillous trophoblast. As part of a series of important discoveries, Perez et al. (2015) went on to find the rhythmic expression of various circadian genes, including *Clock*, *Bmal1*, *Per2*, and *Cry1* in human full-term placenta.

It is incredible that no obvious circadian rhythms were found in the anencephalic fetus despite an intact maternal circadian rhythms were detected through the 24-h period fetal heart rate monitoring for anencephaly (Mirmiran and Lunshof, 1996), which demonstrated that the fetal brain, especially in the SCN, is required for the generation of fetal circadian rhythms (Mirmiran and Lunshof, 1996). It is still unclear when the fetal SCN clock first appeared morphologically, yet through the *in vitro* autoradiography by ^{125}I-labeled melatonin, the SCN is apparent as discrete nuclei in the human fetus and already has melatonin receptors at 18 weeks of gestational age (GA) (Reppert et al., 1988). Besides, it has been demonstrated that the VIP and AVP neurons were first observed at 31 weeks of GA in the ventrolateral part of the fetal SCN (Swaab et al., 1990; Swaab et al., 1994). Therefore, it is currently recognized that the circadian rhythms in humans are formed and developed during the perinatal period (Rivkees, 1997; Sumova and Cecmanova, 2020), while the components of the circadian system like the SCN are established and functional early in human fetus (Serón-Ferré et al., 1993).

4.2 Prenatal circadian rhythms: Complex interaction of maternal, placental, and fetal circadian systems

Pregnancy presents an unusual circadian physiology pattern in which the fetal circadian system is completely embodied within that of the mother (Mark et al., 2017), and the two systems are connected by the placenta and interact with each other through this interface (Mark et al., 2017; Astiz and Oster, 2020; Bates and Herzog, 2020). Generally, placenta is responsible for the bidirectional transference of nutrients, hormones, metabolites, and gases (*i.e.,* oxygen and carbon dioxide) between the mother and fetus (Seron-Ferre et al., 2012; Valenzuela et al., 2015; Astiz and Oster, 2020). Meanwhile, the placenta conveys the maternal circadian timing cues, such as physical activity, feeding behavior, temperature, heart rate, blood pressure, and hormonal levels, to the fetus (Serón-Ferré et al., 2001a; Seron-Ferre et al., 2012). In particular, multiple hormones produced by the mother, including melatonin, dopamine, glucocorticoids, estrogen, and progesterone, have profound effects on the development and entrainment of the fetal circadian rhythms (Mirmiran and Lunshof, 1996; Rivkees, 1997; Seron-Ferre et al., 2007; Mark et al., 2017). In addition, hormones such as human chorionic gonadotropin (hCG), secreted by the placenta, also exhibit obvious circadian characteristics (Waddell et al., 2012; Mark et al., 2017; Bates and Herzog, 2020). It will be very interesting to know how those hormones affect the formation of the fetal circadian rhythms.

4.2.1 Melatonin

Melatonin, known as the hormone of night (Seron-Ferre et al., 2007), can be secreted by various organs, including the pineal gland, ovary, and placenta (Itoh et al., 1999; Lanoix et al., 2008; Reiter et al., 2013; Reiter et al., 2014). However, melatonin is not synthesized by the fetal pineal gland or other organs (Mark et al., 2017), so the fetus must rely on the maternal melatonin for photoperiodic information since the unaltered melatonin readily crosses the placenta and distributes to the fetal tissues (Waddell et al., 2012; Reiter et al., 2014; Valenzuela et al., 2015). During normal human gestation, the nighttime peak melatonin level decreases slightly between the first and second trimesters, but begins to increase after 24 weeks, then increases to significantly high levels after 32 weeks, thereafter reaches its peak at the end of pregnancy, and finally returns to the pre-pregnancy level on the day after parturition (Nakamura et al., 2001; Mark et al., 2017). Late in human pregnancy, uterine contractions become intensest during the night as melatonin levels are at their highest (Nakamura et al., 2001), and the peak melatonin at the end of pregnancy is thought to promote uterine contractions that necessary for delivery (McCarthy et al., 2019).

Studies have demonstrated that the onset of human term delivery is more commonly between the late night and the early morning (Glattre and Bjerkedal, 1983; Cooperstock et al., 1987).

Similar circadian characteristics of delivery were also observed in preterm labors after 28 weeks of GA (Lindow et al., 2000; Iams et al., 2002), but not in those before 28 weeks (Vatish et al., 2010), which might be explained by the immaturity of fetal circadian system or other pathological factors that bypass the physiological circadian process of labor (Vatish et al., 2010). Interestingly, studies revealed that the elevated nocturnal levels of melatonin synergized with oxytocin to trigger and maintain the uterine contractions during labor and that melatonin sensitized the human uterine to oxytocin (Reiter et al., 2014; Carlomagno et al., 2018; Chuffa et al., 2019). Consistently, women who engage in shift work during pregnancy have an increased incidence of spontaneous miscarriages, preterm deliveries, and low birth weight infants (Zhu et al., 2004; Croteau et al., 2006). Disruptions of the melatonin rhythms due to the shift work might be responsible for these adverse pregnancy outcomes (Reiter et al., 2014). In addition, as a free radical scavenger and an antioxidant, melatonin plays an important role in protecting the fetus and placenta from oxidative stress to promote the embryonic development and to treat the preeclampsia, intrauterine growth restriction, and the undernourished pregnancy (Reiter et al., 2014; Valenzuela et al., 2015; Rodrigues Helmo et al., 2018; Chuffa et al., 2019).

4.2.2 Dopamine

As the antiphase and functionally antagonistic of melatonin, dopamine has been proposed as a "light-phase" entrainment signal of the circadian systems (Iuvone and Gan, 1995; Astiz and Oster, 2020). Plasma dopamine levels in humans peak around the waking time (about 08:00) and drop to a nadir in the middle of sleep (about 03:00) (Sowers and Vlachakis, 1984). Increased dopamine concentrations were detected in women's amniotic fluid between the second and third trimesters, and were significantly higher than those in maternal and fetal plasma (Peleg et al., 1986), because dopamine could freely cross through the placenta into the fetal circulatory system (Watanabe et al., 1990). Furthermore, D1-dopamine receptors could be detected in the fetal SCN as early as 22 weeks of GA (Rivkees and Lachowicz, 1997). However, it remains unknown when and how the maternal dopamine entrains the circadian rhythms in fetus during the pregnancy (Bates and Herzog, 2020).

4.2.3 Glucocorticoids

Cortisol, the glucocorticoid stress hormone, is regulated by the circadian of the hypothalamic-pituitary-adrenal (HPA) axis (Mark et al., 2017; Oster et al., 2017; McCarthy et al., 2019). During gestation, cortisol levels in maternal plasma peak in the early morning (from 07:30 to 08:30) and drop to a nadir at night (from 18:30 to 01:30) (Patrick et al., 1980). The maternal plasma cortisol levels increase progressively between 11 and 22 weeks of GA and then stay high until the initiation of delivery (Patrick et al., 1980; Carr et al., 1981). Such elevated maternal cortisol is critical for fetal tissue development, especially the maturation of the brain and lung (Matthews et al., 2004), and helpful for dampening the maternal stress signals to protect the fetus (McCarthy et al., 2019). Conversely, excessive cortisol level is detrimental for the fetal development that delaying the fetal and placental growth and increasing the risk of behavioral and mental disorders later in life (Busada and Cidlowski, 2017; Van den Bergh et al., 2020).

The placental glucocorticoid barrier regulates the glucocorticoids' passage from the mother to the fetus *via* the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) that converts the biologically active glucocorticoids (*i.e.*, cortisol and corticosterone) to their inactive forms (*i.e.*, cortisone and 11-dehydrocorticosterone) (Edwards et al., 1996; Burton and Waddell, 1999). In humans, the glucocorticoids passage from the maternal to fetal circulation is gradually reduced due to the increasing placental 11β-HSD2 expression with advancing gestation (Burton and Waddell, 1999; McTernan et al., 2001). Impressively, glucocorticoid receptors have been identified in the fetal circulation, and maternal glucocorticoids could entrain fetal circadian rhythms through binding to these receptors (Bates and Herzog, 2020). Moreover, studies have found that the suppression of maternal adrenal function with glucocorticoid treatment resulted in a temporary disappearance of the fetal heart rate, breathing, and limb movement rhythms (Verdurmen et al., 2013). Interestingly, these inhibitory effects were dependent on the GA when the glucocorticoid therapy was started and disappeared with the restoration of the maternal HPX axis (Mulder et al., 2004), indicating the fetal rhythms depended on the maternal adrenal functions (Koenen et al., 2005).

4.2.4 Sex hormones

The effects of sex hormones on the entrainments of fetal circadian rhythms are still under investigation. Estrogen and progesterone are two sex hormones that are essential for the successful pregnancy (Mark et al., 2017). During the first 3 months of pregnancy, estrogen and progesterone are synthesized and secreted by the ovary. After that, the placenta replaces the corpus luteum to secrete these two hormones, and estrogen is also produced by the uterus (McCarthy et al., 2019). The levels of estrogen and progesterone increase steadily over the human gestation due to an increased secretion from the ovary and placenta (Mark et al., 2017). During gestation, estrogen levels in maternal plasma peak in the morning and become lowest at midnight (Patrick et al., 1979; Challis et al., 1980), whereas a significant antiphase oscillation of the estrogen occur in the progesterone levels (Junkermann et al., 1982; Magiakou et al., 1996), which might be regulated by the circadian of placental glucocorticoids (Serón-Ferré et al., 1993).

Estrogen promotes the synthesis of progesterone (Babischkin et al., 1997), which regulates maternal immunity to facilitate implantation (Hardy et al., 2006), maintains uterine quiescence during pregnancy (Peters et al., 2017), and causes myometrial contractions to trigger labor at the end of pregnancy (Brown

et al., 2004). Interestingly, these two hormones were found to inhibit the expression of 11β-HSD2 in human placental extracts, which possibly increased the transport of glucocorticoids from the mother to the fetus (Sun et al., 1998), thereby indirectly influencing the fetal circadian rhythms.

Collectively, much less is known about other rhythmic signals such as leptin, placental lactogen, prolactin, or hCG that generated by the mother or placenta on the development and entrainment of fetal circadian rhythms (Astiz and Oster, 2020; Bates and Herzog, 2020). Because the interactions among maternal, placental, and fetal circadian systems are critical to the establishment, maintenance, and success of pregnancy, and the interactions also affect the growth, development, and even postpartum life of the fetus (Mark et al., 2017; Bates and Herzog, 2020), further studies are still needed to elucidate the complex interactions among the three circadian systems and to bridge the above knowledge gaps.

4.3 Postnatal circadian rhythms: Progressive maturation along with ontogenesis

After birth, neonates immediately begin to establish their own physical and physiological independence while losing the protect of the maternal-placental barrier (Joseph et al., 2015; Wong et al., 2022). From now on, the ontogenesis of the newborn begins to be greatly affected by the external environment (Brooks and Canal, 2013; Hazelhoff et al., 2021). Increasing evidence indicates that human postnatal circadian rhythms gradually mature along with the ontogenesis (Rivkees and Hao, 2000; Rivkees, 2007; Bueno and Menna-Barreto, 2016), in which the external environment, especially the light, plays an important role in the development and maturation (Mirmiran and Ariagno, 2000; Nishihara et al., 2002; Challet, 2007). Particularly, it should be pointed out that, in early infancy, the maternal entrainment factors and maternal-fetal interactions retained during pregnancy are more important than the external environment (Löhr and Siegmund, 1999; Rivkees, 2001; Nishihara et al., 2002; Sumova et al., 2012).

4.3.1 Maternal effects

The first thing to be discussed is the role of hormones. During the first few weeks of life, circadian rhythms in human neonates occur as the retentions of the maternal influence *in utero*, but the endogenous rhythms appear only later (Rivkees, 1997; Rivkees and Hao, 2000; Brooks and Canal, 2013). For example, an antiphase oscillation of maternal cortisol circadian rhythms (*i.e.*, the peak of cortisol levels occurred between 12:00 and 16:00) was found in the umbilical artery but not the umbilical vein of the term fetus (Serón-Ferré et al., 2001b), which reflects the activation of the intrinsic fetal HPA axis in response to the falling maternal transport of glucocorticoids during the nadir of the maternal rhythms (Mark et al., 2017). Moreover, the neonatal salivary cortisol levels were higher at night than in the morning within the first 8 weeks of postnatal age (PNA) (Iwata et al., 2013; Kinoshita et al., 2016), which were in consonance with the fetal cortisol rhythms (Serón-Ferré et al., 2001b), reflecting the preservation of fetal adrenal rhythms.

Neonates begin to exhibit the circadian salivary cortisol rhythms analogous to that of adults (*i.e.*, higher cortisol levels in the morning than at night) until 2–3 months of PNA (Price et al., 1983; Spangler, 1991; Mantagos et al., 1998; Joseph et al., 2015). However, an adult-type salivary cortisol circadian of term infants appears to be established actually at 1 month and remains stable throughout the first year of life (Ivars et al., 2015). All in all, these studies prove that the fetal cortisol circadian rhythms are preserved in the first few weeks of life, until the adult-type circadian rhythms are established.

The rhythm of melatonin is another example. (Muñoz-Hoyos et al., 1992; Muñoz-Hoyos et al., 1998) found that the adult-type circadian melatonin rhythms occurred in both the umbilical artery and vein for neonates at birth, which depended on the maternal melatonin crossing the placenta, as melatonin levels in the umbilical artery are positively correlated to those in the maternal serum and a similar correlation between the maternal and neonatal melatonin levels in the first voided urine after delivery (Kivelä et al., 1990). Besides, although the increasing amounts of melatonin and its metabolite 6-sulfatoxymelatonin were detected in the urine of the term neonates during the first week of life (Kivelä et al., 1990; Muñoz-Hoyos et al., 1993), the stable circadian melatonin rhythms were not developed until approximately 9–12 weeks of PNA (Attanasio et al., 1986; Kennaway et al., 1992; Kennaway et al., 1996; Joseph et al., 2015).

The second thing to be discussed is the maternal care, primarily maternal feeding, but it is still the roles of hormones in nature (Löhr and Siegmund, 1999; Nishihara et al., 2002; Park et al., 2020). Various hormones in breast milk, such as glucocorticoids and melatonin, can be absorbed and transferred into the neonatal circulation through the gastrointestinal tract (Arslanoglu et al., 2012; Wong et al., 2022). Interestingly, the cortisol and cortisone concentrations in breast milk follow the circadian of maternal HPA axis activity (van der Voorn et al., 2016; Italianer et al., 2020). Moreover, the cortisone rhythm in human breast milk at 1 month postpartum was associated with the nighttime sleep states of newborns at the age of 3 months (Toorop et al., 2020). Similarly, studies have also demonstrated the presence of pronounced circadian melatonin rhythms in the maternal breast milk (Illnerová et al., 1993; Katzer et al., 2016), which might contribute to the synchronization of postnatal circadian rhythms for neonates and their mothers.

One more thing needs to be pointed out is that, in addition to the maternal influence on the neonatal circadian rhythms, the maternal circadian rhythms are in turn affected by the development of the neonatal circadian rhythms (Nishihara

and Horiuchi, 1998; Nishihara et al., 2000; Nishihara et al., 2002). For example, the ultradian rhythms (*i.e.*, rhythms with period lengths much less than 24 h) (Rivkees, 1997) of rest-activity states were already detected as early as the third week of life for term infants, and the amplitude of this rhythm gradually increased from the 6th to 12th week, then formed circadian rhythms with a 24-h monophasic pattern (Nishihara et al., 2002). During this period, as neonates develop their own circadian rest-activity rhythms, the mothers' rhythms would inevitably be affected by their interrupted sleep at night to take care of their babies (Nishihara and Horiuchi, 1998; Nishihara et al., 2000).

4.3.2 Environmental effects

In the late postnatal period, environmental time cues replace the maternal effects and begin to play a critical role in the development of neonatal circadian rhythms (Rivkees, 1997; Rivkees, 2004; Brooks and Canal, 2013). Light is the most dominant zeitgeber (Löhr and Siegmund, 1999; Challet, 2007; Wong et al., 2022), so the importance of light cannot be overemphasized. The light entrainments are functionally affected by the maturity of the eyes, RHT, and SCN (Brooks and Canal, 2013; Hazelhoff et al., 2021).

For term infants, the structural development of the eyes occurs as early as *in utero*, with the first structure of the eyes beginning to form at 17 days of GA (Van Cruchten et al., 2017), while the development of pupil starts approximately at 17 weeks of GA (Hazelhoff et al., 2021), and thereafter the pupillary light reflex already present at 34 weeks of GA (Robinson and Fielder, 1990). As the sole photoreceptive area in humans (Brooks and Canal, 2013), major classes of photoreceptors in the retina including the ipRGCs, rods, cones, and melanopsin all emerge and develop in the first trimester (Van Cruchten et al., 2017; Hazelhoff et al., 2021).

Covering the eyes of term neonates during the phototherapy for neonatal hyperbilirubinemia would result in significantly increased plasma melatonin levels during the first 72 h of life, indicating the sensitivity of the neonatal pineal glands to the changes of environmental illumination and the functional maturation of the neonatal eyes in transmitting the ambient light cues (Jaldo-Alba et al., 1993). However, it remains unclear when human ipRGCs transmit the light cues to the SCN, but the melanopsin-dependent ipRGCs in mice could provide light signals to the SCN already on the day of birth (Sekaran et al., 2005), and even earlier in late gestation before birth (Rao et al., 2013).

Honestly, only several studies reported the developmental process of human RHT and SCN. RHT has been identified in neonates at 36 weeks of GA (Rivkees, 2004; Rivkees, 2007). On the other hand, it has been found that the SCN of baboons born at term was already responsive to light and could be entrained by the low-intensity (200 lux) lighting (Rivkees et al., 1997). Interestingly, the SCN in preterm baboons functionally responded to light from a stage that was equivalent to 24 weeks of GA for human infants (Hao and Rivkees, 1999). Theoretically, the ambient light signals might be projected from the ipRGCs on retina to the SCN *via* the RHT at least after birth for term neonates (Hazelhoff et al., 2021). Further maturations of the human SCN continues after birth (Rivkees, 2004; Rivkees, 2007).

The numbers of AVP neurons and total neurons in the SCN of term neonates at birth are only 13% and 20% of those in adults, respectively (Swaab et al., 1990). After birth, these nerve cells increase rapidly to a peak at 1–2 years of age, then decrease gradually to the adult levels (Swaab, 1995). However, the development of VIP neurons in the SCN is slower and does not reach the adult levels until about 3 years of age (Swaab et al., 1994). Interestingly, there is a clear sex difference (*i.e.,* 2-fold higher in males than that in females) in the number of VIP neurons after 10 years of age (Swaab et al., 1994), which suggested a possibility that the SCN involves not only in the timing of circadian rhythms, but also in the temporal organization of sexually dimorphic reproductive functions (Swaab, 1995; Hofman, 1997).

The impact of light on the clock gene expression is also a research progress worthy of special attention. The light affects the expression of clock genes, such as *Per1*, *Per2*, and *Cry1*, in the SCN of rodents at different developmental stages after birth (Kováciková et al., 2005; Ciarleglio et al., 2011). Moreover, it is the cycled light rather than the constant light that promotes the development of their biological clocks (Abraham et al., 2006; Ohta et al., 2006; Bode et al., 2011). Impressively, human neonates, especially the preterm neonates who exposed to cycled light would have better weight gains (Mann et al., 1986; Brandon et al., 2002; Vasquez-Ruiz et al., 2014; Brandon et al., 2017), less crying and fussing behaviors (Guyer et al., 2012), less hospital stay (Vasquez-Ruiz et al., 2014; Brandon et al., 2017), earlier rest-activity rhythms (Rivkees, 2004; Rivkees et al., 2004), longer nighttime sleep duration (Guyer et al., 2015), and even more robust salivary melatonin rhythms (Vasquez-Ruiz et al., 2014) compared to those exposed to continuous light or darkness. Systematic reviews also witnessed the beneficial effects of cycled light over continuous bright light or darkness for preterm neonates (Morag and Ohlsson, 2016; Liao et al., 2018). Therefore, as early as the 1990s, the guidelines for perinatal care that proposed by the American College of Obstetricians and Gynecologists and American Academy of Pediatrics were recommended to introduce a regular day-night cycled light into the NICU and neonatal nursery (Mirmiran et al., 2003a; Guyer et al., 2015).

Besides the light cues, studies have pointed out that the environmental noise disrupted the neurodevelopment of newborns and thus affected the development of their circadian rhythms (Wachman and Lahav, 2011; Kuhn et al., 2013). However, music therapy did improve the heart rate, breathing, and sleep of newborns (Arnon et al., 2006; Loewy et al., 2013), which might exert a positive impact on the well-being and quality

TABLE 1 Studies about the sleep-wake rhythms in preterm infants.

Studies	Subjects	Methods of evaluation	Main findings
Guyon et al. (2022)	12 preterm infants (GA: 35.1 ± 2.1 weeks) *vs.* 21 term infants (GA: 39.8 ± 0.8 weeks)	Polysomnography	• Preterm *vs.* term infants: TST↓, AS↓, QS↑, arousal in AS↓, arousal in QS↑ • With advancing PMA for preterm infants: TST and SE during day sleep↓, TST and SE during night sleep↑, AS↓, QS↑, arousal in AS↑, arousal in QS↓
Koch et al. (2021)	65 preterm infants (GA: 30.8 ± 2.1 weeks)	Video recordings	• Preterm infants spend about 43% of the time in AS, 38% in awake, and 19% in QS during the first 5 days of life • Sleep cycle durations of preterm infants range from 16 to 23 min with the average of 19 min
Georgoulas et al. (2021)	175 preterm and term infants (GA: 28–40 weeks)	Direct behavioral observations; EEG	• Preterm *vs.* term infants: AS↑, IS↑, QS↓, awake↓ • With advancing PMA for preterm infants: AS↓, IS↓, QS↑, awake↑
Park et al. (2020)	94 preterm infants (GA: 26.2 ± 1.4 weeks)	Digitized waveforms	• With advancing PMA for preterm infants: AS↓, QS↑, waking states↑ • Delayed feeding progression leads to delayed sleep-wake state development
Cailleau et al. (2020)	10 preterm infants (GA: 27–37 weeks) *vs.* 5 term infants (GA: 39–40 weeks)	Video recordings	• Preterm *vs.* term infants: QS↓ • With advancing PMA for preterm infants: QS↑
Lan et al. (2019)	30 preterm infants (GA: 31.17 ± 2.6 weeks)	Actigraphy	• Sleep-wake patterns of preterm infants are associated with the gender, illness severity, PMA, and body weight • Preterm infants' TST and percentage of sleep time are longer at night than during the day • With advancing PMA for preterm infants: TST↓, SE↓, percentage of sleep time↓, frequency of sleep and wake bouts↑
Cremer et al. (2016)	38 preterm infants (GA: 29.0 ± 2.6 weeks)	Video recordings	• Preterm infants with higher GA have longer awake times • Preterm boys have shorter awake times than girls
Bueno and Menna-Barreto, (2016)	19 preterm infants (GA: 28–36 weeks)	Actigraphy; Sleep and feeding diaries by the nurse	• Preterm infants exhibit the feeding-related 3-h period ultradian activity-rest rhythms after birth • Daily pattern circadian rhythms were observed for most preterm infants since 35 weeks of PMA
Guyer et al. (2015)	34 preterm infants (GA: 30.0 ± 1.8 weeks) *vs.* 21 term infants (GA: 39.7 ± 1.3 weeks)	Actigraphy; Parental sleep diaries	• Preterm *vs.* term infants: TST↑, LSP↑, nighttime sleep↑, nighttime activity↓ • With advancing PMA for preterm infants: TST↓, LSP↑, nighttime sleep↑, daytime sleep↓, activity at daytime↑, activity at nighttime↑
Dorn et al. (2014)	60 preterm infants (GA: 30.0 ± 10.8 weeks)	Actigraphy	• Preterm infants primarily exhibit the 4-h period ultradian activity rhythms, with the most time in the low activity patterns • With advancing PMA for preterm infants: SE↑, activity frequencies↓, low activity patterns↑, middle and high activity patterns↓
Palmu et al. (2013)	12 preterm infants (GA: 24.7–30.3 weeks)	Polysomnography	• Only few premature infants exhibit about 20–50 min period ultradian sleep-wake rhythms due to the unstable respiratory states • Preterm infants have frequent transitions of sleep stages, spend most of time in AS, and the proportion is correlated with PMA
Lee et al. (2010)	35 preterm infants (GA: 24.9–31.9 weeks)	aEEG recordings	• The sleep-wake cycling is more prominent in preterm infants with higher PNA at 34–36 weeks PMA • The appearance of sleep-wake cycling is significantly associated with PNA
Soubasi et al. (2009)	96 preterm infants (GA: 30.18 ± 2 weeks)	aEEG recordings	

(Continued on following page)

TABLE 1 (*Continued*) Studies about the sleep-wake rhythms in preterm infants.

Studies	Subjects	Methods of evaluation	Main findings
			• Preterm infants exhibit definite sleep-wake cycles with advanced GA • The evolution of sleep-wake cycling is correlated with positive significant interaction of PMA and GA
Foreman et al. (2008)	97 preterm infants (GA: 32.72 ± 2.28 weeks)	Video recordings	• With advancing PMA for preterm infants: AS↓, QS↑, drowsy↑, awake↑, defined states↑, diffuse states↓ • Male *vs.* female preterm infants: AS↓, drowsy↑, awake↑, defined states↓, diffuse states↑
Sisman et al. (2005)	31 preterm infants (GA: 25–32 weeks)	aEEG recordings	• The frequency of mature sleep-wake cycling in preterm infants increased with PMA independent of GA
Scher et al. (2005)	33 preterm infants (GA: 23–29 weeks)	EEG-sleep recordings	• Most preterm infants exhibit about 37–100 min period ultradian sleep state rhythms at 25–30 weeks PMA
Hoppenbrouwers et al. (2005)	195 preterm infants (GA: 30.5 ± 3.3 weeks) *vs.* 88 term infants (GA: 39.4 ± 1.0 weeks)	Polysomnography	• Preterm *vs.* term infants: AS↑, QS↓, SE↓ • With advancing PMA for preterm infants: AS↓, QS↑, SE↑ • Preterm infants' sleep-wake architecture is associated with ventilatory support, gestational age, and maternal smoking, but without sex or steroid administration
Holditch-Davis et al. (2004)	134 preterm infants (GA: 28.8 ± 2.6 weeks)	Direct behavioral observations	• With advancing PMA for preterm infants: AS↓, QS↑, quiet and active waking states↑, large body movements↓ • Sleep-wake transitions in preterm infants increased until 40 weeks PMA and changed to decrease after 43 weeks PMA
Mirmiran et al. (2003b)	40 preterm infants (GA: 30.2 ± 1.5 weeks)	Video recordings	• With advancing PMA for preterm infants: AS↓, QS↑
Korte et al. (2001)	10 preterm infants (GA: 34–36 weeks) *vs.* 10 term infants (GA: 37–42 weeks)	Actigraphy; Standardized diaries	• Preterm *vs.* term infants: ultradian activity-rest rhythms↑, circadian activity-rest rhythms↓, no difference in TST • With advancing PMA for preterm infants: nighttime sleep↑, daytime sleep↓
Bach et al. (2000)	38 preterm infants (GA: 34 ± 2 weeks)	EEG; Eye movement recordings	• Cool exposure leads to: TST↓, longest sleep period↓, wakefulness↑, AS↑, QS↓ • Male *vs.* female preterm infants: TST↓, longest sleep period↓, wakefulness↑, AS↑, QS↓
Antonini et al. (2000)	9 preterm infants (GA: 31.3–34.6 weeks)	Sleep diagrams by the mother	• With advancing PNA for preterm infants: daytime sleep↓, nighttime sleep↑, TST unchanged, nighttime sleep > daytime sleep after 8 weeks PNA
Shimada et al. (1999)	44 preterm infants (GA: 31.0 ± 3.4 weeks) *vs.* 40 term infants (GA: 39.6 ± 1.3 weeks)	Sleep diagrams by the mother; Parental sleep questionnaires; Video recordings	• 75% of these preterm infants have an ultradian or irregular sleep-wake rhythms unrelated to feeding for 3–4 weeks after discharge from the hospital • Circadian sleep-wake rhythms in preterm infants were entrained at the mean age of approximately 45 weeks PMA, similar as term infants
Ingersoll and Thoman, (1999)	95 preterm infants (GA: 28.5 ± 2.2 weeks)	Video recordings	• With advancing PMA for preterm infants: QS↑, AS↓, wakefulness↓, bout lengths of QS↑, bout lengths of AS and wakefulness do not change
Sahni et al. (1995)	35 preterm infants (GA: 31.0 ± 2.0 weeks)	Direct behavioral observations; EEG	• Preterm infants spend about 75% of their sleep time in AS and 19% in QS between 30 and 39 weeks PMA • With advancing PMA for preterm infants: AS↓, QS↑
Glotzbach et al. (1995)	17 preterm infants (GA: 31.1 ± 1.2 weeks)	Actigraphy	• Preterm infants exhibit feeding-related ultradian sleep-wake rhythms at about 35 weeks PMA
Borghese et al. (1995)	49 preterm infants (GA: 28.6 ± 2.6 weeks)	Motility monitoring system	• Most preterm infants exhibit both ultradian and diurnal sleep-wake rhythms at 36 weeks PMA

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TABLE 1 (*Continued*) Studies about the sleep-wake rhythms in preterm infants.

Studies	Subjects	Methods of evaluation	Main findings
			• From 36 weeks to 6 months PMA: QS↑, wakefulness↑, AS↓, frequency and degree of within-sleep cyclicity↑
Ardura et al. (1995)	60 preterm infants (GA: 33.4 ± 2.4 weeks) *vs.* 63 term infants (GA: 39.5 ± 1.3 weeks)	Direct behavioral observations	• Preterm *vs.* term infants: TST↑, daytime sleep↑, nighttime sleep↑ • With advancing PMA for preterm infants: TST↓, daytime sleep↓, nighttime sleep does not change
Hayes et al. (1993)	13 preterm infants (GA: 26–36 weeks)	Actigraphy	• Preterm infants exhibit 80 min and 30 min periods ultradian activity state rhythms • With advancing PMA for preterm infants: ultradian periodicities↓, activity bout durations↑
Curzi-Dascalova et al. (1993)	24 preterm infants (GA: 26.3–34.1 weeks)	Polysomnography	• Preterm infants spend most of their sleep time in AS rather QS after 27 weeks PMA • With advancing PMA for preterm infants: AS↓, QS↓, IS↑
Mirmiran and Kok, (1991)	12 preterm infants (GA: 25–32 weeks)	Actigraphy	• Only one of these preterm infants exhibit the 24-h period circadian rest-activity rhythms at 29 weeks PMA
McMillen et al. (1991)	19 preterm infants (GA: 27–35 weeks) *vs.* 22 term infants (GA: 38–42 weeks)	Sleep-wake activity diaries	• PNA at the circadian sleep-wake rhythms entrained are inversely correlated with GA for preterm infants, with 50% of preterm infants begin to exhibit circadian rhythms at 47 weeks PMA • Preterm *vs.* term infants: earlier PMA at circadian rhythms entrained
Mirmiran et al. (1990)	11 preterm infants (GA: 26–32 weeks)	Actigraphy	• Preterm infants exhibit ultradian rest-activity rhythms rather than circadian rhythms at 28–35 weeks PMA
Curzi-Dascalova et al. (1988)	18 preterm infants (GA: 34.2 ± 0.5 weeks) *vs.* 20 term infants (GA: 38.8 ± 0.2 weeks)	Polysomnography	• With advancing PMA for preterm infants: mean sleep cycle duration↑, AS↑, QS↑, IS↓
Anders and Keener, (1985)	24 preterm infants (GA: 27–35 weeks) *vs.* 40 term infants (GA: >37 weeks)	Video recordings	• Preterm *vs.* term infants: TST↑, LSP↑, AS↑, QS↓ • With advancing PMA for preterm infants: TST↑, LSP↑, AS↓, QS↑, wakefulness↑

Abbreviations: aEEG, amplitude-integrated electroencephalography; AS, active sleep; EEG, electroencephalography; GA, gestational age; IS, indeterminate sleep; LSP, longest sustained sleep period; PMA, postmenstrual age; PNA, postnatal age; QS, quiet sleep; SE, sleep efficiency; TST, total sleep time.

of life for neonates, especially for preterm infants in the NICU (Yue et al., 2021). Other environmental factors, such as ambient temperature (Tourneux et al., 2008), comforting touch (Smith et al., 2014), remodeling mattress (Deiriggi, 1990; Visscher et al., 2015), and nursing measures (Collins et al., 2015; Lan et al., 2018) were also found to affect the neonatal rhythms of several physiological parameters, but their roles on the development of circadian rhythms in neonates have not been extensively studied yet (Liao et al., 2018; Gogou et al., 2019).

5 The characteristics of circadian rhythms in preterm infants

Preterm birth is defined as a live birth that occurs before 37 completed weeks of GA (Walani, 2020), which causes the fetus to detach prematurely from the natural protective environment of the uterus (Vohr, 2013; Hazelhoff et al., 2021) and puts an early end of fetal development in the uterus, especially for the brain and lung, which are critical to the neonates' survival after birth (Saigal and Doyle, 2008). Preterm infants have an increased risk of short-term and long-term morbidities (Deng et al., 2021), like the neurological and respiratory conditions (Vogel et al., 2018). Unfortunately, those babies continue to contribute disproportionately to neonatal mortality and even the childhood morbidity, which puts a heavy burden on health resources (Saigal and Doyle, 2008; Vohr, 2013).

Impressively, circadian rhythms in premature infants primarily occur as ultradian or irregular rhythms (Mirmiran et al., 2003a; Rivkees, 2007; Koch et al., 2021). It is hypothesized that the rhythms in preterm neonates appeared to be closely related to their GA (Begum et al., 2006; Darnall et al., 2006), due to the development of the fetal brain is related to the stages of pregnancy (Andescavage et al., 2017). On the other hand, the continuous active brain maturation occurs after birth (Matthews et al., 2018), so their endogenously-driven rhythms also change

TABLE 2 Studies about the cardiorespiratory rhythms in preterm infants.

Studies	Subjects	Methods of evaluation	Main findings
Koch et al. (2021)	65 preterm infants (GA: 30.8 ± 2.1 weeks)	Surface EMG	• The base HR are negatively correlated with GA during the first 5 days of life • Average oscillating period length of HR rhythms: 159 min • Average amplitude of HR rhythms: 5.9 bpm
Hasenstab-Kenney et al. (2020)	40 preterm infants (GA: 27.0 ± 3.1 weeks)	Respiratory inductance plethysmography; Nasal thermistor; ECG	• Pharyngeal irritation leads to: HR↓, duration of cardiac rhythms responses↑, respiratory rhythms changes↑
Hasenstab et al. (2019)	48 preterm infants (GA: 27.7 ± 0.5 weeks)	Respiratory inductance plethysmography; Nasal thermistor; ECG	• Pharyngeal stimulation leads to HR decreased in 32% preterm infants and remained stable in 61% • HR decrease is related to extreme prematurity and resulted in increased respiratory rhythms disturbance
Bauer et al. (2009)	22 preterm infants (GA: 30.3 ± 1.7 weeks)	Indirect calorimetry	• Oxygen consumptions are significantly associated with the HR • Circadian rhythms of oxygen consumptions with two peaks in the afternoon and early morning are detected in most preterm infants early after birth
Gewolb and Vice, (2006)	20 preterm infants (GA: 29.4 ± 2.1 weeks) *vs.* 16 term infants (GA: 39.2 ± 1.1 weeks)	Pharyngeal pressure transducer; Thoracoabdominal strain gauge	• With advancing PMA for preterm infants: percentage of apneic swallows↓, variation of breath interval↓, integration of swallow and respiratory rhythms↑ • Stabilization of suck and suck-swallow rhythms occurs at about 36 weeks PMA, and coordination of respiration and swallow rhythms occurs later
Begum et al. (2006)	124 preterm infants (GA: 23–36 weeks) *vs.* 63 term infants (GA: 37–42 weeks)	ECG; Pulse oximetry	• Circadian cycles are observed among 23.8% neonates in HR, 20% in PR, 27.8% in RR, and 16% in SpO_2 in first 3 days of life • Percentages of circadian PR cycles are negatively correlated with GA, but amplitudes are positively correlated with GA and PMA
Gewolb et al. (2001)	20 preterm infants (GA: 29.4 ± 2.1 weeks)	Pharyngeal pressure transducer; Nasal thermistor; Cardiac monitor	• Swallow rhythms are stable after 32 weeks PMA, percentage of swallows in runs increased with increasing PMA • Stability of suck rhythms and sucks in runs are positively correlated with PMA
Dimitriou et al. (1999)	22 preterm infants (GA: 23–28 weeks)	Indwelling arterial cannula transducer	• Significant circadian and ultradian rhythms of BP are shown on day 2 but not day 7 after birth
Glotzbach et al. (1995)	17 preterm infants (GA: 31.1 ± 1.2 weeks)	ECG	• Preterm infants exhibit feeding-related ultradian HR rhythms at about 35 weeks PMA
D'Souza et al. (1992)	9 preterm infants (GA: 26–29 weeks)	Skin electrodes monitor	• Three preterm infants exhibit circadian HR rhythms at 33–42 weeks PMA
Tenreiro et al. (1991)	20 preterm infants (GA: 24–29 weeks)	Surface electrode monitor	• Circadian and ultradian HR rhythms are appeared and disappeared erratically for the period of 6–17 weeks after birth • Circadian and ultradian rhythmicity of HR increases with regular light-dark and feeding patterns
Mirmiran and Kok, (1991)	12 preterm infants (GA: 25–32 weeks)	Neonatal intensive care monitor	• Five of these preterm infants exhibit the 24-h period circadian HR rhythms at 29–33 weeks PMA
Updike et al. (1985)	6 preterm infants (GA: 34–37 weeks)	Noninvasive electrodes monitor	• Three preterm infants exhibit circadian respiratory pause frequency rhythms with peak occurring between 23:00 to 05:00 during 10–20 days after birth • Two preterm infants exhibit circadian transcutaneous oxygen level rhythms with trough occurring between 00: 30 to 04:30 during 10–20 days after birth

Abbreviations: BP, blood pressure; bpm, beats per minute; ECG, electrocardiography; EMG, electromyography; GA, gestational age; HR, heart rate; PMA, postmenstrual age; PR, pulse rate; RR, respiratory rate; SpO_2, pulse oximeter oxygen saturation.

TABLE 3 Studies about the body temperature rhythms in preterm infants.

Studies	Subjects	Methods of evaluation	Main findings
Koch et al. (2021)	65 preterm infants (GA: 30.8 ± 2.1 weeks)	Zero heat flux method *via* the skin electrode	• Average oscillating period length of ultradian BT rhythms within the first 5 days of life: 290 min • Average amplitude of BT rhythms: 0.147°C
Bueno and Menna-Barreto, (2016)	19 preterm infants (GA: 28–36 weeks)	Wrist skin thermistor record	• Dominant circadian WT rhythms are present in preterm infants since the first 2 weeks of life
Mirmiran et al. (2003b)	40 preterm infants (GA: 30.2 ± 1.5 weeks)	Rectal digital ambulatory record	• Preterm infants mainly exhibit 2–4 h period ultradian BT rhythms at 36 weeks PMA • Preterm infants exhibit 12 and 24 h period circadian BT rhythms at 1–3 months after birth • The amplitude of BT rhythms is correlated with PMA and light-dark patterns
Thomas, (2001)	26 preterm infants (GA: 30.9 ± 2.1 weeks)	Skin transducer monitor	• 21 preterm infants exhibit circadian BT rhythms at mean of 33 weeks PMA • The amplitude of BT rhythms is correlated with PMA for not sick infants, but not for sick infants
Thomas and Burr, (2002)	34 preterm infants (GA: 26–33 weeks)	Abdominal skin thermistor record	• Preterm infants have circadian ST rhythms at 44–46 weeks PMA • The acrophase of circadian ST rhythms is related to parental co-sleeping and hospital stay length
Glotzbach et al. (1995)	17 preterm infants (GA: 31.1 ± 1.2 weeks)	Rectal and abdominal skin thermistor record	• Preterm infants exhibit feeding-related ultradian RT and ST rhythms at about 35 weeks PMA • Amplitudes of RT rhythms of preterm infants at 35–37 weeks PMA are much higher compared with 32–34 weeks PMA
D'Souza et al. (1992)	9 preterm infants (GA: 26–29 weeks)	Skin electrodes monitor	• Four of these preterm infants exhibit light-related circadian ST rhythms at 34–42 weeks PMA
Tenreiro et al. (1991)	20 preterm infants (GA: 24–29 weeks)	Surface electrode monitor	• Circadian and ultradian ST rhythms are appeared and disappeared erratically during 6–17 weeks after birth • Circadian and ultradian rhythmicity of ST increases with regular light-dark and feeding patterns
Mirmiran and Kok, (1991)	12 preterm infants (GA: 25–32 weeks)	Skin transducer monitor	• Seven of these preterm infants exhibit circadian BT rhythms with different periods and out of time synchronization at 29–34 weeks PMA
Mirmiran et al. (1990)	11 preterm infants (GA: 26–32 weeks)	Rectal sensor monitor	• Five preterm infants exhibit circadian RT rhythms with high values at night and low values during the day at 28–34 weeks PMA
Updike et al. (1985)	6 preterm infants (GA: 34–37 weeks)	Skin thermistor record	• Five preterm infants exhibit circadian ST rhythms with trough occurring between 23:00 to 04:30 during 10–20 days after birth

Abbreviations: BT, body temperature; GA, gestational age; PMA, postmenstrual age; RT, rectal temperature; ST, skin temperature; WT, wrist temperature.

with the postmenstrual age (PMA) (Mirmiran et al., 2003a; Darnall et al., 2006). However, due to the remarkable heterogeneity in terms of methodological designs, the characteristics of the circadian rhythms in preterm infants have not been consistently described, and some studies have even found conflicting results (Mirmiran et al., 2003a). For comprehensively and precisely understanding the circadian rhythms in preterm infants, relevant advances are summarized in Tables 1–4 and discussed as follows:

5.1 Sleep-wake rhythms

It is well established that the sleep is essential for normal brain development and health throughout the whole life (Peirano et al., 2003; Gogou et al., 2019). Premature newborns spend more than 70% of their first several weeks sleeping after birth (Ardura et al., 1995; Wong et al., 2022), thereby maintaining the proper sleep homeostasis is even more important for their neurological development and functional maturation (Bennet et al., 2018; Uchitel et al., 2021). The direct behavioral observations, parental sleep questionnaires, video recordings, polysomnography, actigraphy, and electroencephalography (EEG) (Table 1) have been developed to investigate the sleep-wake states of neonates (Mirmiran et al., 2003a; Collins et al., 2015; Gogou et al., 2019).

Based on the behavioral, cardiopulmonary, and EEG patterns (Darnall et al., 2006; Dereymaeker et al., 2017), the sleep states of preterm infants are generally classified as: active sleep (AS), the precursor of adult rapid eye movement (REM) sleep; quiet sleep (QS), the precursor of adult non-REM sleep; and indeterminate sleep (IS), the transition between AS and QS patterns (Mirmiran et al., 2003a; Liao et al., 2018). More specifically, the AS could promote the synapse formation, neuronal differentiation and migration, and the development of brain functional connectivity networks (Kurth et al., 2017; Gogou et al., 2019), whilst the QS promote the myelination, replenishment of energy reserves, and

TABLE 4 Studies about the hormonal rhythms in preterm infants.

Studies	Subjects	Methods of evaluation	Main findings
Biran et al. (2019)	209 preterm and term infants (GA: 24.0–41.9 weeks)	Plasma melatonin and urine 6-sulfatoxymelatonin levels by RIA	• No obvious rhythms of plasma melatonin and urine 6-sulfatoxymelatonin excretion were found in these neonates during first 55 days of life
Ivars et al. (2017)	51 preterm infants (GA: 23.3–31.9 weeks) *vs.* 130 term infants (GA: 37–42 weeks)	Salivary cortisol levels by RIA	• Salivary cortisol circadian rhythms in preterm infants are established by 1 month CA and persisted throughout the first year • The establishment of salivary cortisol circadian rhythms is correlated with GA and delayed by topical corticosteroid medication
Dorn et al. (2014)	60 preterm infants (GA: 33.0 ± 10.8 weeks)	Salivary cortisol levels by ELISA	• No circadian or ultradian rhythms of salivary cortisol are found in preterm infants during the first 3 weeks of life except one at 34.3 weeks PMA • Salivary cortisol levels in day 1 are higher than day 7 and 14 after birth, nighttime cortisol levels are higher than daytime
Kidd et al. (2005)	11 preterm infants (GA: 26–29 weeks)	Salivary cortisol levels by RIA	• No circadian salivary cortisol rhythms are found during the first 4 weeks of life • Five infants exhibit unsustainable adult-type rhythms after 39 weeks PMA • Salivary cortisol levels are negatively correlated with PNA
Antonini et al. (2000)	9 preterm infants (GA: 31.3–34.6 weeks)	Salivary cortisol levels by RIA	• Salivary cortisol circadian rhythms in preterm infants are emerged and persisted at approximately 8–12 weeks after birth
Jett et al. (1997)	14 preterm infants (GA: 25.6 ± 1.3 weeks)	Plasma cortisol levels by RIA	• No circadian rhythm of plasma cortisol is found in preterm infants during the first 4 days of life
Mantagos et al. (1996)	23 preterm infants (GA: 33–36 weeks)	Plasma melatonin levels by RIA	• No circadian rhythm of plasma melatonin is found in preterm infants under cyclic or constant light conditions during the first 4 days of life
Commentz et al. (1996)	64 preterm and term male infants (GA: 26–42 weeks)	Urine melatonin and 6-hydroxymelatonin sulfate levels by RIA	• No circadian rhythm of urine melatonin and 6-hydroxymelatonin sulfate excretion are found in these infants during the first 7 days of life • Urine melatonin and 6-hydroxymelatonin sulfate excretion in these infants are negatively correlated with GA
Economou et al. (1993)	60 preterm and term infants (GA: 33.5 ± 1.5 weeks)	Serum cortisol levels by IFA	• A free running serum cortisol rhythm is found in healthy preterm and term infants during the first 4 weeks of life • Sick preterm and term infants exhibit higher serum cortisol levels at 20:00, while healthy infants exhibit lower levels at 20:00
Kennaway et al. (1992)	14 preterm infants (GA: 29–35 weeks) *vs.* 17 term infants (GA > 37 weeks)	Urine 6-sulfatoxymelatonin levels by RIA	• Appearance of rhythmic urine 6-sulfatoxymelaton in preterm infants are delayed by 9 weeks than term infants and 2–3 weeks after correcting for GA • Urine 6-sulfatoxymelaton excretion in preterm infants is gradually increased during the first 52 weeks after birth but lower than term infants

Abbreviations: CA, corrected age; ELISA, enzyme linked immune sorbent assay; GA, gestational age; IFA, immunofluorescence assay; PMA, postmenstrual age; PNA, postnatal age; RIA, radioimmunoassay.

cognitive development in premature infants (Liao et al., 2018; Gogou et al., 2019).

As summarized in Table 1, Curzi-Dascalova et al. (1993), found the AS and QS states can be discerned in preterm infants as early as 27 weeks of GA. The results varied due to the different GA of the enrolled cases, but most studies revealed that preterm infants experienced more total sleep time and AS, while less QS than term ones (Anders and Keener, 1985; Ardura et al., 1995; Sahni et al., 1995; Hoppenbrouwers et al., 2005; Guyer et al., 2015; Georgoulas et al., 2021), which might reflect the accelerated neurological maturation of preterm infants (Mirmiran et al., 2003a; Bennet et al., 2018). Besides, preterm infants had fewer total arousals and, more specifically, fewer arousals in the AS (Guyon et al., 2022), which seemed to cause a higher risk of sudden infant death syndrome (Mirmiran et al., 2003a; Bennet et al., 2018).

With developmental maturity, preterm infants have more sleep during nighttime but less during daytime (Antonini et al.,

2000; Korte et al., 2001; Guyer et al., 2015; Lan et al., 2019; Guyon et al., 2022). Meanwhile, as the PMA increased, the AS proportion comes out of a decreasing trend, but it is not true for the QS, IS, wakefulness, and activity, which all experience an increasing trend (Anders and Keener, 1985; Curzi-Dascalova et al., 1988; Curzi-Dascalova et al., 1993; Borghese et al., 1995; Sahni et al., 1995; Ingersoll and Thoman, 1999; Mirmiran et al., 2003b; Holditch-Davis et al., 2004; Hoppenbrouwers et al., 2005; Foreman et al., 2008; Dorn et al., 2014; Guyer et al., 2015; Lan et al., 2019; Cailleau et al., 2020; Park et al., 2020; Georgoulas et al., 2021; Guyon et al., 2022). In addition, other factors like sex, illness severity, body weight, ventilatory support, maternal smoking, and ambient temperature also affect the sleep-wake patterns (Bach et al., 2000; Hoppenbrouwers et al., 2005; Foreman et al., 2008; Lan et al., 2019).

It is well understood that the sleep homeostasis in humans are regulated by two independent but synergistic processes (Borbély, 1982; Deboer, 2018): a Clock-dependent circadian process (Process C), controlled by the SCN circadian oscillator, determines the alternation of different sleep propensity (Cremer et al., 2016); and a Sleep-dependent homeostatic process (Process S) that is determined by the prior sleep pressure, which comes from the adenosine buildup in the basal forebrain during wakefulness (Deboer, 2018; Wong et al., 2022). However, due to the immature development of the central nervous system, especially the SCN, Process C and Process S are not stably present in preterm infants or even in term ones (Salzarulo and Fagioli, 1992; Schwichtenberg et al., 2016). As a result, preterm infants experience many sleep and wake episodes within the 24-h period, and those ultradian sleep-wake rhythms persist for several months until the Process C and Process S are gradually developed (Mirmiran et al., 2003a; Cremer et al., 2016).

As shown in Table 1, preterm infants exhibit ultradian or irregular sleep-wake rhythms with different periods in the early postnatal life (Mirmiran et al., 1990; Hayes et al., 1993; Borghese et al., 1995; Shimada et al., 1999; Scher et al., 2005; Dorn et al., 2014; Koch et al., 2021), which might be explained by the environmental factors, such as feeding patterns (Glotzbach et al., 1995; Thomas, 2000; Bueno and Menna-Barreto, 2016) and respiratory states (Palmu et al., 2013). As for when the sleep-wake rhythms begin to occur and entrain, Scher et al. (2005), observed the ultradian sleep-wake rhythms as early as 25 weeks of PMA. Mirmiran and Kok, (1991) found the circadian sleep-wake rhythms began to appear after 29 weeks of PMA. However, McMillen et al. (1991), found that the entrainment of circadian sleep-wake rhythms did not occur in 50% of the preterm infants at 47 weeks of PMA, and all cases did not begin to develop the circadian rhythms until approximately 54 weeks of PMA.

Besides, several studies also demonstrated that a definite sleep-wake cycling existed in preterm infants with the advanced GA and became more prominent as the PMA increased (Sisman et al., 2005; Soubasi et al., 2009; Lee et al., 2010). Therefore, it could be concluded that with the continuous development of the brain and neural functions, circadian sleep-wake rhythms in preterm infants are consolidated and eventually developed to a 24-h pattern, just as those in adults (Mirmiran et al., 2003a; Bennet et al., 2018).

5.2 Cardiorespiratory rhythms

Many physiological biomarkers of the cardiopulmonary system in adults, such as the heart rate, blood pressure, and respiratory rate, exhibit distinct circadian rhythms (Elstad et al., 2018). A complex network that composed of the brainstem respiratory center, autonomic nervous system, and a variety of central and peripheral chemoreceptors and mechanoreceptors is responsible for regulating the rhythmic oscillations of the cardiorespiratory system (Darnall et al., 2006; Longin et al., 2006). Due to the immaturity of this network (Hunt, 2006), cardiorespiratory events like apnea, periodic breathing, and bradycardia are common in premature infants (Hodgman et al., 1990; Darnall et al., 2006), which leads to the erratic cardiopulmonary rhythms with marked individual differences (Begum et al., 2006). Clinically, the incidence and duration of cardiorespiratory events are associated with the GA and PMA (Hellmeyer et al., 2012; Fairchild et al., 2016; Patel et al., 2016).

As shown in Table 2, some, but not all, preterm infants experienced circadian or ultradian rhythms for the heart rate, pulse rate, respiratory rate, blood pressure, and oxygen consumption at the first few weeks after birth (Begum et al., 2006; Mirmiran and Kok, 1991; Bauer et al., 2009; D'Souza et al., 1992; Updike et al., 1985). Interestingly, unlike the ultradian sleep-wake rhythms gradually grew into circadian rhythms after birth, these cardiopulmonary rhythms in premature infants appeared and disappeared erratically (Tenreiro et al., 1991; Dimitriou et al., 1999), *e.g.*, presence on day 2 but absence on day 7 after birth for the heart rate rhythms, which might be caused by the residual of maternal effects (Dimitriou et al., 1999). Tenreiro et al. (1991) also proposed that the circadian components of these cardiopulmonary rhythms gradually and erratically came into phases with one another, while the regular light-dark and feeding patterns seemed to promote the presence of the dominant circadian rhythms, which developed as the increased coupling between the component oscillators.

In addition, the well-developed laryngeal reflexes and coordination of pharyngoesophageal-cardiorespiratory (PECR) responses are essential for the development and maintenance of cardiorespiratory rhythms (Gewolb and Vice, 2006; Hasenstab-Kenney et al., 2020). As shown in Table 2, pharyngeal stimulations cause a decrease of heart rate in premature infants with uncoordinated suck-swallow-respiration rhythms due to the immature laryngeal reflexes and PECR responses, which would aggravate the disturbance of cardiac and respiratory rhythms (Hasenstab et al., 2019; Hasenstab-Kenney et al., 2020).

(Gewolb et al., 2001; Gewolb and Vice, 2006) found that the development and establishment of suck-swallow rhythms were associated with their PMA. The swallow rhythms appeared at 32 weeks of PMA first (Gewolb et al., 2001), followed by the stabilization of suck and suck-swallow rhythms between 36 and 40 weeks of PMA (Gewolb and Vice, 2006), then the suck-swallow-respiration rhythms began to coordinate and to integrate as the adaptation of feeding patterns and the maturation of neurodevelopment (Darnall et al., 2006).

5.3 Body temperature rhythms

The human body temperature is precisely regulated by a network that consists of the skin thermal sensors, hypothalamic thermoregulatory center, autonomic nervous system, and several thermoregulation effector systems including brown adipose tissue, peripheral vasomotricity, and sweat glands (Bach et al., 1996; Jost et al., 2017). Due to the immaturity of the regulatory network, especially the dysfunction of the autonomic nervous system, their body temperature during the first few days of life is susceptible to the rapidly changed external environment temperature (Jost et al., 2017). Therefore, premature infants are typically nursed in the incubators to treat the autonomic dysregulation of body temperature (Thomas, 2001). Interestingly, Bueno and Menna-Barreto (2016), found a positive correlation between the wrist temperature and environment temperature inside the incubator, but no significant association between the period or potency for them. Similarly, Thomas (2001) demonstrated that the circadian of incubator temperature did not appear to be the primary determinant of the body temperature rhythms.

As summarized in Table 3, due to the heterogeneity of the body temperature monitoring, the GA of preterm infants, and sample size, the body temperature rhythms have not yet been consistently described. Several studies observed the ultradian body temperature rhythms within the first few days of life (Glotzbach et al., 1995; Mirmiran et al., 2003b; Koch et al., 2021), and the circadian rhythms by approximately 1–3 months of PNA (Mirmiran et al., 2003b; Bueno and Menna-Barreto, 2016). Interestingly, Thomas and Burr (2002), found that the acrophase of circadian abdominal skin temperature rhythms was related to the parental co-sleeping and length of hospital stay for preterm infants at 44–46 weeks of PMA. However, some studies demonstrated that the body temperature rhythms were only found in some, but not all preterm infants (Mirmiran et al., 1990; Mirmiran and Kok, 1991; D'Souza et al., 1992; Updike et al., 1985; Thomas, 2001). For example, Tenreiro et al. (1991) found that the ultradian and circadian rhythms of skin temperature appeared and disappeared erratically during 6–17 weeks of PNA, which was similar to the cardiopulmonary rhythms.

5.4 Hormonal rhythms

As summarized in Table 4, due to the difficulties in sample collection and analysis, studies on hormonal rhythms in preterm infants are still very limited until now, and nearly all focused on the cortisol and melatonin rhythms. With regard to the cortisol, due to the immature of HPA axis (Bolt et al., 2002), no significant circadian or ultradian rhythms were observed during the early postnatal periods (Economou et al., 1993; Jett et al., 1997; Kidd et al., 2005; Dorn et al., 2014). Nevertheless, studies have found that healthy preterm infants had higher nighttime cortisol levels than daytime at birth, and that cortisol levels tended to decrease gradually after birth (Kidd et al., 2005; Dorn et al., 2014). Impressively, premature infants with perinatal stress like respiratory distress experienced higher cortisol levels at nighttime after birth compared with those healthy preterm and term neonates (Economou et al., 1993; Gunes et al., 2006).

It remains unclear when premature infants develop the circadian cortisol rhythms. Antonini et al. (2000) found the salivary cortisol circadian rhythms emerged and persisted at approximately 8–12 weeks of PNA, which was in line with term infants. However, Ivars et al. (2017) found that the cortisol rhythms were established by 1 month of corrected age, persisted throughout the first year of life, but delayed by topical corticosteroid medication. In addition, Ivars et al. (2017) also suggested that the establishment of cortisol rhythms was related to the GA rather than PNA, because the maturation of adrenal cortex was depend on the GA of preterm infants (Bolt et al., 2002).

Circadian melatonin rhythms could not be detected in preterm infants under different ambient illumination conditions during the early postnatal life (Commentz et al., 1996; Mantagos et al., 1996; Biran et al., 2019). Several studies demonstrated that the blood melatonin and urine 6-sulfatoxymelatonin levels were positively correlated with the GA (Biran et al., 2019) and birth weight of preterm infants (Muñoz-Hoyos et al., 2007), but the serum melatonin levels and urine 6-sulfatoxymelaton excretion increased during the first 7 days and even 52 weeks of PNA (Kennaway et al., 1992; Commentz et al., 1996; Muñoz-Hoyos et al., 2007), which might be attributed to the gradual maturation of the pineal gland where the melatonin is mainly synthesized (Commentz et al., 1997).

However, Commentz et al. (1996) found the urine melatonin and 6-hydroxymelatonin sulfate excretion in male preterm infants during 2–7 days of PNA were negatively associated with the GA, indicating that the melatonin levels might be related to the sex. As for the establishment of circadian melatonin rhythms, Kennaway et al. (1992) observed the appearance of urine 6-sulfatoxymelaton circadian rhythms was approximately at 18–21 weeks of PNA, which was delayed by 9 weeks than those term infants and 2–3 weeks after correcting for GA.

6 The effects and mechanisms of caffeine on circadian rhythms

The potential association between caffeine consumption and circadian rhythms has attracted extensive attention in the past decades (Landolt, 2015). However, the underlying mechanisms remain largely elusive. Various research attempts in the non-human field also reinforce this impression (Spaeth et al., 2014). In this section, we briefly introduce the up-to-date progress that achieved in human and non-human mammals, while the effects on premature infants will be delineated in the next section.

6.1 The effects of caffeine on circadian rhythms

In humans, several clinical observational studies with small sample size have witnessed the alterations of circadian sleep-wake (Landolt et al., 1995a; Landolt et al., 1995b; McHill et al., 2014; Weibel et al., 2021), body temperature (Wright et al., 1997; Wright et al., 2000; McHill et al., 2014), blood pressure (Green and Suls, 1996; Guessous et al., 2014), heart rates (Green and Suls, 1996; Kohler et al., 2006; Crooks et al., 2019), melatonin (Wright et al., 1997; Wright et al., 2000; Burke et al., 2015), and cortisol rhythms (Lovallo et al., 2005; Rieth et al., 2016) in adults who consumed caffeine by comparison with placebo controls.

In rodents, caffeine disrupted the mesors, amplitudes, and acrophases of the circadian heart rate, temperature, motor activity, and sleep-wake rhythms (Pelissier et al., 1999; Pelissier-Alicot et al., 2002; Vivanco et al., 2013; Panagiotou et al., 2019). Caffeine also potentiated the light-induced phase shift, which responded to the rest-activity circadian rhythms, indicating that caffeine enhanced the clock sensitivity to light (Antle et al., 2001; Vivanco et al., 2013; van Diepen et al., 2014; Jha et al., 2017; Ruby et al., 2018). In addition, caffeine lengthened the period and amplitude of circadian clocks in mammalian cells *in vitro* and in mice *ex vivo* and *in vivo* (Oike et al., 2011; Narishige et al., 2014; Burke et al., 2015). At the cellular level, caffeine also altered the expression of circadian clock genes, such as *Clock*, *Bmal1*, and *Per1* in the liver and jejunum of mice under *ad libitum* feeding conditions (Sherman et al., 2011).

6.2 The mechanisms of caffeine on circadian rhythms

Caffeine influences the circadian rhythms by modulating the endogenous cAMP/Ca^{2+} signaling pathway, the core components of the mammalian circadian pacemaker (Harvey et al., 2020; O'Neill et al., 2008), through a variety of complex mechanisms (Aguilar-Roblero et al., 2007; Narishige et al., 2014; Burke et al., 2015; Landolt, 2015; Jagannath et al., 2021) (Figure 3). Basically, caffeine antagonizes all types of adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3 receptors) and mainly functions by non-specifically antagonizing the A_1 and A_{2A} receptors (Nehlig et al., 1992; Cappelletti et al., 2015; Rodak et al., 2021; Yang et al., 2021). The blockade of adenosine receptors indirectly regulates the production of cAMP by inhibition (A_1 and A_3 receptors) or stimulation (A_{2A} and A_{2B} receptors) of adenylate cyclase (Nehlig et al., 1992; Kumar and Lipshultz, 2019; Yang et al., 2021). Caffeine also prevents the degradation and increases the intracellular cAMP levels by non-selectively inhibiting phosphodiesterase (Nehlig et al., 1992; Cappelletti et al., 2015; Kumar and Lipshultz, 2019; Yang et al., 2021). In addition, caffeine mobilizes intracellular Ca^{2+} transmission from the endoplasmic reticulum through activating the ryanodine receptor channels (Aguilar-Roblero et al., 2007; Kumar and Lipshultz, 2019) and the inositol triphosphate receptors (Yang et al., 2021).

The increased cytosolic cAMP/Ca^{2+} activates the protein kinase A (PKA) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), thereby leading to the phospho-dependent activation of cAMP response element binding protein (CREB), which in concert with its coactivators to activate the cAMP response element (CRE) (Narishige et al., 2014; Harvey et al., 2020; Reichert et al., 2022). Besides, the increased intracellular Ca^{2+} levels also result in the phosphorylation of extracellular regulated protein kinases (ERK), which drives to form the activator protein 1 (AP-1) transcription factor (Jagannath et al., 2021). Then, interestingly, CRE and AP-1 together drive the *Per* gene transcription (Narishige et al., 2014; Jagannath et al., 2021), which in turn participates in the transcriptional feedback loops that regulate circadian rhythms (Figure 3).

In addition, caffeine affects the release of neurotransmitters, such as γ-aminobutyric acid, dopamine, glutamate, acetylcholine, norepinephrine, and serotonin, between synaptic neurons in almost all brain areas by blocking the adenosine receptors (Nehlig et al., 1992; Cappelletti et al., 2015; Yang et al., 2021) (Figure 3), thereby significantly influencing the sleep-wake rhythms (Kumar and Lipshultz, 2019).

7 The effects of caffeine on circadian rhythms in preterm infants

Caffeine is widely prescribed to treat or prevent the AOP (Eichenwald, 2020; van Dam et al., 2020) and has recently been attempted to prevent the encephalopathy (Williamson et al., 2021; Yang et al., 2021) for preterm neonates in the NICU. Therefore, studies on the caffeine treatment in preterm infants mainly focus on the respiratory and neurodevelopmental outcomes (Schmidt et al., 2006; Schmidt et al., 2007), while less attention has been paid to its effects on their circadian rhythms.

In fact, the ultradian or irregular circadian rhythms due to the neurodevelopmental immaturity of preterm infants with

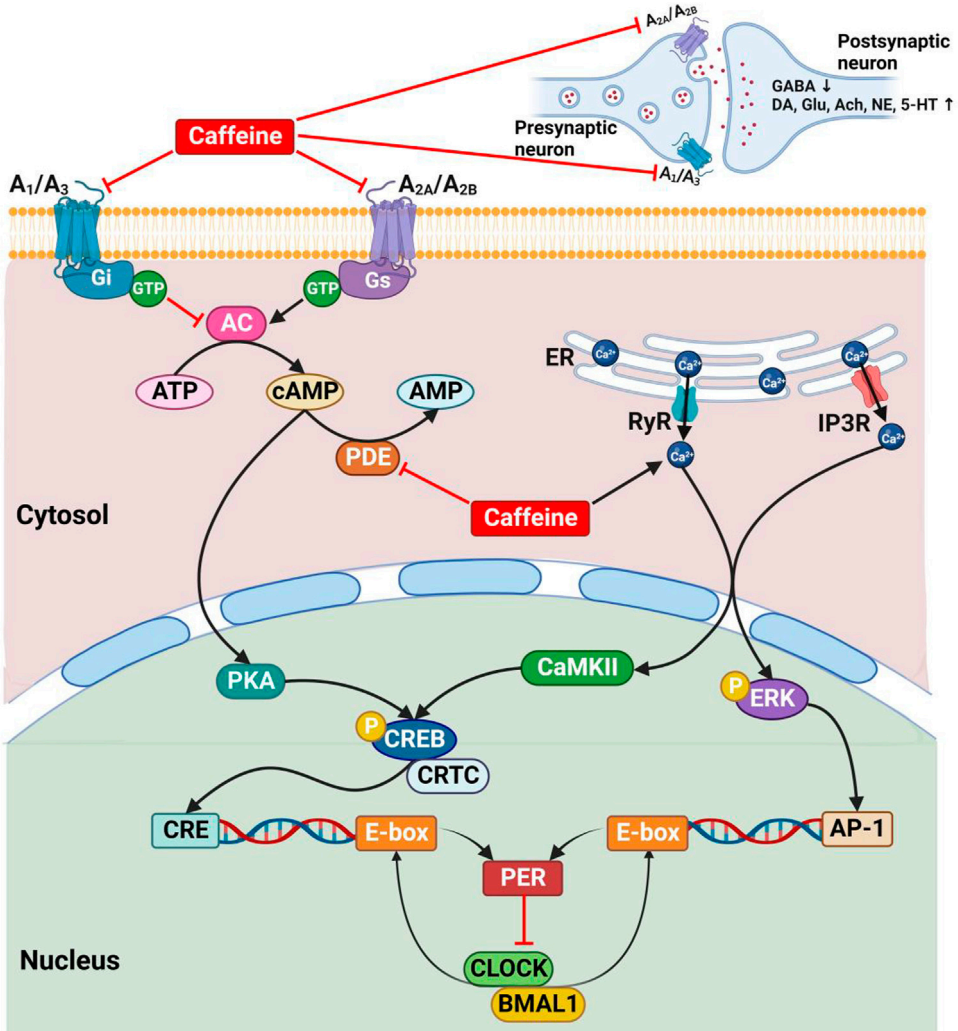

FIGURE 3
The mechanisms of caffeine on circadian rhythms. A_1, A_3, A_{2A}, and A_{2B}, adenosine receptors; AC, adenylate cyclase; Ach, acetylcholine; AMP, adenosine monophosphate; ATP, adenosine triphosphate; AP-1, activator protein 1; BMAL1, brain and muscle ARNT-like 1; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; cAMP, cyclic adenosine monophosphate; CLOCK, circadian locomotor output cycles kaput; CRE, cAMP response element; CREB, cAMP responsive element binding protein; CRTC, CREB regulated transcription coactivator; DA, dopamine; ER, endoplasmic reticulum; ERK, extracellular regulated protein kinases; GABA, γ-aminobutyric acid; Gi, inhibitory adenylate cyclase G protein; Glu, glutamate; Gs, stimulating adenylate cyclase G protein; GTP, guanosine triphosphate; IP3R, inositol triphosphate receptor; NE, norepinephrine; P, phosphorylation; PDE, phosphodiesterase; PER, period; PKA, protein kinase A; RyR, ryanodine receptor; 5-HT, serotonin.

different GA during the early postnatal life (Begum et al., 2006; Darnall et al., 2006) are more likely to mask caffeine's effects. Moreover, preterm infants with different PNA and/or PMA experience different circadian characteristics (Mirmiran et al., 2003a; Darnall et al., 2006), so whether the response to caffeine therapy are partly related to the maturation of the circadian system in preterm infants remains to be explored.

Thus, relevant advances are summarized here to delineate those effects of caffeine on the circadian rhythms in preterm infants. Besides, theophylline and aminophylline, another two methylxanthines and fully metabolized in the body to produce the main metabolite caffeine (Bory et al., 1979; Pacifici, 2014), are also commonly used in the treatment of AOP (Henderson-Smart and De Paoli, 2010; Henderson-Smart and Steer, 2010; Eichenwald et al., 2016). The real effects of theophylline and aminophylline are thus thought to be related to caffeine in nature (Bory et al., 1979). Collectively, studies involving the effects of caffeine, theophylline, and aminophylline on the circadian rhythms in preterm infants are summarized in Tables 5, 6 and described as follows:

TABLE 5 Studies about the effects of methylxanthine on sleep-wake rhythms in preterm infants.

Studies	Subjects	Treatments	Methods of evaluation	Main findings
Seppä-Moilanen et al. (2021)	21 preterm infants (GA: 28.4–33.6 weeks)	Caffeine citrate (loading: 20 mg/kg; maintenance: 5 mg/kg/day)	Polysomnography	• Caffeine do not affect the sleep-arousal characteristics of preterm infants on the second day of treatment
Koch et al. (2020)	52 preterm infants (GA: 29.9 ± 1.96 weeks) *vs.* 12 preterm infants (GA: 33.4 ± 1.75 weeks)	Caffeine citrate (loading: 20 mg/kg; maintenance: 5–10 mg/kg/day) *vs.* no-caffeine	Videographic recordings	• In caffeine cohort with GA ≥ 28 weeks: AS↓ and wakefulness↑ as caffeine concentrations and PNA increased over the first 5 days of life • In caffeine cohort with GA < 28 weeks: no clear caffeine effects on sleep-wake behavior • In no-caffeine cohort: no PNA effects on sleep-wake behavior
Hassanein et al. (2015)	20 preterm infants (GA: 31.70 ± 1.16 weeks)	Caffeine citrate (loading: 20 mg/kg)	aEEG recordings	• A loading dose of caffeine leads to AS↓, QS↓, drowsiness↓, quite alert↑, active alert↑, crying↑
Lee et al. (2010)	35 preterm infants (GA: 24.9–31.9 weeks)	Aminophylline (loading: 5 mg/kg; maintenance: 1.5 mg/kg/8 h)	aEEG recordings	• The sleep-wake cycling is more prominent in preterm infants receiving aminophylline at 34–36 weeks PMA • Aminophylline use is associated with the appearance of sleep-wake cycling in preterm infants
Hayes et al. (2007)	14 preterm infants (GA: 28.6 ± 2.3 weeks) *vs.* 13 preterm infants (GA: 30.3 ± 1.5 weeks) *vs.* 10 preterm infants (GA: 32.4 ± 1.5 weeks)	Caffeine *vs.* theophylline *vs.* untreated control	Videographic recordings; Actigraphy	• Methylxanthine duration is associated with: AS↓, wakefulness↑, sleep-related movements↑ • Methylxanthine *vs.* untreated: arousal rate↓, wakefulness↓, sleep-related movement↓ at night (from 24:00 to 05:00)
Chardon et al. (2004)	11 preterm infants (GA: 31.1 ± 1.8 weeks) *vs.* 11 preterm infants (GA: 30.3 ± 2.0 weeks)	Caffeine citrate (4.0 ± 0.5 mg/kg/day) *vs.* no-caffeine	Actigraphy; EEG; Eye movement monitors; Visual observations	• Caffeine has no significant effects on the TST, AS, QS, and IS for preterm infants during the inter-feeding intervals (2–3 h)
Curzi-Dascalova et al. (2002)	10 preterm infants (GA: 32.6 ± 0.21 weeks) *vs.* 5 preterm infants (GA: 32.7 ± 0.3 weeks)	Caffeine citrate (loading: 20 mg/kg; maintenance: 5 mg/kg/day) *vs.* no-caffeine	Polysomnography	• Caffeine has no significant effects on the AS, QS, IS, wakefulness, and state transitions for preterm infants during daytime (from 09:00 to 19:00) between 33 and 34 weeks PMA
Thoman et al. (1985)	4 preterm infants (GA: 28–30 weeks) *vs.* 5 preterm infants (GA: 29–35 weeks) *vs.* 28 term infants (GA: 37–42 weeks)	Theophylline *vs.* no-theophylline *vs.* untreated control	Direct behavioral observations	• Theophylline *vs.* no-theophylline for preterm infants at 2–5 weeks post-term: waking activity↑, alert↑, drowse or transition↑, AS↓ • Theophylline preterm *vs.* untreated term infants: waking activity↑, alert↑, drowse or transition↑, AS↓, QS↓
Gabriel et al. (1978)	6 preterm infants (GA: 30.4–32.9 weeks)	Aminophylline (loading: 5.5 mg/kg; maintenance: 1.1 mg/kg/8 h)	Polysomnography	• Sleep cycles of AS, QS, and IS are unaffected during short-term theophylline treatment and after drug withdrawal
Dietrich et al. (1978)	9 preterm infants (GA: 26–32 weeks)	Aminophylline (loading: 5.8 mg/kg; maintenance: 1.4 mg/kg/8 h)	Direct behavioral observations; EEG	• During theophylline therapy *vs.* before theophylline therapy: AS↑, QS↓, IS↓, wakefulness↑

Abbreviations: aEEG, amplitude-integrated electroencephalography; AS, active sleep; EEG, electroencephalography; GA, gestational age; IS, indeterminate sleep; PMA, postmenstrual age; PNA, postnatal age; QS, quiet sleep; TST, total sleep time.

7.1 The effects on sleep-wake rhythms

The well-studied effects of caffeine on sleep-wake rhythms in preterm infants are still limited as the sample sizes were small and the study designs were heterogeneous (Table 5). Some studies revealed that the sleep-wake patterns were not significantly changed after short-term treatment with caffeine or theophylline during short observation periods (Gabriel et al.,

TABLE 6 Studies about the effects of methylxanthine on cardiorespiratory rhythms in preterm infants.

Studies	Subjects	Treatments	Methods of evaluation	Main findings
Seppä-Moilanen et al. (2021)	21 preterm infants (GA: 28.4–33.6 weeks)	Caffeine citrate (loading: 20 mg/kg; maintenance: 5 mg/kg/day)	Polysomnography	• Caffeine leads to SpO_2↑, while HRV not changed on the second day of treatment
Williams et al. (2020)	32 preterm infants (GA: 27.27–31.49 weeks)	Caffeine citrate (loading: 20 mg/kg)	EMG	• A loading dose of caffeine leads to RR↑
Shivakumar et al. (2019)	185 preterm infants (GA: 29.5 ± 1.6 weeks)	Caffeine citrate (loading: 20 mg/kg; maintenance: 5 mg/kg/day) *vs.* aminophylline (loading: 5 mg/kg; maintenance: 1.5 mg/kg/8 h)	Echocardiography	• Aminophylline leads to HR↑, while caffeine has no significant increase in HR after 48 h of continued therapy compared with pretreatment values
Huvanandana et al. (2019)	40 preterm infants (GA: 23.6–33.3 weeks)	Caffeine base (loading: 10 mg/kg)	Intra-arterial blood pressure monitor; ECG	• A loading dose of caffeine leads to mean arterial pressure variability↑, pulse pressure variability↑, HRV↓
Dix et al. (2018)	34 preterm infants (GA: 28.8 ± 2.1 weeks)	Caffeine base (loading: 10 mg/kg)	Physiological parameter monitor	• A loading dose of caffeine leads to HR↑ and MABP↑ over time, while RR and SaO_2 not changed
Dekker et al. (2017)	13 preterm infants (GA: 26–28 weeks) *vs.* 10 preterm infants (GA: 27–29 weeks)	Caffeine base (loading: 10 mg/kg)	Pulse oximeter	• A loading dose of caffeine leads to HR↑, while RR and SpO_2 not changed
Parikka et al. (2015)	17 preterm infants (GA: 23.7–31.9 weeks)	Caffeine citrate (loading: 20 mg/kg)	Pulse oximeter	• A loading dose of caffeine leads to HR↑, while RR not changed
Hassanein et al. (2015)	20 preterm infants (GA: 31.70 ± 1.16 weeks)	Caffeine citrate (loading: 20 mg/kg)	Continuous cardiovascular and respiratory monitoring	• A loading dose of caffeine leads to HR↑, MABP↑, SpO_2↑
Ulanovsky et al. (2014)	21 preterm infants (GA: 30.3 ± 2.5 weeks)	Caffeine citrate (loading: 15–20 mg/kg; maintenance: 5–10 mg/kg/day)	Cardiac monitor	• A loading dose of caffeine has no significant effects on HRV
Supcun et al. (2010)	51 preterm infants (GA: 24–33 weeks)	Caffeine base (loading: 10 mg/kg)	Cardiac monitor	• A loading dose of caffeine leads to MABP↑, while HR and SaO_2 not changed
Soloveychik et al. (2009)	43 preterm infants (GA: 27.62 ± 2.94 weeks)	Caffeine citrate (5, 10, 20 mg/kg)	Continuous cardiovascular monitoring	• A dose of caffeine leads to BP↑, HR↑
Hoecker et al. (2006)	16 preterm infants (GA: 24–33 weeks)	Caffeine citrate (loading: 25 mg/kg/4 h; maintenance: 10 mg/kg/day)	Continuous cardiovascular and respiratory monitoring	• Two divided loading dose of caffeine lead to HR↑, diastolic BP↑, while RR not changed
von Poblotzki et al. (2003)	16 preterm infants (GA: 24.0–29.5 weeks)	Theophylline (5 mg/kg)	Continuous cardiorespiratory monitoring	• A dose of theophylline leads to HR↑, while RR and SpO_2 not changed
Hoecker et al. (2002)	16 preterm infants (GA: 31 ± 1.2 weeks)	Caffeine base (loading: 25 mg/kg; maintenance: 5 mg/kg/day)	Continuous cardiorespiratory monitoring	• A loading dose of caffeine has no significant effects on BP and HR
Bauer et al. (2001)	18 preterm infants (GA: 28–33 weeks)	Caffeine citrate (loading: 10 mg/kg; maintenance: 5 mg/kg/day)	Continuous cardiorespiratory monitoring	• The RR, HR, and SaO_2 are not significant changed at 48 h after caffeine treatment
Dani et al. (2000)	20 preterm infants (GA: 30.4 ± 3.0 weeks)	Caffeine citrate (loading: 10 mg/kg; maintenance: 2.5 mg/kg/day) *vs.* aminophylline (loading: 5 mg/kg; maintenance: 1.25 mg/kg/12 h)	Pulse oximeter; Continuous cardiorespiratory monitoring	• The HR, MABP, and SaO_2 are not significant changed after caffeine or aminophylline treatment for at least 3 days
Carnielli et al. (2000)	18 preterm infants (GA: 32.7 ± 1.1 weeks)	Aminophylline (loading: 5 mg/kg; maintenance: 1.25 mg/kg/12 h)	Continuous cardiorespiratory monitoring	• A loading dose of theophylline leads to HR↑, RR↑
Govan et al. (1995)	20 preterm infants (GA: 28.0 ± 2.0 weeks)	Aminophylline (loading: 6 mg/kg)	Pulsed Doppler; Intra-arterial blood pressure monitor	• A loading dose of theophylline leads to HR↑, while MABP not changed
Chang and Gray, (1994)	10 preterm infants (GA: 27–32 weeks)	Aminophylline (loading: 7.5 mg/kg)	Cardiorespiratory monitor	• A loading dose of theophylline leads to HR↑, while MABP not changed
Bucher et al. (1994)	13 preterm infants (GA: 26–34 weeks)	Aminophylline (loading: 6 mg/kg)	Pulse oximeter; ECG	• A loading dose of theophylline leads to HR↑, while SaO_2 not changed
McDonnell et al. (1992)	10 preterm infants (GA: 23–31 weeks)	Aminophylline (loading: 6.2 mg/kg)	Pulse oximeter; Intra-arterial blood pressure monitor	• A loading dose of theophylline leads to HR↑, while MABP not changed

(Continued on following page)

TABLE 6 (*Continued*) Studies about the effects of methylxanthine on cardiorespiratory rhythms in preterm infants.

Studies	Subjects	Treatments	Methods of evaluation	Main findings
Pryds and Schneider, (1991)	16 preterm infants (GA: 25–34 weeks)	Aminophylline (loading: 10 mg/kg)	Intra-arterial blood pressure monitor	• A loading dose of theophylline has no significant effects on MABP
Walther et al. (1990)	10 preterm infants (GA: 29.6 ± 3.0 weeks)	Caffeine citrate (loading: 20 mg/kg; maintenance: 5 mg/kg/day)	ECG; Oscillometry	• Caffeine leads to MABP↑ during first 3 days treatment, while HR not changed
Saliba et al. (1989)	7 preterm infants (GA: 31.3 ± 2.0 weeks)	Caffeine citrate (20 mg/kg) or saline	ECG; Oscillometry	• A loading dose of caffeine leads to HR↑, while MABP not changed
Walther et al. (1986)	10 preterm infants (GA: 30.7 ± 0.8 weeks)	Aminophylline (loading: 6.8 mg/kg; maintenance: 2 mg/kg/8 h)	Pulsed Doppler; Echocardiography	• Theophylline leads to HR↑ during first 7 days treatment, while MABP not changed

Abbreviations: BP, blood pressure; bpm, beats per minute; ECG, electrocardiography; EMG, electromyography; GA, gestational age; HR, heart rate; HRV, heart rate variability; MABP, mean arterial blood pressure; PMA, postmenstrual age; PR, pulse rate; RR, respiratory rate; SaO_2, arterial oxygen saturation; SpO_2, pulse oximeter oxygen saturation.

1978; Curzi-Dascalova et al., 2002; Chardon et al., 2004; Seppä-Moilanen et al., 2021).

However, some other studies observed significant effects of caffeine on the sleep-wake rhythms, although these effects were not entirely consistent (Dietrich et al., 1978; Thoman et al., 1985; Hayes et al., 2007; Hassanein et al., 2015; Koch et al., 2020). For example, Koch et al. (2020), found that the AS decreased while the wakefulness increased but QS unchanged as caffeine concentrations and the PNA increased over the first 5 days of life in preterm infants more than 28 weeks of GA, but no clear effects on the sleep-wake states were found in preterm infants less than 28 weeks of GA, and no such PNA effects were found in no-caffeine cohort. Hassanein et al. (2015) also detected significant decreases in the AS, QS, and drowsiness, while increases in the quite alert, active alert, and crying in preterm infants half an hour after caffeine administration. Similar methylxanthine-induced changes in the AS and wakefulness states were also observed in studies conducted by Hayes et al. (2007) and by Thoman et al. (1985). However, Dietrich et al. (1978) found the AS and wakefulness increased while the QS and IS decreased during theophylline therapy.

In addition, Lee et al. (2010) discovered that the appearance of sleep-wake cycling was associated with the aminophylline use and more prominent. However, in the prospective follow-up study of the CAP trial (Marcus et al., 2014), no significant differences in sleep states were found in preterm infants aged 5–12 years who had been treated with caffeine after birth compared with the placebo group, which possibly due to the apparent discrepancy in total recording and sleep time between the two groups.

This is also true for some animal studies. Denenberg et al. (1982) found that theophylline reduced the AS, while increased wakefulness, delayed the development of QS, and affected the intermediate states of sleep-wake and AS-QS transitions in newborn rabbits. Montandon et al. (2009) also discovered that the sleep time was reduced, sleep onset latency was increased, and non-REM sleep was fragmented in adult rats treated with caffeine compared to controls during the neonatal period.

Due to the heterogeneous designs and inconsistent results of the above studies, it is difficult to draw clear conclusions. Nonetheless, it can be summarized that caffeine affects the sleep patterns in preterm infants, especially the AS and wakefulness, and the effects might persist into the childhood and even the adulthood. If this hypothesis holds true, then the inhibition of adenosine receptors by caffeine would exactly explain the altered sleep-wake states in preterm infants, as the association between caffeine, adenosine, and sleep has been well documented in adults (Huang et al., 2011; Porkka-Heiskanen and Kalinchuk, 2011; Huang et al., 2014a; Urry and Landolt, 2015; Reichert et al., 2022). In addition, the alteration of sleep-wake patterns might be partially responsible for the caffeine-induced increase in cerebral cortical activity (Supcun et al., 2010; Hassanein et al., 2015) and decrease in apneic episodes (Dietrich et al., 1978; Montandon et al., 2009; Seppä-Moilanen et al., 2019; Seppä-Moilanen et al., 2021).

7.2 The effects on cardiorespiratory rhythms

Current studies have confirmed that caffeine acts both peripherally and centrally to stimulate respiration mainly via inhibiting the adenosine A_1 and A_{2A} receptors (Abdel-Hady et al., 2015; Eichenwald et al., 2016; Dobson and Hunt, 2018). Caffeine activates the medullary respiratory center, improves sensitivity to carbon dioxide, increases respiratory muscle strength, enhances diaphragmatic contractility, and induces bronchodilation (Kassim et al., 2009; Parikka et al., 2015; Dekker et al., 2017; Sanchez-Solis et al., 2020; Williams et al., 2020), which synergistically cause the increased minute ventilation and oxygen consumption, while cause the decreased apnea, periodic breathing, and intermittent hypoxia

(Seppä-Moilanen et al., 2021; Seppä-Moilanen et al., 2019; Dobson et al., 2017; Rhein et al., 2014; von Poblotzki et al., 2003; Bauer et al., 2001; Carnielli et al., 2000).

In addition, caffeine or theophylline therapy increases the cardiac output, stroke volume, and metabolic rate (Walther et al., 1986; Walther et al., 1990; Carnielli et al., 2000; Bauer et al., 2001; Soloveychik et al., 2009; Shivakumar et al., 2019), but decreases blood flow velocities in cerebral and intestinal arteries (Pryds and Schneider, 1991; McDonnell et al., 1992; Bucher et al., 1994; Chang and Gray, 1994; Govan et al., 1995; Lundstrøm et al., 1995; Lane et al., 1999; Hoecker et al., 2002; Hoecker et al., 2006; Dix et al., 2018; Hwang et al., 2018; Abdel Wahed et al., 2019) for preterm infants, which appeared to be related to the enhanced endothelial function through antagonism of adenosine receptors, inhibition of phosphodiesterase, and through promotion of intracellular calcium concentrations (Higashi, 2019). Although the clinical significance remains unclear, this reduced perfusion activity was a reminder that caffeine might have adverse effects on the developing brain and gastrointestinal tract (McDonnell et al., 1992; Lane et al., 1999; Hoecker et al., 2002; Hoecker et al., 2006; Atik et al., 2017; Abdel Wahed et al., 2019).

Unlike the cardiopulmonary system, the effects of caffeine on the cardiorespiratory rhythms in preterm infants have not been specifically studied. Nonetheless, the effects of caffeine on the heart rate, respiratory rate, blood pressure, and oxygen saturation have been examined. As summarized in Table 6, some studies found that a loading of caffeine or theophylline increases the heart rate (Hassanein et al., 2015; Dekker et al., 2017; Parikka et al., 2015; von Poblotzki et al., 2003; Carnielli et al., 2000; Soloveychik et al., 2009; Dix et al., 2018; Govan et al., 1995; Chang and Gray, 1994; Bucher et al., 1994; McDonnell et al., 1992; Saliba et al., 1989), blood pressure (Soloveychik et al., 2009; Supcun et al., 2010; Hassanein et al., 2015; Dix et al., 2018; Huvanandana et al., 2019), respiratory rate (Williams et al., 2020), and oxygen saturation (Hassanein et al., 2015), which were in line with those studies with multiple caffeine dosing (Walther et al., 1986; Walther et al., 1990; Hoecker et al., 2006; Shivakumar et al., 2019). Those findings reflected the complex effects, directly or indirectly like the enhanced autonomic nervous system responsiveness (Huvanandana et al., 2019), of caffeine on the cardiopulmonary system. However, several other studies did not find similar effects (Pryds and Schneider, 1991; Dani et al., 2000; Bauer et al., 2001; Hoecker et al., 2002; Ulanovsky et al., 2014).

Unfortunately, no research has touched this area yet in premature infants until now. It is worth mentioning that neonatal caffeine treatment upregulates adenosine receptors in cardiorespiratory related nuclei of the rat brain (Gaytan et al., 2006; Gaytan and Pasaro, 2012), and this effect persists into the adulthood (Bairam et al., 2009), which underscores the urgent to study the potential long-term effects of caffeine on the cardiorespiratory system in preterm infants (Montandon et al., 2008). In view of the complex and profound effects of caffeine in this field, systematic and in-depth research is still necessary.

7.3 The effects on other rhythms

Two studies recorded the body temperature of preterm infants and incubator temperature during short-term caffeine administration. Chardon et al. (2004) found that caffeine has no significant effect on the skin temperature and incubator temperature. However, Bauer et al. (2001) observed that a lower incubator temperature was sufficient to maintain a normal body temperature for preterm infants after caffeine treatment, which might be related to the increased metabolism caused by methylxanthines (Bucher et al., 1994; Carnielli et al., 2000; Bauer et al., 2001). However, the effects of caffeine on circadian body temperature rhythms have not been extensively studied. Similarly, although caffeine has been shown to affect melatonin (Wright et al., 1997; Wright et al., 2000; Burke et al., 2015) and cortisol (Lovallo et al., 2005; Rieth et al., 2016) rhythms in adults, these effects in premature infants still need to be addressed.

Collectively, the relevant research on the circadian rhythms in premature infants receiving caffeine therapy is still scarce. Although existing studies have suggested the possible effects of caffeine on the circadian rhythms, heterogeneity in study designs and inconsistency in conclusions weaken the power of those evidence. More research is needed in the future to confirm the effects of caffeine and the underlying mechanisms. The story should not end here.

8 Circadian-based caffeine therapeutic strategies for AOP: New possibility opens up

It is estimated that more than 15 million neonates are born preterm globally each year, and the preterm birth appears to be increasing in most countries (Vogel et al., 2018; Walani, 2020; Deng et al., 2021). Premature babies may have various problems like AOP. Unfortunately, the tough challenges are always there for the current AOP therapy, such as significant interindividual variability in the response to caffeine (Saroha and Patel, 2020; He et al., 2021). Intriguingly, one most recent study revealed that the *Clock* gene polymorphisms were significantly associated with the response to caffeine therapy in preterm infants (Guo et al., 2022). Although the molecular action mechanism through which there is a better response is unknown, these results show that the circadian rhythms might play a critical role in response to the therapy. In this way, a new possibility opens up in this area of research (Figure 4), and we tentatively propose three initiatives.

8.1 Considering the circadian changes

As discussed above, the efficacy of caffeine appeared to interact with the circadian rhythms in premature infants.

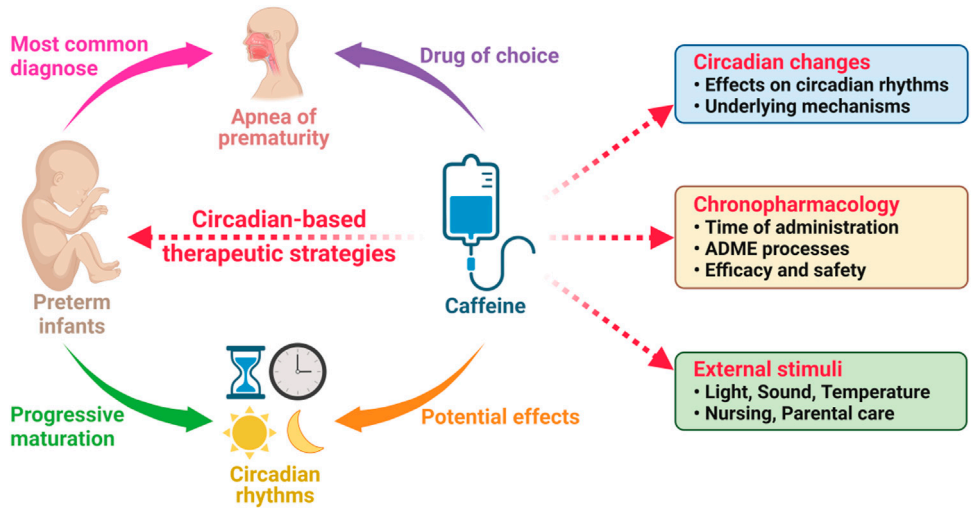

FIGURE 4
The circadian-based therapeutic strategies of caffeine in preterm infants with apnea of prematurity. ADME, absorption, distribution, metabolism, and excretion.

Studies have demonstrated the significant effects and underlying mechanisms of caffeine in adults and in animals (Landolt, 2015), but it remains unclear whether the similar mechanisms also exist in those preterm infants. The effects of caffeine on the circadian rhythms, especially the sleep-wake rhythms, are advised to be considered into the strategy of the caffeine therapy (Figure 4).

In addition, studies have revealed that several circadian-related problems like sleep, breathing, and blood pressure in premature infants may persist into childhood and even adulthood (Weisman et al., 2011; Huang et al., 2014b; Sipola-Leppanen et al., 2015; Caravale et al., 2017; Durankus et al., 2020). Based on the existing evidence, it is feasible to propose that caffeine's effects on circadian rhythms may ameliorate those problems and promote the maturation of circadian rhythms in preterm infants to the level of normal term infants.

8.2 Considering the chronopharmacology

The concept of chronopharmacology holds that the ADME processes and the sensitivity of a biological target to a drug are determined by the endogenous biological circadian oscillations (Ohdo et al., 2019; Bicker et al., 2020; Dong et al., 2020; Dobrek, 2021). Variable efficacy and safety profiles would be exhibited for many drugs if they are administered at different times of the day (Dallmann et al., 2016; Cederroth et al., 2019; Nahmias and Androulakis, 2021). For preterm infants, interestingly, several circadian-related gene polymorphisms were found to be significantly associated with the response to caffeine therapy for AOP (Guo et al., 2022). It remains unclear whether caffeine administrated at different times of the day would cause changes in the ADME processes and the therapeutic effects, but it really opens a possibility to applicate the chronopharmacology in the NICU.

Although less research is currently available, there are rare but thought-provoking reports that arouse our strong interests (Smolensky et al., 1987; Pelissier-Alicot et al., 2002), which will lead us into a wonderland in the future. For examples, Pelissier-Alicot et al. (2002) found that the pharmacokinetic profiles of caffeine in rats, such as the clearance, volume of distribution, and area under the plasma concentration-time curve (AUC), depended strongly on the time of day of administration, while the daily rhythmicity of heart rate, body temperature, and locomotor activity in rats also changed with the dosing time of caffeine. Similarly, Smolensky et al. (1987) demonstrated that the pharmacokinetic profiles and therapeutic effects of theophylline in asthmatic children varied with the dosing time. These findings attract us that the circadian rhythms might play a critical role in the ADME processes as well as the efficacy and safety of caffeine therapy in preterm infants.

Currently, caffeine is now commonly administered once daily in preterm infants (Long et al., 2021). The question is whether we are willing to make positive attempts to tailor the dosing time according to the principles of chronopharmacology. If the significant association between circadian-related gene polymorphisms and response to caffeine therapy in preterm infants (Guo et al., 2022) were true and phenotypically manifested, then the administration at different time points of the day is more likely to witness those potentially altered pharmacokinetics of and clinical response to caffeine.

Maintaining normal circadian rhythms are necessary to stay health. Essentially, caffeine interferes with these rhythms to a

certain extent, and its arousal effects are very important for the AOP management among various pharmacological mechanisms. Therefore, whether to apply caffeine in accordance with the circadian rhythms to maintain the stabilities of these rhythms as much as possible, or to subtly counteract these rhythms to amplify its arousal effect and achieve a better therapeutic effect, all these aspects deserve our in-depth consideration (Figure 4).

8.3 Considering the other external stimuli

If the homeostasis of circadian rhythms were necessary for health, then correcting the possible adverse effects due to preterm birth is a matter that needs to be taken seriously in the NICU, including the effects on the treatment drugs being used. As discussed above, several external stimuli or known as zeitgebers, such as light, sound, temperature, nursing, and parental care, *etc.*, play important roles in the maturation of circadian rhythms. Cycled light (Abraham et al., 2006; Ohta et al., 2006; Bode et al., 2011), music therapy (Arnon et al., 2006; Loewy et al., 2013), appropriate incubator temperature (Tourneux et al., 2008), comfortable nursing (Collins et al., 2015; Lan et al., 2018), and even the adequate parental care (Löhr and Siegmund, 1999; Nishihara et al., 2002; Park et al., 2020) are helpful for the development and maturation of the circadian rhythms in neonates.

Therefore, the beneficial effects of those external stimuli on the circadian rhythms for premature infants cannot be ignored in the NICU, taking the application of caffeine to manage the AOP for example (Figure 4). Coordinating all treatment strategies with the principles of circadian rhythms will be a constructive attempt to improve the disease management and care for premature infants. Assuredly, we have to admit that only rare evidence is available currently, and the realization of the therapeutic strategies cannot be achieved overnight. However, any kind of discussions, attempts, and efforts in this field should well be encouraged in the future.

9 Conclusion

Due to the tough challenges and potential role of circadian rhythms in the response to current caffeine therapy for the AOP management, a comprehensive review was conducted here. Studies have revealed that the human circadian system begins to form in early pregnancy, receives the maternal circadian signals through the placenta before birth, and progressively matures under the influence of the external cues and the mother after birth. Preterm infants experience the ultradian or irregular rhythms during the early postnatal life, which are progressively developed into circadian rhythms as the maturation of neurodevelopment. Caffeine alters the circadian rhythms in humans and animals, and its promising role in preterm infants has also been revealed. The proposed novel circadian-based therapeutic strategies could open new possibilities in the clinical practice to promote the precision caffeine therapy. Arguably, as studies going on, it is believed that in the near future, these initiatives will remain powerful approaches to enhance our biological understanding of the relationship between preterm infants, circadian rhythms, and caffeine therapy.

Author contributions

H-RD, FC: Conceptualization. H-RD, H-LG, Y-HH: data curation. H-RD: Writing—original draft. JX, X-SD, RC, FC: Supervision, Writing—review and editing. FC: Project administration, Funding acquisition. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abdel Wahed, M. A., Issa, H. M., Khafagy, S. M., and Abdel Raouf, S. M. (2019). Effect of caffeine on superior mesenteric artery blood flow velocities in preterm neonates. *J. Matern. Fetal. Neonatal Med.* 32 (3), 357–361. doi:10.1080/14767058.2017.1378337

Abdel-Hady, H., Nasef, N., Shabaan, A. E., and Nour, I. (2015). Caffeine therapy in preterm infants. *World J. Clin. Pediatr.* 4 (4), 81–93. doi:10.5409/wjcp.v4.i4.81

Abraham, D., Dallmann, R., Steinlechner, S., Albrecht, U., Eichele, G., and Oster, H. (2006). Restoration of circadian rhythmicity in circadian clock-deficient mice in constant light. *J. Biol. Rhythms* 21 (3), 169–176. doi:10.1177/0748730406288040

Aguilar-Roblero, R., Mercado, C., Alamilla, J., Laville, A., and Díaz-Muñoz, M. (2007). Ryanodine receptor Ca2+-release channels are an output pathway for the circadian clock in the rat suprachiasmatic nuclei. *Eur. J. Neurosci.* 26 (3), 575–582. doi:10.1111/j.1460-9568.2007.05679.x

Allada, R., and Bass, J. (2021). Circadian mechanisms in medicine. *N. Engl. J. Med.* 384 (6), 550–561. doi:10.1056/NEJMra1802337

Anders, T. F., and Keener, M. (1985). Developmental course of nighttime sleep-wake patterns in full-term and premature infants during the first year of life. I. *Sleep* 8 (3), 173–192. doi:10.1093/sleep/8.3.173

Andescavage, N. N., du Plessis, A., McCarter, R., Serag, A., Evangelou, I., Vezina, G., et al. (2017). Complex trajectories of brain development in the healthy human fetus. *Cereb. Cortex* 27 (11), 5274–5283. doi:10.1093/cercor/bhw306

Antle, M. C., Steen, N. M., and Mistlberger, R. E. (2001). Adenosine and caffeine modulate circadian rhythms in the Syrian hamster. *Neuroreport* 12 (13), 2901–2905. doi:10.1097/00001756-200109170-00029

Antonini, S. R., Jorge, S. M., and Moreira, A. C. (2000). The emergence of salivary cortisol circadian rhythm and its relationship to sleep activity in preterm infants. *Clin. Endocrinol.* 52 (4), 423–426. doi:10.1046/j.1365-2265.2000.00935.x

Ardura, J., Andrés, J., Aldana, J., and Revilla, M. A. (1995). Development of sleep-wakefulness rhythm in premature babies. *Acta Paediatr.* 84 (5), 484–489. doi:10.1111/j.1651-2227.1995.tb13679.x

Arendt, J. (2018). Approaches to the pharmacological management of jet lag. *Drugs* 78 (14), 1419–1431. doi:10.1007/s40265-018-0973-8

Arnon, S., Shapsa, A., Forman, L., Regev, R., Bauer, S., Litmanovitz, I., et al. (2006). Live music is beneficial to preterm infants in the neonatal intensive care unit environment. *Birth* 33 (2), 131–136. doi:10.1111/j.0730-7659.2006.00090.x

Arslanoglu, S., Bertino, E., Nicocia, M., and Moro, G. E. (2012). WAPM working group on nutrition: potential chronobiotic role of human milk in sleep regulation. *J. Perinat. Med.* 40 (1), 1–8. doi:10.1515/jpm.2011.134

Astiz, M., and Oster, H. (2020). Feto-maternal crosstalk in the development of the circadian clock system. *Front. Neurosci.* 14, 631687. doi:10.3389/fnins.2020.631687

Atik, A., Harding, R., De Matteo, R., Kondos-Devcic, D., Cheong, J., Doyle, L. W., et al. (2017). Caffeine for apnea of prematurity: Effects on the developing brain. *Neurotoxicology* 58, 94–102. doi:10.1016/j.neuro.2016.11.012

Attanasio, A., Rager, K., and Gupta, D. (1986). Ontogeny of circadian rhythmicity for melatonin, serotonin, and N-acetylserotonin in humans. *J. Pineal Res.* 3 (3), 251–256. doi:10.1111/j.1600-079x.1986.tb00747.x

Babischkin, J. S., Grimes, R. W., Pepe, G. J., and Albrecht, E. D. (1997). Estrogen stimulation of P450 cholesterol side-chain cleavage activity in cultures of human placental syncytiotrophoblasts. *Biol. Reprod.* 56 (1), 272–278. doi:10.1095/biolreprod56.1.272

Bach, V., Telliez, F., Krim, G., and Libert, J. P. (1996). Body temperature regulation in the newborn infant: interaction with sleep and clinical implications. *Neurophysiol. Clin.* 26 (6), 379–402. doi:10.1016/s0987-7053(97)89152-6

Bach, V., Telliez, F., Leke, A., and Libert, J. P. (2000). Gender-related sleep differences in neonates in thermoneutral and cool environments. *J. Sleep. Res.* 9 (3), 249–254. doi:10.1046/j.1365-2869.2000.00206.x

Bairam, A., Joseph, V., Lajeunesse, Y., and Kinkead, R. (2009). Altered expression of adenosine A1 and A2A receptors in the carotid body and nucleus tractus solitarius of adult male and female rats following neonatal caffeine treatment. *Brain Res.* 1287, 74–83. doi:10.1016/j.brainres.2009.06.064

Bates, K., and Herzog, E. D. (2020). Maternal-fetal circadian communication during pregnancy. *Front. Endocrinol.* 11, 198. doi:10.3389/fendo.2020.00198

Bauer, J., Janecke, A., Gerss, J., Masjosthusmann, K., Werner, C., and Hoffmann, G. (2009). Circadian variation on oxygen consumption in preterm infants. *J. Perinat. Med.* 37 (4), 413–417. doi:10.1515/JPM.2009.067

Bauer, J., Maier, K., Linderkamp, O., and Hentschel, R. (2001). Effect of caffeine on oxygen consumption and metabolic rate in very low birth weight infants with idiopathic apnea. *Pediatrics* 107 (4), 660–663. doi:10.1542/peds.107.4.660

Begum, E., Bonno, M., Obata, M., Yamamoto, H., Kawai, M., and Komada, Y. (2006). Emergence of physiological rhythmicity in term and preterm neonates in a neonatal intensive care unit. *J. Circadian Rhythms* 4, 11. doi:10.1186/1740-3391-4-11

Bennet, L., Walker, D. W., and Horne, R. S. C. (2018). Waking up too early - the consequences of preterm birth on sleep development. *J. Physiol.* 596 (23), 5687–5708. doi:10.1113/JP274950

Bicker, J., Alves, G., Falcao, A., and Fortuna, A. (2020). Timing in drug absorption and disposition: the past, present, and future of chronopharmacokinetics. *Br. J. Pharmacol.* 177 (10), 2215–2239. doi:10.1111/bph.15017

Biran, V., Decobert, F., Bednarek, N., Boizeau, P., Benoist, J. F., Claustrat, B., et al. (2019). Melatonin levels in preterm and term infants and their mothers. *Int. J. Mol. Sci.* 20 (9), 2077. doi:10.3390/ijms20092077

Bode, B., Taneja, R., Rossner, M. J., and Oster, H. (2011). Advanced light-entrained activity onsets and restored free-running suprachiasmatic nucleus circadian rhythms in per2/dec mutant mice. *Chronobiol. Int.* 28 (9), 737–750. doi:10.3109/07420528.2011.607374

Bolt, R. J., Van Weissenbruch, M. M., Popp-Snijders, C., Sweep, F. G., Lafeber, H. N., and Delemarre-van de Waal, H. A. (2002). Maturity of the adrenal cortex in very preterm infants is related to gestational age. *Pediatr. Res.* 52 (3), 405–410. doi:10.1203/00006450-200209000-00017

Borbély, A. A. (1982). A two process model of sleep regulation. *Hum. Neurobiol.* 1 (3), 195–204.

Borghese, I. F., Minard, K. L., and Thoman, E. B. (1995). Sleep rhythmicity in premature infants: implications for development status. *Sleep* 18 (7), 523–530. doi:10.1093/sleep/18.7.523

Bory, C., Baltassat, P., Porthault, M., Bethenod, M., Frederich, A., and Aranda, J. V. (1979). Metabolism of theophylline to caffeine in premature newborn infants. *J. Pediatr.* 94 (6), 988–993. doi:10.1016/s0022-3476(79)80246-2

Brandon, D. H., Holditch-Davis, D., and Belyea, M. (2002). Preterm infants born at less than 31 weeks' gestation have improved growth in cycled light compared with continuous near darkness. *J. Pediatr.* 140 (2), 192–199. doi:10.1067/mpd.2002.121932

Brandon, D. H., Silva, S. G., Park, J., Malcolm, W., Kamhawy, H., and Holditch-Davis, D. (2017). Timing for the introduction of cycled light for extremely preterm infants: A randomized controlled trial. *Res. Nurs. Health* 40 (4), 294–310. doi:10.1002/nur.21797

Brooks, E., and Canal, M. M. (2013). Development of circadian rhythms: role of postnatal light environment. *Neurosci. Biobehav. Rev.* 37 (4), 551–560. doi:10.1016/j.neubiorev.2013.02.012

Brown, A. G., Leite, R. S., and Strauss, J. F., 3rd (2004). Mechanisms underlying "functional" progesterone withdrawal at parturition. *Ann. N. Y. Acad. Sci.* 1034, 36–49. doi:10.1196/annals.1335.004

Bucher, H. U., Wolf, M., Keel, M., von Siebenthal, K., and Duc, G. (1994). Effect of aminophylline on cerebral haemodynamics and oxidative metabolism in premature infants. *Eur. J. Pediatr.* 153 (2), 123–128. doi:10.1007/BF01959223

Bueno, C., and Menna-Barreto, L. (2016). Development of sleep/wake, activity and temperature rhythms in newborns maintained in a neonatal intensive care unit and the impact of feeding schedules. *Infant Behav. Dev.* 44, 21–28. doi:10.1016/j.infbeh.2016.05.004

Burke, T. M., Markwald, R. R., McHill, A. W., Chinoy, E. D., Snider, J. A., Bessman, S. C., et al. (2015). Effects of caffeine on the human circadian clock *in vivo* and *in vitro*. *Sci. Transl. Med.* 7 (305), 305ra146. doi:10.1126/scitranslmed.aac5125

Burton, P. J., and Waddell, B. J. (1999). Dual function of 11beta-hydroxysteroid dehydrogenase in placenta: modulating placental glucocorticoid passage and local steroid action. *Biol. Reprod.* 60 (2), 234–240. doi:10.1095/biolreprod60.2.234

Busada, J. T., and Cidlowski, J. A. (2017). Mechanisms of glucocorticoid action during development. *Curr. Top. Dev. Biol.* 125, 147–170. doi:10.1016/bs.ctdb.2016.12.004

Cailleau, L., Weber, R., Cabon, S., Flamant, C., Roue, J. M., Favrais, G., et al. (2020). Quiet sleep organization of very preterm infants is correlated with postnatal maturation. *Front. Pediatr.* 8, 559658. doi:10.3389/fped.2020.559658

Cappelletti, S., Piacentino, D., Sani, G., and Aromatario, M. (2015). Caffeine: cognitive and physical performance enhancer or psychoactive drug? *Curr. Neuropharmacol.* 13 (1), 71–88. doi:10.2174/1570159X13666141210215655

Caravale, B., Sette, S., Cannoni, E., Marano, A., Riolo, E., Devescovi, A., et al. (2017). Sleep characteristics and temperament in preterm children at two years of age. *J. Clin. Sleep. Med.* 13 (9), 1081–1088. doi:10.5664/jcsm.6728

Carlomagno, G., Minini, M., Tilotta, M., and Unfer, V. (2018). From implantation to birth: Insight into molecular melatonin functions. *Int. J. Mol. Sci.* 19 (9), 2802. doi:10.3390/ijms19092802

Carnielli, V. P., Verlato, G., Benini, F., Rossi, K., Cavedagni, M., Filippone, M., et al. (2000). Metabolic and respiratory effects of theophylline in the preterm infant. *Arch. Dis. Child. Fetal Neonatal Ed.* 83 (1), F39–F43. doi:10.1136/fn.83.1.f39

Carr, B. R., Parker, C. R., Jr., Madden, J. D., MacDonald, P. C., and Porter, J. C. (1981). Maternal plasma adrenocorticotropin and cortisol relationships throughout human pregnancy. *Am. J. Obstet. Gynecol.* 139 (4), 416–422. doi:10.1016/0002-9378(81)90318-5

Cederroth, C. R., Albrecht, U., Bass, J., Brown, S. A., Dyhrfjeld-Johnsen, J., Gachon, F., et al. (2019). Medicine in the fourth dimension. *Cell Metab.* 30 (2), 238–250. doi:10.1016/j.cmet.2019.06.019

Challet, E. (2007). Minireview: Entrainment of the suprachiasmatic clockwork in diurnal and nocturnal mammals. *Endocrinology* 148 (12), 5648–5655. doi:10.1210/en.2007-0804

Challis, J. R., Patrick, J. E., Campbell, K., Natale, R., and Richardson, B. (1980). Diurnal changes in maternal plasma oestrone and oestradiol at 30 to 31, 34 to 35 and 38 to 39 weeks gestational age. *Br. J. Obstet. Gynaecol.* 87 (11), 983–988. doi:10.1111/j.1471-0528.1980.tb04462.x

Chang, J., and Gray, P. H. (1994). Aminophylline therapy and cerebral blood flow velocity in preterm infants. *J. Paediatr. Child. Health* 30 (2), 123–125. doi:10.1111/j.1440-1754.1994.tb00594.x

Chardon, K., Bach, V., Telliez, F., Cardot, V., Tourneux, P., Leke, A., et al. (2004). Effect of caffeine on peripheral chemoreceptor activity in premature neonates: interaction with sleep stages. *J. Appl. Physiol.* 96 (6), 2161–2166. doi:10.1152/japplphysiol.01160.2003

Chuffa, L. G. A., Lupi, L. A., Cucielo, M. S., Silveira, H. S., Reiter, R. J., and Seiva, F. R. F. (2019). Melatonin promotes uterine and placental health: Potential molecular mechanisms. *Int. J. Mol. Sci.* 21 (1), 300. doi:10.3390/ijms21010300

Ciarleglio, C. M., Axley, J. C., Strauss, B. R., Gamble, K. L., and McMahon, D. G. (2011). Perinatal photoperiod imprints the circadian clock. *Nat. Neurosci.* 14 (1), 25–27. doi:10.1038/nn.2699

Clark, I., and Landolt, H. P. (2017). Coffee, caffeine, and sleep: a systematic review of epidemiological studies and randomized controlled trials. *Sleep. Med. Rev.* 31, 70–78. doi:10.1016/j.smrv.2016.01.006

Collins, C. L., Barfield, C., Davis, P. G., and Horne, R. S. (2015). Randomized controlled trial to compare sleep and wake in preterm infants less than 32weeks of gestation receiving two different modes of non-invasive respiratory support. *Early Hum. Dev.* 91 (12), 701–704. doi:10.1016/j.earlhumdev.2015.09.011

Commentz, J. C., Henke, A., Dammann, O., Hellwege, H. H., and Willig, R. P. (1996). Decreasing melatonin and 6-hydroxymelatonin sulfate excretion with advancing gestational age in preterm and term newborn male infants. *Eur. J. Endocrinol.* 135 (2), 184–187. doi:10.1530/eje.0.1350184

Commentz, J. C., Uhlig, H., Henke, A., Hellwege, H. H., and Willig, R. P. (1997). Melatonin and 6-hydroxymelatonin sulfate excretion is inversely correlated with gonadal development in children. *Horm. Res.* 47 (3), 97–101. doi:10.1159/000185442

Cooperstock, M., England, J. E., and Wolfe, R. A. (1987). Circadian incidence of premature rupture of the membranes in term and preterm births. *Obstet. Gynecol.* 69 (6), 936–941.

Cremer, M., Jost, K., Gensmer, A., Pramana, I., Delgado-Eckert, E., Frey, U., et al. (2016). Immediate effects of phototherapy on sleep in very preterm neonates: an observational study. *J. Sleep. Res.* 25 (5), 517–523. doi:10.1111/jsr.12408

Crooks, E., Hansen, D. A., Satterfield, B. C., Layton, M. E., and Van Dongen, H. P. A. (2019). Cardiac autonomic activity during sleep deprivation with and without caffeine administration. *Physiol. Behav.* 210, 112643. doi:10.1016/j.physbeh.2019.112643

Croteau, A., Marcoux, S., and Brisson, C. (2006). Work activity in pregnancy, preventive measures, and the risk of delivering a small-for-gestational-age infant. *Am. J. Public Health* 96 (5), 846–855. doi:10.2105/AJPH.2004.058552

Curzi-Dascalova, L., Aujard, Y., Gaultier, C., and Rajguru, M. (2002). Sleep organization is unaffected by caffeine in premature infants. *J. Pediatr.* 140 (6), 766–771. doi:10.1067/mpd.2002.124383

Curzi-Dascalova, L., Figueroa, J. M., Eiselt, M., Christova, E., Virassamy, A., d'Allest, A. M., et al. (1993). Sleep state organization in premature infants of less than 35 weeks' gestational age. *Pediatr. Res.* 34 (5), 624–628. doi:10.1203/00006450-199311000-00013

Curzi-Dascalova, L., Peirano, P., and Morel-Kahn, F. (1988). Development of sleep states in normal premature and full-term newborns. *Dev. Psychobiol.* 21 (5), 431–444. doi:10.1002/dev.420210503

D'Souza, S. W., Tenreiro, S., Minors, D., Chiswick, M. L., Sims, D. G., and Waterhouse, J. (1992). Skin temperature and heart rate rhythms in infants of extreme prematurity. *Arch. Dis. Child.* 67 (7), 784–788. doi:10.1136/adc.67.7_spec_no.784

Dai, H. R., Liu, Y., Lu, K. Y., He, X., Guo, H. L., Hu, Y. H., et al. (2022). Population pharmacokinetic modeling of caffeine in preterm infants with apnea of prematurity: New findings from concomitant erythromycin and AHR genetic polymorphisms. *Pharmacol. Res.* 184, 106416. doi:10.1016/j.phrs.2022.106416

Dallmann, R., Okyar, A., and Levi, F. (2016). Dosing-time makes the poison: Circadian regulation and pharmacotherapy. *Trends Mol. Med.* 22 (5), 430–445. doi:10.1016/j.molmed.2016.03.004

Dani, C., Bertini, G., Reali, M. F., Tronchin, M., Wiechmann, L., Martelli, E., et al. (2000). Brain hemodynamic changes in preterm infants after maintenance dose caffeine and aminophylline treatment. *Biol. Neonate* 78 (1), 27–32. doi:10.1159/000014243

Darnall, R. A., Ariagno, R. L., and Kinney, H. C. (2006). The late preterm infant and the control of breathing, sleep, and brainstem development: a review. *Clin. Perinatol.* 33 (4), 883–914. doi:10.1016/j.clp.2006.10.004

Davis, P. G. (2020). When to start and stop caffeine and why respiratory status matters. *Semin. Fetal Neonatal Med.* 25 (6), 101175. doi:10.1016/j.siny.2020.101175

Deboer, T. (2018). Sleep homeostasis and the circadian clock: Do the circadian pacemaker and the sleep homeostat influence each other's functioning? *Neurobiol. Sleep. Circadian Rhythms* 5, 68–77. doi:10.1016/j.nbscr.2018.02.003

Deguchi, T. (1975). Ontogenesis of a biological clock for serotonin:acetyl coenzyme A N-acetyltransferase in pineal gland of rat. *Proc. Natl. Acad. Sci. U. S. A.* 72 (7), 2814–2818. doi:10.1073/pnas.72.7.2814

Deiriggi, P. M. (1990). Effects of waterbed flotation on indicators of energy expenditure in preterm infants. *Nurs. Res.* 39 (3), 140–146. doi:10.1097/00006199-199005000-00003

Dekker, J., Hooper, S. B., van Vonderen, J. J., Witlox, R., Lopriore, E., and Te Pas, A. B. (2017). Caffeine to improve breathing effort of preterm infants at birth: a randomized controlled trial. *Pediatr. Res.* 82 (2), 290–296. doi:10.1038/pr.2017.45

Denenberg, V. H., Zeidner, L. P., Thoman, E. B., Kramer, P., Rowe, J. C., Philipps, A. F., et al. (1982). Effects of theophylline on behavioral state development in the newborn rabbit. *J. Pharmacol. Exp. Ther.* 221 (3), 604–608.

Deng, K., Liang, J., Mu, Y., Liu, Z., Wang, Y., Li, M., et al. (2021). Preterm births in China between 2012 and 2018: an observational study of more than 9 million women. *Lancet. Glob. Health* 9 (9), e1226–e1241. doi:10.1016/S2214-109X(21)00298-9

Dereymaeker, A., Pillay, K., Vervisch, J., De Vos, M., Van Huffel, S., Jansen, K., et al. (2017). Review of sleep-EEG in preterm and term neonates. *Early Hum. Dev.* 113, 87–103. doi:10.1016/j.earlhumdev.2017.07.003

Dietrich, J., Krauss, A. N., Reidenberg, M., Drayer, D. E., and Auld, P. A. (1978). Alterations in state in apneic pre-term infants receiving theophylline. *Clin. Pharmacol. Ther.* 24 (4), 474–478. doi:10.1002/cpt1978244474

Dimitriou, G., Greenough, A., Kavvadia, V., and Mantagos, S. (1999). Blood pressure rhythms during the perinatal period in very immature, extremely low birthweight neonates. *Early Hum. Dev.* 56 (1), 49–56. doi:10.1016/s0378-3782(99)00034-1

Dix, L. M. L., van Bel, F., Baerts, W., and Lemmers, P. M. A. (2018). Effects of caffeine on the preterm brain: An observational study. *Early Hum. Dev.* 120, 17–20. doi:10.1016/j.earlhumdev.2018.03.008

Dobrek, L. (2021). Chronopharmacology in therapeutic drug monitoring-dependencies between the rhythmics of pharmacokinetic processes and drug concentration in blood. *Pharmaceutics* 13 (11), 1915. doi:10.3390/pharmaceutics13111915

Dobson, N. R., and Hunt, C. E. (2018). Caffeine: an evidence-based success story in VLBW pharmacotherapy. *Pediatr. Res.* 84 (3), 333–340. doi:10.1038/s41390-018-0089-6

Dobson, N. R., Rhein, L. M., Darnall, R. A., Corwin, M. J., Heeren, T. C., Eichenwald, E., et al. (2017). Caffeine decreases intermittent hypoxia in preterm infants nearing term-equivalent age. *J. Perinatol.* 37 (10), 1135–1140. doi:10.1038/jp.2017.82

Dong, D., Yang, D., Lin, L., Wang, S., and Wu, B. (2020). Circadian rhythm in pharmacokinetics and its relevance to chronotherapy. *Biochem. Pharmacol.* 178, 114045. doi:10.1016/j.bcp.2020.114045

Dorn, F., Wirth, L., Gorbey, S., Wege, M., Zemlin, M., Maier, R. F., et al. (2014). Influence of acoustic stimulation on the circadian and ultradian rhythm of premature infants. *Chronobiol. Int.* 31 (9), 1062–1074. doi:10.3109/07420528.2014.948183

Du Pre, B. C., Van Veen, T. A., Young, M. E., Vos, M. A., Doevendans, P. A., and Van Laake, L. W. (2014). Circadian rhythms in cell maturation. *Physiol. (Bethesda)* 29 (1), 72–83. doi:10.1152/physiol.00036.2013

Durankus, F., Aladag Ciftdemir, N., Vatansever Ozbek, U., Duran, R., and Acunas, B. (2020). Comparison of sleep problems between term and preterm born preschool children. *Sleep. Med.* 75, 484–490. doi:10.1016/j.sleep.2020.09.013

Economou, G., Andronikou, S., Challa, A., Cholevas, V., and Lapatsanis, P. D. (1993). Cortisol secretion in stressed babies during the neonatal period. *Horm. Res.* 40 (5-6), 217–221. doi:10.1159/000183798

Edwards, C. R., Benediktsson, R., Lindsay, R. S., and Seckl, J. R. (1996). 11 beta-hydroxysteroid dehydrogenases: key enzymes in determining tissue-specific glucocorticoid effects. *Steroids* 61 (4), 263–269. doi:10.1016/0039-128x(96)00033-5

Eichenwald, E. C., Watterberg, K. L., Aucott, S., Benitz, W. E., Cummings, J. J., Goldsmith, J., et al. (2016). Apnea of prematurity. *Pediatrics* 137 (1), e20153757. doi:10.1542/peds.2015-3757

Eichenwald, E. C. (2020). National and international guidelines for neonatal caffeine use: are they evidenced-based? *Semin. Fetal Neonatal Med.* 25 (6), 101177. doi:10.1016/j.siny.2020.101177

Einspieler, C., Prayer, D., and Marschik, P. B. (2021). Fetal movements: the origin of human behaviour. *Dev. Med. Child. Neurol.* 63 (10), 1142–1148. doi:10.1111/dmcn.14918

Elstad, M., O'Callaghan, E. L., Smith, A. J., Ben-Tal, A., and Ramchandra, R. (2018). Cardiorespiratory interactions in humans and animals: rhythms for life. *Am. J. Physiol. Heart Circ. Physiol.* 315 (1), H6–H17. doi:10.1152/ajpheart.00701.2017

Fairchild, K., Mohr, M., Paget-Brown, A., Tabacaru, C., Lake, D., Delos, J., et al. (2016). Clinical associations of immature breathing in preterm infants: part 1-central apnea. *Pediatr. Res.* 80 (1), 21–27. doi:10.1038/pr.2016.43

Faramarzi, F., Shiran, M., Rafati, M., Farhadi, R., Salehifar, E., and Nakhshab, M. (2021). Prediction of pharmacokinetic values of two various dosages of caffeine in premature neonates with apnea. *Indian J. Pharmacol.* 53 (2), 108–114. doi:10.4103/ijp.IJP_504_19

Foreman, S. W., Thomas, K. A., and Blackburn, S. T. (2008). Individual and gender differences matter in preterm infant state development. *J. Obstet. Gynecol. Neonatal Nurs.* 37 (6), 657–665. doi:10.1111/j.1552-6909.2008.00292.x

Frigato, E., Lunghi, L., Ferretti, M. E., Biondi, C., and Bertolucci, C. (2009). Evidence for circadian rhythms in human trophoblast cell line that persist in hypoxia. *Biochem. Biophys. Res. Commun.* 378 (1), 108–111. doi:10.1016/j.bbrc.2008.11.006

Froy, O., and Miskin, R. (2007). The interrelations among feeding, circadian rhythms and ageing. *Prog. Neurobiol.* 82 (3), 142–150. doi:10.1016/j.pneurobio.2007.03.002

Froy, O. (2007). The relationship between nutrition and circadian rhythms in mammals. *Front. Neuroendocrinol.* 28 (2-3), 61–71. doi:10.1016/j.yfrne.2007.03.001

Gabriel, M., Witolla, C., and Albani, M. (1978). Sleep and aminophylline treatment of apnea in preterm infants. *Eur. J. Pediatr.* 128 (3), 145–149. doi:10.1007/BF00444299

Gaytan, S. P., and Pasaro, R. (2012). Neonatal caffeine treatment up-regulates adenosine receptors in brainstem and hypothalamic cardio-respiratory related nuclei of rat pups. *Exp. Neurol.* 237 (2), 247–259. doi:10.1016/j.expneurol.2012.06.028

Gaytan, S. P., Saadani-Makki, F., Bodineau, L., Frugiere, A., Larnicol, N., and Pasaro, R. (2006). Effect of postnatal exposure to caffeine on the pattern of adenosine A1 receptor distribution in respiration-related nuclei of the rat brainstem. *Auton. Neurosci.* 126-127, 339–346. doi:10.1016/j.autneu.2006.03.009

Gentle, S. J., Travers, C. P., and Carlo, W. A. (2018). Caffeine controversies. *Curr. Opin. Pediatr.* 30 (2), 177–181. doi:10.1097/MOP.0000000000000588

Georgoulas, A., Jones, L., Laudiano-Dray, M. P., Meek, J., Fabrizi, L., and Whitehead, K. (2021). Sleep-wake regulation in preterm and term infants. *Sleep* 44 (1), zsaa148. doi:10.1093/sleep/zsaa148

Gewolb, I. H., and Vice, F. L. (2006). Maturational changes in the rhythms, patterning, and coordination of respiration and swallow during feeding in preterm and term infants. *Dev. Med. Child. Neurol.* 48 (7), 589–594. doi:10.1017/S001216220600123X

Gewolb, I. H., Vice, F. L., Schwietzer-Kenney, E. L., Taciak, V. L., and Bosma, J. F. (2001). Developmental patterns of rhythmic suck and swallow in preterm infants. *Dev. Med. Child. Neurol.* 43 (1), 22–27. doi:10.1017/s0012162201000044

Glattre, E., and Bjerkedal, T. (1983). The 24-hour rhythmicity of birth. A populational study. *Acta Obstet. Gynecol. Scand.* 62 (1), 31–36. doi:10.3109/00016348309155754

Glotzbach, S. F., Edgar, D. M., and Ariagno, R. L. (1995). Biological rhythmicity in preterm infants prior to discharge from neonatal intensive care. *Pediatrics* 95 (2), 231–237. doi:10.1542/peds.95.2.231

Gogou, M., Haidopoulou, K., and Pavlou, E. (2019). Sleep and prematurity: sleep outcomes in preterm children and influencing factors. *World J. Pediatr.* 15 (3), 209–218. doi:10.1007/s12519-019-00240-8

Gonzalez de Mejia, E., and Ramirez-Mares, M. V. (2014). Impact of caffeine and coffee on our health. *Trends Endocrinol. Metab.* 25 (10), 489–492. doi:10.1016/j.tem.2014.07.003

Govan, J. J., Ohlsson, A., Ryan, M. L., Myhr, T., and Fong, K. (1995). Aminophylline and Doppler time-averaged mean velocity in the middle cerebral artery in preterm neonates. *J. Paediatr. Child. Health* 31 (5), 461–464. doi:10.1111/j.1440-1754.1995.tb00858.x

Green, P. J., and Suls, J. (1996). The effects of caffeine on ambulatory blood pressure, heart rate, and mood in coffee drinkers. *J. Behav. Med.* 19 (2), 111–128. doi:10.1007/BF01857602

Guessous, I., Eap, C. B., and Bochud, M. (2014). Blood pressure in relation to coffee and caffeine consumption. *Curr. Hypertens. Rep.* 16 (9), 468. doi:10.1007/s11906-014-0468-2

Gunes, T., Koklu, E., Ozturk, M. A., Koklu, S., and Cetin, N. (2006). Evaluation of serum cortisol levels in a relatively large and mature group of ventilated and nonventilated preterm infants with respiratory distress syndrome. *Am. J. Perinatol.* 23 (6), 335–339. doi:10.1055/s-2006-948222

Guo, H. L., Long, J. Y., Hu, Y. H., Liu, Y., He, X., Li, L., et al. (2022). Caffeine therapy for apnea of prematurity: Role of the circadian CLOCK gene polymorphism. *Front. Pharmacol.* 12, 724145. doi:10.3389/fphar.2021.724145

Guyer, C., Huber, R., Fontijn, J., Bucher, H. U., Nicolai, H., Werner, H., et al. (2012). Cycled light exposure reduces fussing and crying in very preterm infants. *Pediatrics* 130 (1), e145–e151. doi:10.1542/peds.2011-2671

Guyer, C., Huber, R., Fontijn, J., Bucher, H. U., Nicolai, H., Werner, H., et al. (2015). Very preterm infants show earlier emergence of 24-hour sleep-wake rhythms compared to term infants. *Early Hum. Dev.* 91 (1), 37–42. doi:10.1016/j.earlhumdev.2014.11.002

Guyon, A., Ravet, F., Champavert, A., Thieux, M., Patural, H., Plancoulaine, S., et al. (2022). Maturation of arousals during day and night in preterm infants. *Children* 9 (2), 223. doi:10.3390/children9020223

Hao, H., and Rivkees, S. A. (1999). The biological clock of very premature primate infants is responsive to light. *Proc. Natl. Acad. Sci. U. S. A.* 96 (5), 2426–2429. doi:10.1073/pnas.96.5.2426

Hardy, D. B., Janowski, B. A., Corey, D. R., and Mendelson, C. R. (2006). Progesterone receptor plays a major antiinflammatory role in human myometrial cells by antagonism of nuclear factor-kappaB activation of cyclooxygenase 2 expression. *Mol. Endocrinol.* 20 (11), 2724–2733. doi:10.1210/me.2006-0112

Harvey, J. R. M., Plante, A. E., and Meredith, A. L. (2020). Ion channels controlling circadian rhythms in suprachiasmatic nucleus excitability. *Physiol. Rev.* 100 (4), 1415–1454. doi:10.1152/physrev.00027.2019

Hasenstab, K. A., Nawaz, S., Lang, I. M., Shaker, R., and Jadcherla, S. R. (2019). Pharyngoesophageal and cardiorespiratory interactions: potential implications for premature infants at risk of clinically significant cardiorespiratory events. *Am. J. Physiol. Gastrointest. Liver Physiol.* 316 (2), G304–G12. doi:10.1152/ajpgi.00303.2018

Hasenstab-Kenney, K. A., Bellodas Sanchez, J., Prabhakar, V., Lang, I. M., Shaker, R., and Jadcherla, S. R. (2020). Mechanisms of bradycardia in premature infants: Aerodigestive-cardiac regulatory-rhythm interactions. *Physiol. Rep.* 8 (13), e14495. doi:10.14814/phy2.14495

Hassanein, S. M., Gad, G. I., Ismail, R. I., and Diab, M. (2015). Effect of caffeine on preterm infants' cerebral cortical activity: an observational study. *J. Matern. Fetal. Neonatal Med.* 28 (17), 2090–2095. doi:10.3109/14767058.2014.978757

Hayes, M. J., Akilesh, M. R., Fukumizu, M., Gilles, A. A., Sallinen, B. A., Troese, M., et al. (2007). Apneic preterms and methylxanthines: arousal deficits, sleep fragmentation and suppressed spontaneous movements. *J. Perinatol.* 27 (12), 782–789. doi:10.1038/sj.jp.7211820

Hayes, M. J., Plante, L., Kumar, S. P., and Delivoria-Papadopoulos, M. (1993). Spontaneous motility in premature infants: features of behavioral activity and rhythmic organization. *Dev. Psychobiol.* 26 (5), 279–291. doi:10.1002/dev.420260505

Hazelhoff, E. M., Dudink, J., Meijer, J. H., and Kervezee, L. (2021). Beginning to see the light: Lessons learned from the development of the circadian system for optimizing light conditions in the neonatal intensive care unit. *Front. Neurosci.* 15, 634034. doi:10.3389/fnins.2021.634034

He, X., Qiu, J. C., Lu, K. Y., Guo, H. L., Li, L., Jia, W. W., et al. (2021). Therapy for apnoea of prematurity: a retrospective study on effects of standard dose and genetic

variability on clinical response to caffeine citrate in Chinese preterm infants. *Adv. Ther.* 38 (1), 607–626. doi:10.1007/s12325-020-01544-2

Hellmeyer, L., Herz, K., Liedtke, B., Wohlmuth, P., Schmidt, S., and Hackeloeer, B. J. (2012). The underestimation of immaturity in late preterm infants. *Arch. Gynecol. Obstet.* 286 (3), 619–626. doi:10.1007/s00404-012-2366-7

Henderson-Smart, D. J., and De Paoli, A. G. (2010). Methylxanthine treatment for apnoea in preterm infants. *Cochrane Database Syst. Rev.* (12), CD000140. doi:10.1002/14651858.CD000140.pub2

Henderson-Smart, D. J., and Steer, P. A. (2010). Caffeine versus theophylline for apnea in preterm infants. *Cochrane Database Syst. Rev.* (1), CD000273. doi:10.1002/14651858.CD000273

Higashi, Y. (2019). Coffee and endothelial function: A coffee paradox? *Nutrients* 11 (9), 2104. doi:10.3390/nu11092104

Hodgman, J. E., Gonzalez, F., Hoppenbrouwers, T., and Cabal, L. A. (1990). Apnea, transient episodes of bradycardia, and periodic breathing in preterm infants. *Am. J. Dis. Child.* 144 (1), 54–57. doi:10.1001/archpedi.1990.02150250064032

Hoecker, C., Nelle, M., Beedgen, B., Rengelshausen, J., and Linderkamp, O. (2006). Effects of a divided high loading dose of caffeine on circulatory variables in preterm infants. *Arch. Dis. Child. Fetal Neonatal Ed.* 91 (1), F61–F64. doi:10.1136/adc.2005.073866

Hoecker, C., Nelle, M., Poeschl, J., Beedgen, B., and Linderkamp, O. (2002). Caffeine impairs cerebral and intestinal blood flow velocity in preterm infants. *Pediatrics* 109 (5), 784–787. doi:10.1542/peds.109.5.784

Hofman, M. A. (1997). Lifespan changes in the human hypothalamus. *Exp. Gerontol.* 32 (4-5), 559–575. doi:10.1016/s0531-5565(96)00162-3

Holditch-Davis, D., Scher, M., Schwartz, T., and Hudson-Barr, D. (2004). Sleeping and waking state development in preterm infants. *Early Hum. Dev.* 80 (1), 43–64. doi:10.1016/j.earlhumdev.2004.05.006

Hoppenbrouwers, T., Hodgman, J. E., Rybine, D., Fabrikant, G., Corwin, M., Crowell, D., et al. (2005). Sleep architecture in term and preterm infants beyond the neonatal period: the influence of gestational age, steroids, and ventilatory support. *Sleep* 28 (11), 1428–1436. doi:10.1093/sleep/28.11.1428

Hsieh, E. M., Hornik, C. P., Clark, R. H., Laughon, M. M., Benjamin, D. K., Jr., Smith, P. B., et al. (2014). Medication use in the neonatal intensive care unit. *Am. J. Perinatol.* 31 (9), 811–821. doi:10.1055/s-0033-1361933

Hsieh, P. N., Zhang, L., and Jain, M. K. (2018). Coordination of cardiac rhythmic output and circadian metabolic regulation in the heart. *Cell. Mol. Life Sci.* 75 (3), 403–416. doi:10.1007/s00018-017-2606-x

Huang, S., Jiao, X., Lu, D., Pei, X., Qi, D., and Li, Z. (2020). Recent advances in modulators of circadian rhythms: an update and perspective. *J. Enzyme Inhib. Med. Chem.* 35 (1), 1267–1286. doi:10.1080/14756366.2020.1772249

Huang, Y. S., Paiva, T., Hsu, J. F., Kuo, M. C., and Guilleminault, C. (2014). Sleep and breathing in premature infants at 6 months post-natal age. *BMC Pediatr.* 14, 303. doi:10.1186/s12887-014-0303-6

Huang, Z. L., Urade, Y., and Hayaishi, O. (2011). The role of adenosine in the regulation of sleep. *Curr. Top. Med. Chem.* 11 (8), 1047–1057. doi:10.2174/156802611795347654

Huang, Z. L., Zhang, Z., and Qu, W. M. (2014). Roles of adenosine and its receptors in sleep-wake regulation. *Int. Rev. Neurobiol.* 119, 349–371. doi:10.1016/B978-0-12-801022-8.00014-3

Hunt, C. E. (2006). Ontogeny of autonomic regulation in late preterm infants born at 34-37 weeks postmenstrual age. *Semin. Perinatol.* 30 (2), 73–76. doi:10.1053/j.semperi.2006.02.005

Huvanandana, J., Thamrin, C., McEwan, A. L., Hinder, M., and Tracy, M. B. (2019). Cardiovascular impact of intravenous caffeine in preterm infants. *Acta Paediatr.* 108 (3), 423–429. doi:10.1111/apa.14382

Hwang, J., Kim, Y. S., Shin, J. H., and Choi, B. M. (2018). Hemodynamic effects on systemic blood flow and ductal shunting flow after loading dose of intravenous caffeine in preterm infants according to the patency of ductus arteriosus. *J. Korean Med. Sci.* 33 (4), e25. doi:10.3346/jkms.2018.33.e25

Iams, J. D., Newman, R. B., Thom, E. A., Goldenberg, R. L., Mueller-Heubach, E., Moawad, A., et al. (2002). Frequency of uterine contractions and the risk of spontaneous preterm delivery. *N. Engl. J. Med.* 346 (4), 250–255. doi:10.1056/NEJMoa002868

Illnerová, H., Buresová, M., and Presl, J. (1993). Melatonin rhythm in human milk. *J. Clin. Endocrinol. Metab.* 77 (3), 838–841. doi:10.1210/jcem.77.3.8370707

Ingersoll, E. W., and Thoman, E. B. (1999). Sleep/wake states of preterm infants: stability, developmental change, diurnal variation, and relation with caregiving activity. *Child. Dev.* 70 (1), 1–10. doi:10.1111/1467-8624.00001

Italianer, M. F., Naninck, E. F. G., Roelants, J. A., van der Horst, G. T. J., Reiss, I. K. M., Goudoever, J. B. V., et al. (2020). Circadian variation in human milk composition, a systematic review. *Nutrients* 12 (8), 2328. doi:10.3390/nu12082328

Itoh, M. T., Ishizuka, B., Kuribayashi, Y., Amemiya, A., and Sumi, Y. (1999). Melatonin, its precursors, and synthesizing enzyme activities in the human ovary. *Mol. Hum. Reprod.* 5 (5), 402–408. doi:10.1093/molehr/5.5.402

Iuvone, P. M., and Gan, J. (1995). Functional interaction of melatonin receptors and D1 dopamine receptors in cultured chick retinal neurons. *J. Neurosci.* 15 (2), 2179–2185. doi:10.1523/jneurosci.15-03-02179.1995

Ivars, K., Nelson, N., Theodorsson, A., Theodorsson, E., Strom, J. O., and Morelius, E. (2015). Development of salivary cortisol circadian rhythm and reference intervals in full-term infants. *PLoS One* 10 (6), e0129502. doi:10.1371/journal.pone.0129502

Ivars, K., Nelson, N., Theodorsson, A., Theodorsson, E., Strom, J. O., and Morelius, E. (2017). Development of salivary cortisol circadian rhythm in preterm infants. *PLoS One* 12 (8), e0182685. doi:10.1371/journal.pone.0182685

Iwata, O., Okamura, H., Saitsu, H., Saikusa, M., Kanda, H., Eshima, N., et al. (2013). Diurnal cortisol changes in newborn infants suggesting entrainment of peripheral circadian clock *in utero* and at birth. *J. Clin. Endocrinol. Metab.* 98 (1), E25–E32. doi:10.1210/jc.2012-2750

Jagannath, A., Peirson, S. N., and Foster, R. G. (2013). Sleep and circadian rhythm disruption in neuropsychiatric illness. *Curr. Opin. Neurobiol.* 23 (5), 888–894. doi:10.1016/j.conb.2013.03.008

Jagannath, A., Varga, N., Dallmann, R., Rando, G., Gosselin, P., Ebrahimjee, F., et al. (2021). Adenosine integrates light and sleep signalling for the regulation of circadian timing in mice. *Nat. Commun.* 12 (1), 2113. doi:10.1038/s41467-021-22179-z

Jaldo-Alba, F., Muñóz-Hoyos, A., Molina-Carballo, A., Molina-Font, J. A., Acuña-Castroviejo, D., and Munoz-Hoyos, A. (1993). Light deprivation increases plasma levels of melatonin during the first 72 h of life in human infants. *Acta Endocrinol.* 129 (5), 442–445. doi:10.1530/acta.0.1290442

Jett, P. L., Samuels, M. H., McDaniel, P. A., Benda, G. I., Lafranchi, S. H., Reynolds, J. W., et al. (1997). Variability of plasma cortisol levels in extremely low birth weight infants. *J. Clin. Endocrinol. Metab.* 82 (9), 2921–2925. doi:10.1210/jcem.82.9.4206

Jha, P. K., Bouaouda, H., Gourmelen, S., Dumont, S., Fuchs, F., Goumon, Y., et al. (2017). Sleep deprivation and caffeine treatment potentiate photic resetting of the master circadian clock in a diurnal rodent. *J. Neurosci.* 37 (16), 4343–4358. doi:10.1523/JNEUROSCI.3241-16.2017

Jha, P. K., Bouaouda, H., Kalsbeek, A., and Challet, E. (2021). Distinct feedback actions of behavioural arousal to the master circadian clock in nocturnal and diurnal mammals. *Neurosci. Biobehav. Rev.* 123, 48–60. doi:10.1016/j.neubiorev.2020.12.011

Joseph, D., Chong, N. W., Shanks, M. E., Rosato, E., Taub, N. A., Petersen, S. A., et al. (2015). Getting rhythm: how do babies do it? *Arch. Dis. Child. Fetal Neonatal Ed.* 100 (1), F50–F54. doi:10.1136/archdischild-2014-306104

Jost, K., Pramana, I., Delgado-Eckert, E., Kumar, N., Datta, A. N., Frey, U., et al. (2017). Dynamics and complexity of body temperature in preterm infants nursed in incubators. *PLoS One* 12 (4), e0176670. doi:10.1371/journal.pone.0176670

Junkermann, H., Mangold, H., Vecsei, P., and Runnebaum, B. (1982). Circadian rhythm of serum progesterone levels in human pregnancy and its relation to the rhythm of cortisol. *Acta Endocrinol. (Copenh).* 101 (1), 98–104. doi:10.1530/acta.0.1010098

Kassim, Z., Greenough, A., and Rafferty, G. F. (2009). Effect of caffeine on respiratory muscle strength and lung function in prematurely born, ventilated infants. *Eur. J. Pediatr.* 168 (12), 1491–1495. doi:10.1007/s00431-009-0961-9

Katzer, D., Pauli, L., Mueller, A., Reutter, H., Reinsberg, J., Fimmers, R., et al. (2016). Melatonin concentrations and antioxidative capacity of human breast milk according to gestational age and the time of day. *J. Hum. Lact.* 32 (4), NP105–NP10. doi:10.1177/0890334415625217

Kennaway, D. J., Goble, F. C., and Stamp, G. E. (1996). Factors influencing the development of melatonin rhythmicity in humans. *J. Clin. Endocrinol. Metab.* 81 (4), 1525–1532. doi:10.1210/jcem.81.4.8636362

Kennaway, D. J., Stamp, G. E., and Goble, F. C. (1992). Development of melatonin production in infants and the impact of prematurity. *J. Clin. Endocrinol. Metab.* 75 (2), 367–369. doi:10.1210/jcem.75.2.1639937

Kidd, S., Midgley, P., Nicol, M., Smith, J., and McIntosh, N. (2005). Lack of adult-type salivary cortisol circadian rhythm in hospitalized preterm infants. *Horm. Res.* 64 (1), 20–27. doi:10.1159/000087324

Kinoshita, M., Iwata, S., Okamura, H., Saikusa, M., Hara, N., Urata, C., et al. (2016). Paradoxical diurnal cortisol changes in neonates suggesting preservation of foetal adrenal rhythms. *Sci. Rep.* 6, 35553. doi:10.1038/srep35553

Kinouchi, K., Mikami, Y., Kanai, T., and Itoh, H. (2021). Circadian rhythms in the tissue-specificity from metabolism to immunity: insights from omics studies. *Mol. Asp. Med.* 80, 100984. doi:10.1016/j.mam.2021.100984

Kivelä, A., Kauppila, A., Leppäluoto, J., Vakkuri, O., and Kivela, A. (1990). Melatonin in infants and mothers at delivery and in infants during the first week of life. *Clin. Endocrinol.* 32 (5), 593–598. doi:10.1111/j.1365-2265.1990.tb00902.x

Koch, G., Datta, A. N., Jost, K., Schulzke, S. M., van den Anker, J., and Pfister, M. (2017). Caffeine citrate dosing adjustments to assure stable caffeine concentrations in preterm neonates. *J. Pediatr.* 191, 50–56. doi:10.1016/j.jpeds.2017.08.064

Koch, G., Jost, K., Schulzke, S. M., Koch, R., Pfister, M., and Datta, A. N. (2021). The rhythm of a preterm neonate's life: ultradian oscillations of heart rate, body temperature and sleep cycles. *J. Pharmacokinet. Pharmacodyn.* 48 (3), 401–410. doi:10.1007/s10928-020-09735-8

Koch, G., Schonfeld, N., Jost, K., Atkinson, A., Schulzke, S. M., Pfister, M., et al. (2020). Caffeine preserves quiet sleep in preterm neonates. *Pharmacol. Res. Perspect.* 8 (3), e00596. doi:10.1002/prp2.596

Koenen, S. V., Mulder, E. J., Wijnberger, L. D., and Visser, G. H. (2005). Transient loss of the diurnal rhythms of fetal movements, heart rate, and its variation after maternal betamethasone administration. *Pediatr. Res.* 57 (1), 662–666. doi:10.1203/01.PDR.0000159762.50504.1F

Kohler, M., Pavy, A., and van den Heuvel, C. (2006). The effects of chewing versus caffeine on alertness, cognitive performance and cardiac autonomic activity during sleep deprivation. *J. Sleep. Res.* 15 (4), 358–368. doi:10.1111/j.1365-2869.2006.00547.x

Korte, J., Wulff, K., Oppe, C., and Siegmund, R. (2001). Ultradian and circadian activity-rest rhythms of preterm neonates compared to full-term neonates using actigraphic monitoring. *Chronobiol. Int.* 18 (4), 697–708. doi:10.1081/cbi-100106082

Kováciková, Z., Sládek, M., Laurinová, K., Bendová, Z., Illnerová, H., Sumová, A., et al. (2005). Ontogenesis of photoperiodic entrainment of the molecular core clockwork in the rat suprachiasmatic nucleus. *Brain Res.* 1064 (1-2), 83–89. doi:10.1016/j.brainres.2005.10.022

Kreutzer, K., and Bassler, D. (2014). Caffeine for apnea of prematurity: a neonatal success story. *Neonatology* 105 (4), 332–336. doi:10.1159/000360647

Krzyżaniak, N., Pawłowska, I., and Bajorek, B. (2016). Review of drug utilization patterns in NICUs worldwide. *J. Clin. Pharm. Ther.* 41 (6), 612–620. doi:10.1111/jcpt.12440

Kuhn, P., Zores, C., Langlet, C., Escande, B., Astruc, D., and Dufour, A. (2013). Moderate acoustic changes can disrupt the sleep of very preterm infants in their incubators. *Acta Paediatr.* 102 (10), 949–954. doi:10.1111/apa.12330

Kumar, V. H. S., and Lipshultz, S. E. (2019). Caffeine and clinical outcomes in premature neonates. *Child. (Basel)* 6 (11), 118. doi:10.3390/children6110118

Kurth, S., Riedner, B. A., Dean, D. C., O'Muircheartaigh, J., Huber, R., Jenni, O. G., et al. (2017). Traveling slow oscillations during sleep: A marker of brain connectivity in childhood. *Sleep* 40 (9), zsx121. doi:10.1093/sleep/zsx121

Lan, H. Y., Yang, L., Hsieh, K. H., Yin, T., Chang, Y. C., and Liaw, J. J. (2018). Effects of a supportive care bundle on sleep variables of preterm infants during hospitalization. *Res. Nurs. Health* 41 (3), 281–291. doi:10.1002/nur.21865

Lan, H. Y., Yin, T., Chen, J. L., Chang, Y. C., and Liaw, J. J. (2019). Factors associated with preterm infants' circadian sleep/wake patterns at the hospital. *Clin. Nurs. Res.* 28 (4), 456–472. doi:10.1177/1054773817724960

Landolt, H. P. (2015). CIRCADIAN RHYTHMS. Caffeine, the circadian clock, and sleep. *Science* 349 (6254), 1289. doi:10.1126/science.aad2958

Landolt, H. P., Dijk, D. J., Gaus, S. E., and Borbély, A. A. (1995). Caffeine reduces low-frequency delta activity in the human sleep EEG. *Neuropsychopharmacology* 12 (3), 229–238. doi:10.1016/0893-133X(94)00079-F

Landolt, H. P., Werth, E., Borbély, A. A., and Dijk, D. J. (1995). Caffeine intake (200 mg) in the morning affects human sleep and EEG power spectra at night. *Brain Res.* 675 (1-2), 67–74. doi:10.1016/0006-8993(95)00040-w

Lane, A. J., Coombs, R. C., Evans, D. H., and Levin, R. J. (1999). Effect of caffeine on neonatal splanchnic blood flow. *Arch. Dis. Child. Fetal Neonatal Ed.* 80 (2), F128–F129. doi:10.1136/fn.80.2.f128

Lanoix, D., Beghdadi, H., Lafond, J., and Vaillancourt, C. (2008). Human placental trophoblasts synthesize melatonin and express its receptors. *J. Pineal Res.* 45 (1), 50–60. doi:10.1111/j.1600-079X.2008.00555.x

Lee, H. J., Kim, H. S., Kim, S. Y., Sim, G. H., Kim, E. S., Choi, C. W., et al. (2010). Effects of postnatal age and aminophylline on the maturation of amplitude-integrated electroencephalography activity in preterm infants. *Neonatology* 98 (3), 245–253. doi:10.1159/000277936

Liao, J. H., Hu, R. F., Su, L. J., Wang, S., Xu, Q., Qian, X. F., et al. (2018). Nonpharmacological interventions for sleep promotion on preterm infants in neonatal intensive care unit: A systematic review. *Worldviews Evid. Based. Nurs.* 15 (5), 386–393. doi:10.1111/wvn.12315

Lindow, S. W., Jha, R. R., and Thompson, J. W. (2000). 24 hour rhythm to the onset of preterm labour. *BJOG* 107 (9), 1145–1148. doi:10.1111/j.1471-0528.2000.tb11114.x

Loewy, J., Stewart, K., Dassler, A. M., Telsey, A., and Homel, P. (2013). The effects of music therapy on vital signs, feeding, and sleep in premature infants. *Pediatrics* 131 (5), 902–918. doi:10.1542/peds.2012-1367

Logan, R. W., and McClung, C. A. (2019). Rhythms of life: circadian disruption and brain disorders across the lifespan. *Nat. Rev. Neurosci.* 20 (1), 49–65. doi:10.1038/s41583-018-0088-y

Löhr, B., and Siegmund, R. (1999). Ultradian and circadian rhythms of sleep-wake and food-intake behavior during early infancy. *Chronobiol. Int.* 16 (2), 129–148. doi:10.3109/07420529909019081

Long, J. Y., Guo, H. L., He, X., Hu, Y. H., Xia, Y., Cheng, R., et al. (2021). Caffeine for the pharmacological treatment of apnea of prematurity in the NICU: dose selection conundrum, therapeutic drug monitoring and genetic factors. *Front. Pharmacol.* 12, 681842. doi:10.3389/fphar.2021.681842

Longin, E., Gerstner, T., Schaible, T., Lenz, T., and Konig, S. (2006). Maturation of the autonomic nervous system: differences in heart rate variability in premature vs. term infants. *J. Perinat. Med.* 34 (4), 303–308. doi:10.1515/JPM.2006.058

Lovallo, W. R., Whitsett, T. L., al'Absi, M., Sung, B. H., Vincent, A. S., and Wilson, M. F. (2005). Caffeine stimulation of cortisol secretion across the waking hours in relation to caffeine intake levels. *Psychosom. Med.* 67 (5), 734–739. doi:10.1097/01.psy.0000181270.20036.06

Lundstrøm, K. E., Larsen, P. B., Brendstrup, L., Skov, L., and Greisen, G. (1995). Cerebral blood flow and left ventricular output in spontaneously breathing, newborn preterm infants treated with caffeine or aminophylline. *Acta Paediatr.* 84 (1), 6–9. doi:10.1111/j.1651-2227.1995.tb13474.x

Lunshof, S., Boer, K., Wolf, H., van Hoffen, G., Bayram, N., and Mirmiran, M. (1998). Fetal and maternal diurnal rhythms during the third trimester of normal pregnancy: outcomes of computerized analysis of continuous twenty-four-hour fetal heart rate recordings. *Am. J. Obstet. Gynecol.* 178 (2), 247–254. doi:10.1016/s0002-9378(98)80008-2

Magiakou, M. A., Mastorakos, G., Rabin, D., Margioris, A. N., Dubbert, B., Calogero, A. E., et al. (1996). The maternal hypothalamic-pituitary-adrenal axis in the third trimester of human pregnancy. *Clin. Endocrinol.* 44 (4), 419–428. doi:10.1046/j.1365-2265.1996.683505.x

Mann, N. P., Haddow, R., Stokes, L., Goodley, S., and Rutter, N. (1986). Effect of night and day on preterm infants in a newborn nursery: randomised trial. *Br. Med. J.* 293 (6557), 1265–1267. doi:10.1136/bmj.293.6557.1265

Mantagos, S., Moustogiannis, A., Makri, M., Vagenakis, A., and VagenAkis, A. (1996). The effect of light on plasma melatonin levels in premature infants. *J. Pediatr. Endocrinol. Metab.* 9 (3), 387–392. doi:10.1515/jpem.1996.9.3.387

Mantagos, S., Moustogiannis, A., and Vagenakis, A. G. (1998). Diurnal variation of plasma cortisol levels in infancy. *J. Pediatr. Endocrinol. Metab.* 11 (4), 549–553. doi:10.1515/jpem.1998.11.4.549

Marcus, C. L., Meltzer, L. J., Roberts, R. S., Traylor, J., Dix, J., D'Ilario, J., et al. (2014). Long-term effects of caffeine therapy for apnea of prematurity on sleep at school age. *Am. J. Respir. Crit. Care Med.* 190 (7), 791–799. doi:10.1164/rccm.201406-1092OC

Mark, P. J., Crew, R. C., Wharfe, M. D., and Waddell, B. J. (2017). Rhythmic three-Part Harmony: The complex interaction of maternal, placental and fetal circadian systems. *J. Biol. Rhythms* 32 (6), 534–549. doi:10.1177/0748730417728671

Matthews, L. G., Walsh, B. H., Knutsen, C., Neil, J. J., Smyser, C. D., Rogers, C. E., et al. (2018). Brain growth in the NICU: critical periods of tissue-specific expansion. *Pediatr. Res.* 83 (5), 976–981. doi:10.1038/pr.2018.4

Matthews, S. G., Owen, D., Kalabis, G., Banjanin, S., Setiawan, E. B., Dunn, E. A., et al. (2004). Fetal glucocorticoid exposure and hypothalamo-pituitary-adrenal (HPA) function after birth. *Endocr. Res.* 30 (4), 827–836. doi:10.1081/erc-200044091

McCarthy, R., Jungheim, E. S., Fay, J. C., Bates, K., Herzog, E. D., and England, S. K. (2019). Riding the rhythm of melatonin through pregnancy to deliver on time. *Front. Endocrinol.* 10, 616. doi:10.3389/fendo.2019.00616

McDonnell, M., Ives, N. K., and Hope, P. L. (1992). Intravenous aminophylline and cerebral blood flow in preterm infants. *Arch. Dis. Child.* 67 (4), 416–418. doi:10.1136/adc.67.4_spec_no.416

McHill, A. W., Smith, B. J., and Wright, K. P., Jr. (2014). Effects of caffeine on skin and core temperatures, alertness, and recovery sleep during circadian misalignment. *J. Biol. Rhythms* 29 (2), 131–143. doi:10.1177/0748730414523078

McMillen, I. C., Kok, J. S., Adamson, T. M., Deayton, J. M., and Nowak, R. (1991). Development of circadian sleep-wake rhythms in preterm and full-term infants. *Pediatr. Res.* 29 (1), 381–384. doi:10.1203/00006450-199104000-00010

McTernan, C. L., Draper, N., Nicholson, H., Chalder, S. M., Driver, P., Hewison, M., et al. (2001). Reduced placental 11beta-hydroxysteroid dehydrogenase type 2 mRNA levels in human pregnancies complicated by intrauterine growth restriction: an analysis of possible mechanisms. *J. Clin. Endocrinol. Metab.* 86 (10), 4979–4983. doi:10.1210/jcem.86.10.7893

Mirmiran, M., and Ariagno, R. L. (2000). Influence of light in the NICU on the development of circadian rhythms in preterm infants. *Semin. Perinatol.* 24 (4), 247–257. doi:10.1053/sper.2000.8593

Mirmiran, M., Baldwin, R. B., and Ariagno, R. L. (2003). Circadian and sleep development in preterm infants occurs independently from the influences of environmental lighting. *Pediatr. Res.* 53 (6), 933–938. doi:10.1203/01.PDR.0000061541.94620.12

Mirmiran, M., and Kok, J. H. (1991). Circadian rhythms in early human development. *Early Hum. Dev.* 26 (2), 121–128. doi:10.1016/0378-3782(91)90016-v

Mirmiran, M., Kok, J. H., de Kleine, M. J., Koppe, J. G., Overdijk, J., and Witting, W. (1990). Circadian rhythms in preterm infants: a preliminary study. *Early Hum. Dev.* 23 (2), 139–146. doi:10.1016/0378-3782(90)90137-8

Mirmiran, M., and Lunshof, S. (1996). Perinatal development of human circadian rhythms. *Prog. Brain Res.* 111, 217–226. doi:10.1016/s0079-6123(08)60410-0

Mirmiran, M., Maas, Y. G., and Ariagno, R. L. (2003). Development of fetal and neonatal sleep and circadian rhythms. *Sleep. Med. Rev.* 7 (4), 321–334. doi:10.1053/smrv.2002.0243

Montandon, G., Horner, R. L., Kinkead, R., and Bairam, A. (2009). Caffeine in the neonatal period induces long-lasting changes in sleep and breathing in adult rats. *J. Physiol.* 587 (22), 5493–5507. doi:10.1113/jphysiol.2009.171918

Montandon, G., Kinkead, R., and Bairam, A. (2008). Adenosinergic modulation of respiratory activity: developmental plasticity induced by perinatal caffeine administration. *Respir. Physiol. Neurobiol.* 164 (1-2), 87–95. doi:10.1016/j.resp.2008.07.013

Morag, I., and Ohlsson, A. (2016). Cycled light in the intensive care unit for preterm and low birth weight infants. *Cochrane Database Syst. Rev.* (8), CD006982. doi:10.1002/14651858.CD006982.pub3

Moschino, L., Zivanovic, S., Hartley, C., Trevisanuto, D., Baraldi, E., and Roehr, C. C. (2020). Caffeine in preterm infants: where are we in 2020? *ERJ Open Res.* 6 (1), 00330-2019. doi:10.1183/23120541.00330-2019

Mulder, E. J., Koenen, S. V., Blom, I., and Visser, G. H. (2004). The effects of antenatal betamethasone administration on fetal heart rate and behaviour depend on gestational age. *Early Hum. Dev.* 76 (1), 65–77. doi:10.1016/j.earlhumdev.2003.10.007

Muñoz-Hoyos, A., Bonillo-Perales, A., Avila-Villegas, R., González-Ripoll, M., Uberos, J., Florido-Navío, J., et al. (2007). Melatonin levels during the first week of life and their relation with the antioxidant response in the perinatal period. *Neonatology* 92 (3), 209–216. doi:10.1159/000102957

Muñoz-Hoyos, A., Jaldo-Alba, F., Molina-Carballo, A., Rodríguez-Cabezas, T., Molina-Font, J. A., Acuña-Castroviejo, D., et al. (1993). Absence of plasma melatonin circadian rhythm during the first 72 hours of life in human infants. *J. Clin. Endocrinol. Metab.* 77 (3), 699–703. doi:10.1210/jcem.77.3.8370692

Muñoz-Hoyos, A., Molina-Carballo, A., Macías, M., Rodríguez-Cabezas, T., Martín-Medina, E., Narbona-López, E., et al. (1998). Comparison between tryptophan methoxyindole and kynurenine metabolic pathways in normal and preterm neonates and in neonates with acute fetal distress. *Eur. J. Endocrinol.* 139 (1), 89–95. doi:10.1530/eje.0.1390089

Muñoz-Hoyos, A., Rodriguez-Cabezas, T., Molina-Carballo, A., Martinez-Sempere, J. J., Ruiz-Cosano, C., Acuña-Castroviejo, D., et al. (1992). Melatonin concentration in the umbilical artery and vein in human preterm and term neonates and neonates with acute fetal distress. *J. Pineal Res.* 13 (4), 184–191. doi:10.1111/j.1600-079x.1992.tb00074.x

Nahmias, Y., and Androulakis, I. P. (2021). Circadian effects of drug responses. *Annu. Rev. Biomed. Eng.* 23, 203–224. doi:10.1146/annurev-bioeng-082120-034725

Nakamura, Y., Tamura, H., Kashida, S., Takayama, H., Yamagata, Y., Karube, A., et al. (2001). Changes of serum melatonin level and its relationship to feto-placental unit during pregnancy. *J. Pineal Res.* 30 (1), 29–33. doi:10.1034/j.1600-079x.2001.300104.x

Narishige, S., Kuwahara, M., Shinozaki, A., Okada, S., Ikeda, Y., Kamagata, M., et al. (2014). Effects of caffeine on circadian phase, amplitude and period evaluated in cells *in vitro* and peripheral organs *in vivo* in PER2::LUCIFERASE mice. *Br. J. Pharmacol.* 171 (24), 5858–5869. doi:10.1111/bph.12890

Nehlig, A., Daval, J. L., and Debry, G. (1992). Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Res. Brain Res. Rev.* 17 (2), 139–170. doi:10.1016/0165-0173(92)90012-b

Nishihara, K., and Horiuchi, S. (1998). Changes in sleep patterns of young women from late pregnancy to postpartum: relationships to their infants' movements. *Percept. Mot. Ski.* 87 (1), 1043–1056. doi:10.2466/pms.1998.87.3.1043

Nishihara, K., Horiuchi, S., Eto, H., and Uchida, S. (2000). Mothers' wakefulness at night in the post-partum period is related to their infants' circadian sleep-wake rhythm. *Psychiatry Clin. Neurosci.* 54 (3), 305–306. doi:10.1046/j.1440-1819.2000.00689.x

Nishihara, K., Horiuchi, S., Eto, H., and Uchida, S. (2002). The development of infants' circadian rest-activity rhythm and mothers' rhythm. *Physiol. Behav.* 77 (1), 91–98. doi:10.1016/s0031-9384(02)00846-6

O'Neill, J. S., Maywood, E. S., Chesham, J. E., Takahashi, J. S., and Hastings, M. H. (2008). cAMP-dependent signaling as a core component of the mammalian circadian pacemaker. *Science* 320 (5878), 949–953. doi:10.1126/science.1152506

Ohdo, S., Koyanagi, S., and Matsunaga, N. (2019). Chronopharmacological strategies focused on chrono-drug discovery. *Pharmacol. Ther.* 202, 72–90. doi:10.1016/j.pharmthera.2019.05.018

Ohta, H., Mitchell, A. C., and McMahon, D. G. (2006). Constant light disrupts the developing mouse biological clock. *Pediatr. Res.* 60 (3), 304–308. doi:10.1203/01.pdr.0000233114.18403.66

Oike, H., Kobori, M., Suzuki, T., and Ishida, N. (2011). Caffeine lengthens circadian rhythms in mice. *Biochem. Biophys. Res. Commun.* 410 (3), 654–658. doi:10.1016/j.bbrc.2011.06.049

Ono, D., Honma, K. I., and Honma, S. (2021). Corrigendum: Roles of neuropeptides, VIP and AVP, in the mammalian central circadian clock. *Front. Neurosci.* 15, 810796. doi:10.3389/fnins.2021.810796

Oster, H., Challet, E., Ott, V., Arvat, E., de Kloet, E. R., Dijk, D. J., et al. (2017). The functional and clinical significance of the 24-hour rhythm of circulating glucocorticoids. *Endocr. Rev.* 38 (1), 3–45. doi:10.1210/er.2015-1080

Pacifici, G. M. (2014). Clinical pharmacology of theophylline in preterm infants: effects, metabolism and pharmacokinetics. *Curr. Pediatr. Rev.* 10 (4), 297–303. doi:10.2174/1573396311666150113213352

Palmu, K., Kirjavainen, T., Stjerna, S., Salokivi, T., and Vanhatalo, S. (2013). Sleep wake cycling in early preterm infants: comparison of polysomnographic recordings with a novel EEG-based index. *Clin. Neurophysiol.* 124 (9), 1807–1814. doi:10.1016/j.clinph.2013.03.010

Panagiotou, M., Meijer, M., Meijer, J. H., and Deboer, T. (2019). Effects of chronic caffeine consumption on sleep and the sleep electroencephalogram in mice. *J. Psychopharmacol.* 33 (1), 122–131. doi:10.1177/0269881118806300

Parikka, V., Beck, J., Zhai, Q., Leppasalo, J., Lehtonen, L., and Soukka, H. (2015). The effect of caffeine citrate on neural breathing pattern in preterm infants. *Early Hum. Dev.* 91 (10), 565–568. doi:10.1016/j.earlhumdev.2015.06.007

Park, J., Silva, S. G., Thoyre, S. M., and Brandon, D. H. (2020). Sleep-wake states and feeding progression in preterm infants. *Nurs. Res.* 69 (1), 22–30. doi:10.1097/NNR.0000000000000395

Patel, M., Mohr, M., Lake, D., Delos, J., Moorman, J. R., Sinkin, R. A., et al. (2016). Clinical associations with immature breathing in preterm infants: part 2-periodic breathing. *Pediatr. Res.* 80 (1), 28–34. doi:10.1038/pr.2016.58

Patrick, J., Challis, J., Campbell, K., Carmichael, L., Natale, R., and Richardson, B. (1980). Circadian rhythms in maternal plasma cortisol and estriol concentrations at 30 to 31, 34 to 35, and 38 to 39 weeks' gestational age. *Am. J. Obstet. Gynecol.* 136 (3), 325–334. doi:10.1016/0002-9378(80)90857-1

Patrick, J., and Challis, J. (1980). Measurement of human fetal breathing movements in healthy pregnancies using a real-time scanner. *Semin. Perinatol.* 4 (4), 275–286.

Patrick, J., Challis, J., Natale, R., and Richardson, B. (1979). Circadian rhythms in maternal plasma cortisol, estrone, estradiol, and estriol at 34 to 35 weeks' gestation. *Am. J. Obstet. Gynecol.* 135 (6), 791–798. doi:10.1016/0002-9378(79)90393-4

Peirano, P., Algarı´n, C., and Uauy, R. (2003). Sleep-wake states and their regulatory mechanisms throughout early human development. *J. Pediatr.* 143 (4), 70–79. doi:10.1067/s0022-3476(03)00404-9

Peleg, D., Munsick, R. A., Diker, D., Goldman, J. A., and Ben-Jonathan, N. (1986). Distribution of catecholamines between fetal and maternal compartments during human pregnancy with emphasis on L-dopa and dopamine. *J. Clin. Endocrinol. Metab.* 62 (5), 911–914. doi:10.1210/jcem-62-5-911

Pelissier, A. L., Gantenbein, M., and Bruguerolle, B. (1999). Caffeine-induced modifications of heart rate, temperature, and motor activity circadian rhythms in rats. *Physiol. Behav.* 67 (1), 81–88. doi:10.1016/s0031-9384(99)00038-4

Pelissier-Alicot, A. L., Schreiber-Deturmeny, E., Simon, N., Gantenbein, M., and Bruguerolle, B. (2002). Time-of-day dependent pharmacodynamic and pharmacokinetic profiles of caffeine in rats. *Naunyn. Schmiedeb. Arch. Pharmacol.* 365 (4), 318–325. doi:10.1007/s00210-001-0527-5

Perez, S., Murias, L., Fernandez-Plaza, C., Diaz, I., Gonzalez, C., Otero, J., et al. (2015). Evidence for clock genes circadian rhythms in human full-term placenta. *Syst. Biol. Reprod. Med.* 61 (6), 360–366. doi:10.3109/19396368.2015.1069420

Peters, G. A., Yi, L., Skomorovska-Prokvolit, Y., Patel, B., Amini, P., Tan, H., et al. (2017). Inflammatory stimuli increase progesterone receptor-A stability and

transrepressive activity in myometrial cells. *Endocrinology* 158 (1), 158–169. doi:10.1210/en.2016-1537

Porkka-Heiskanen, T., and Kalinchuk, A. V. (2011). Adenosine, energy metabolism and sleep homeostasis. *Sleep. Med. Rev.* 15 (2), 123–135. doi:10.1016/j.smrv.2010.06.005

Price, D. A., Close, G. C., and Fielding, B. A. (1983). Age of appearance of circadian rhythm in salivary cortisol values in infancy. *Arch. Dis. Child.* 58 (6), 454–456. doi:10.1136/adc.58.6.454

Pryds, O., and Schneider, S. (1991). Aminophylline reduces cerebral blood flow in stable, preterm infants without affecting the visual evoked potential. *Eur. J. Pediatr.* 150 (5), 366–369. doi:10.1007/BF01955942

Rao, S., Chun, C., Fan, J., Kofron, J. M., Yang, M. B., Hegde, R. S., et al. (2013). A direct and melanopsin-dependent fetal light response regulates mouse eye development. *Nature* 494 (7436), 243–246. doi:10.1038/nature11823

Reichert, C. F., Deboer, T., and Landolt, H. P. (2022). Adenosine, caffeine, and sleep-wake regulation: state of the science and perspectives. *J. Sleep. Res.* 31 (4), e13597. doi:10.1111/jsr.13597

Reiter, R. J., Tan, D. X., Korkmaz, A., and Rosales-Corral, S. A. (2014). Melatonin and stable circadian rhythms optimize maternal, placental and fetal physiology. *Hum. Reprod. Update* 20 (2), 293–307. doi:10.1093/humupd/dmt054

Reiter, R. J., Tan, D. X., Rosales-Corral, S., and Manchester, L. C. (2013). The universal nature, unequal distribution and antioxidant functions of melatonin and its derivatives. *Mini Rev. Med. Chem.* 13 (3), 373–384. doi:10.2174/1389557511313030006

Reppert, S. M. (1992). Pre-natal development of a hypothalamic biological clock. *Prog. Brain Res.* 93, 119–131. doi:10.1016/s0079-6123(08)64568-9

Reppert, S. M., Weaver, D. R., Rivkees, S. A., and Stopa, E. G. (1988). Putative melatonin receptors in a human biological clock. *Science* 242 (4875), 78–81. doi:10.1126/science.2845576

Rhein, L. M., Dobson, N. R., Darnall, R. A., Corwin, M. J., Heeren, T. C., Poets, C. F., et al. (2014). Effects of caffeine on intermittent hypoxia in infants born prematurely: a randomized clinical trial. *JAMA Pediatr.* 168 (3), 250–257. doi:10.1001/jamapediatrics.2013.4371

Ribas-Latre, A., and Eckel-Mahan, K. (2016). Interdependence of nutrient metabolism and the circadian clock system: importance for metabolic health. *Mol. Metab.* 5 (3), 133–152. doi:10.1016/j.molmet.2015.12.006

Rieth, N., Vibarel-Rebot, N., Buisson, C., Jaffre, C., and Collomp, K. (2016). Caffeine and saliva steroids in young healthy recreationally trained women: impact of regular caffeine intake. *Endocrine* 52 (2), 391–394. doi:10.1007/s12020-015-0780-x

Rivkees, S. A. (2003). Developing circadian rhythmicity in infants. *Pediatrics* 112 (2), 373–381. doi:10.1542/peds.112.2.373

Rivkees, S. A. (1997). Developing circadian rhythmicity. Basic and clinical aspects. *Pediatr. Clin. North Am.* 44 (2), 467–487. doi:10.1016/s0031-3955(05)70486-7

Rivkees, S. A. (2004). Emergence and influences of circadian rhythmicity in infants. *Clin. Perinatol.* 31 (2), 217–228. doi:10.1016/j.clp.2004.04.011

Rivkees, S. A., and Hao, H. (2000). Developing circadian rhythmicity. *Semin. Perinatol.* 24 (4), 232–242. doi:10.1053/sper.2000.8598

Rivkees, S. A., Hofman, P. L., and Fortman, J. (1997). Newborn primate infants are entrained by low intensity lighting. *Proc. Natl. Acad. Sci. U. S. A.* 94 (1), 292–297. doi:10.1073/pnas.94.1.292

Rivkees, S. A., and Lachowicz, J. E. (1997). Functional D1 and D5 dopamine receptors are expressed in the suprachiasmatic, supraoptic, and paraventricular nuclei of primates. *Synapse* 26 (1), 1–10. doi:10.1002/(SICI)1098-2396(199705)26:1<1::AID-SYN1>3.0.CO;2-D

Rivkees, S. A., Mayes, L., Jacobs, H., and Gross, I. (2004). Rest-activity patterns of premature infants are regulated by cycled lighting. *Pediatrics* 113 (4), 833–839. doi:10.1542/peds.113.4.833

Rivkees, S. A. (2001). Mechanisms and clinical significance of circadian rhythms in children. *Curr. Opin. Pediatr.* 13 (4), 352–357. doi:10.1097/00008480-200108000-00012

Rivkees, S. A. (2007). The development of circadian rhythms: From animals to humans. *Sleep. Med. Clin.* 2 (3), 331–341. doi:10.1016/j.jsmc.2007.05.010

Robinson, J., and Fielder, A. R. (1990). Pupillary diameter and reaction to light in preterm neonates. *Arch. Dis. Child.* 65 (1), 35–38. doi:10.1136/adc.65.1_spec_no.35

Rodak, K., Kokot, I., and Kratz, E. M. (2021). Caffeine as a factor influencing the functioning of the human body-friend or foe? *Nutrients* 13 (9), 3088. doi:10.3390/nu13093088

Rodrigues Helmo, F., Etchebehere, R. M., Bernardes, N., Meirelles, M. F., Galvao Petrini, C., Penna Rocha, L., et al. (2018). Melatonin treatment in fetal and neonatal diseases. *Pathol. Res. Pract.* 214 (12), 1940–1951. doi:10.1016/j.prp.2018.10.016

Roenneberg, T., and Merrow, M. (2016). The circadian clock and human health. *Curr. Biol.* 26 (10), R432–R443. doi:10.1016/j.cub.2016.04.011

Ruan, W., Yuan, X., and Eltzschig, H. K. (2021). Circadian rhythm as a therapeutic target. *Nat. Rev. Drug Discov.* 20 (4), 287–307. doi:10.1038/s41573-020-00109-w

Ruby, C. L., Verbanes, N. M., Palmer, K. N., Zisk, C. F., Bunion, D. J., and Marinos, L. N. (2018). Caffeine delays light-entrained activity and potentiates circadian photic phase-resetting in mice. *J. Biol. Rhythms* 33 (5), 523–534. doi:10.1177/0748730418789236

Sahni, R., Schulze, K. F., Stefanski, M., Myers, M. M., and Fifer, W. P. (1995). Methodological issues in coding sleep states in immature infants. *Dev. Psychobiol.* 28 (2), 85–101. doi:10.1002/dev.420280203

Saigal, S., and Doyle, L. W. (2008). An overview of mortality and sequelae of preterm birth from infancy to adulthood. *Lancet* 371 (9608), 261–269. doi:10.1016/S0140-6736(08)60136-1

Saliba, E., Autret, E., Gold, F., Pourcelot, L., and Laugier, J. (1989). Caffeine and cerebral blood flow velocity in preterm infants. *Dev. Pharmacol. Ther.* 13 (2-4), 134–138. doi:10.1159/000457595

Salzarulo, P., and Fagioli, I. (1992). Post-natal development of sleep organization in man: speculations on the emergence of the 'S process. *Neurophysiol. Clin.* 22 (2), 107–115. doi:10.1016/s0987-7053(05)80748-8

Sanchez-Solis, M., Garcia-Marcos, P. W., Aguera-Arenas, J., Mondejar-Lopez, P., and Garcia-Marcos, L. (2020). Impact of early caffeine therapy in preterm newborns on infant lung function. *Pediatr. Pulmonol.* 55 (1), 102–107. doi:10.1002/ppul.24540

Saroha, V., and Patel, R. M. (2020). Caffeine for preterm infants: Fixed standard dose, adjustments for age or high dose? *Semin. Fetal Neonatal Med.* 25 (6), 101178. doi:10.1016/j.siny.2020.101178

Scher, M. S., Johnson, M. W., and Holditch-Davis, D. (2005). Cyclicity of neonatal sleep behaviors at 25 to 30 weeks' postconceptional age. *Pediatr. Res.* 57 (6), 879–882. doi:10.1203/01.PDR.0000157678.84132.A8

Schmidt, B., Roberts, R. S., Davis, P., Doyle, L. W., Barrington, K. J., Ohlsson, A., et al. (2006). Caffeine therapy for apnea of prematurity. *N. Engl. J. Med.* 354 (20), 2112–2121. doi:10.1056/NEJMoa054065

Schmidt, B., Roberts, R. S., Davis, P., Doyle, L. W., Barrington, K. J., Ohlsson, A., et al. (2007). Long-term effects of caffeine therapy for apnea of prematurity. *N. Engl. J. Med.* 357 (19), 1893–1902. doi:10.1056/NEJMoa073679

Schwichtenberg, A. J., Christ, S., Abel, E., and Poehlmann-Tynan, J. A. (2016). Circadian sleep patterns in toddlers born preterm: Longitudinal associations with developmental and health concerns. *J. Dev. Behav. Pediatr.* 37 (5), 358–369. doi:10.1097/DBP.0000000000000287

Sekaran, S., Lupi, D., Jones, S. L., Sheely, C. J., Hattar, S., Yau, K. W., et al. (2005). Melanopsin-dependent photoreception provides earliest light detection in the mammalian retina. *Curr. Biol.* 15 (12), 1099–1107. doi:10.1016/j.cub.2005.05.053

Seppä-Moilanen, M., Andersson, S., and Kirjavainen, T. (2021). Caffeine is a respiratory stimulant without effect on sleep in the short-term in late-preterm infants. *Pediatr. Res.* 92, 776–782. doi:10.1038/s41390-021-01794-y

Seppä-Moilanen, M., Andersson, S., Rantakari, K., Mikkola, K., and Kirjavainen, T. (2019). Caffeine and supplemental oxygen effectively suppress periodic breathing with only minor effects during long episodes of apnoea in preterm infants. *Acta Paediatr.* 108 (3), 443–451. doi:10.1111/apa.14541

Serón-Ferré, M., Ducsay, C. A., and Valenzuela, G. J. (1993). Circadian rhythms during pregnancy. *Endocr. Rev.* 14 (5), 594–609. doi:10.1210/edrv-14-5-594

Seron-Ferre, M., Mendez, N., Abarzua-Catalan, L., Vilches, N., Valenzuela, F. J., Reynolds, H. E., et al. (2012). Circadian rhythms in the fetus. *Mol. Cell. Endocrinol.* 349 (1), 68–75. doi:10.1016/j.mce.2011.07.039

Serón-Ferré, M., Riffo, R., Valenzuela, G. J., and Germain, A. M. (2001). Twenty-four-hour pattern of cortisol in the human fetus at term. *Am. J. Obstet. Gynecol.* 184 (6), 1278–1283. doi:10.1067/mob.2001.113322

Serón-Ferré, M., Torres-Farfán, C., Forcelledo, M. L., and Valenzuela, G. J. (2001). The development of circadian rhythms in the fetus and neonate. *Semin. Perinatol.* 25 (6), 363–370. doi:10.1053/sper.2001.29037

Seron-Ferre, M., Valenzuela, G. J., and Torres-Farfan, C. (2007). Circadian clocks during embryonic and fetal development. *Birth Defects Res. C Embryo Today* 81 (3), 204–214. doi:10.1002/bdrc.20101

Sherman, H., Gutman, R., Chapnik, N., Meylan, J., le Coutre, J., and Froy, O. (2011). Caffeine alters circadian rhythms and expression of disease and metabolic markers. *Int. J. Biochem. Cell Biol.* 43 (5), 829–838. doi:10.1016/j.biocel.2011.02.008

Shimada, M., Takahashi, K., Segawa, M., Higurashi, M., Samejim, M., and Horiuchi, K. (1999). Emerging and entraining patterns of the sleep-wake rhythm in preterm and term infants. *Brain Dev.* 21 (7), 468–473. doi:10.1016/s0387-7604(99)00054-6

Shirwaikar, R. D. (2018). Estimation of caffeine regimens: a machine learning approach for enhanced clinical decision making at a neonatal intensive care unit (NICU). *Crit. Rev. Biomed. Eng.* 46 (2), 93–115. doi:10.1615/CritRevBiomedEng.2018025933

Shivakumar, M., Nayak, K., Lewis, L. E. S., Kamath, A., and Purkayastha, J. (2019). Acute hemodynamic effects of methylxanthine therapy in preterm neonates: Effect of variations in subgroups. *J. Trop. Pediatr.* 65 (3), 264–272. doi:10.1093/tropej/fmy044

Sipola-Leppanen, M., Karvonen, R., Tikanmaki, M., Matinolli, H. M., Martikainen, S., Pesonen, A. K., et al. (2015). Ambulatory blood pressure and its variability in adults born preterm. *Hypertension* 65 (3), 615–621. doi:10.1161/HYPERTENSIONAHA.114.04717

Sisman, J., Campbell, D. E., and Brion, L. P. (2005). Amplitude-integrated EEG in preterm infants: maturation of background pattern and amplitude voltage with postmenstrual age and gestational age. *J. Perinatol.* 25 (6), 391–396. doi:10.1038/sj.jp.7211291

Smith, J. R., McGrath, J., Brotto, M., and Inder, T. (2014). A randomized-controlled trial pilot study examining the neurodevelopmental effects of a 5-week M Technique intervention on very preterm infants. *Adv. Neonatal Care* 14 (3), 187–200. doi:10.1097/ANC.0000000000000093

Smolensky, M. H., Scott, P. H., Harrist, R. B., Hiatt, P. H., Wong, T. K., Baenziger, J. C., et al. (1987). Administration-time-dependency of the pharmacokinetic behavior and therapeutic effect of a once-a-day theophylline in asthmatic children. *Chronobiol. Int.* 4 (3), 435–447. doi:10.3109/07420528709083532

Snel, J., and Lorist, M. M. (2011). Effects of caffeine on sleep and cognition. *Prog. Brain Res.* 190, 105–117. doi:10.1016/B978-0-444-53817-8.00006-2

Soloveychik, V., Bin-Nun, A., Ionchev, A., Sriram, S., and Meadow, W. (2009). Acute hemodynamic effects of caffeine administration in premature infants. *J. Perinatol.* 29 (3), 205–208. doi:10.1038/jp.2008.193

Soubasi, V., Mitsakis, K., Nakas, C. T., Petridou, S., Sarafidis, K., Griva, M., et al. (2009). The influence of extrauterine life on the aEEG maturation in normal preterm infants. *Early Hum. Dev.* 85 (12), 761–765. doi:10.1016/j.earlhumdev.2009.10.004

Sowers, J. R., and Vlachakis, N. (1984). Circadian variation in plasma dopamine levels in man. *J. Endocrinol. Invest.* 7 (4), 341–345. doi:10.1007/BF03351014

Spaeth, A. M., Goel, N., and Dinges, D. F. (2014). Cumulative neurobehavioral and physiological effects of chronic caffeine intake: individual differences and implications for the use of caffeinated energy products. *Nutr. Rev.* 72 (S1), 34–47. doi:10.1111/nure.12151

Spangler, G. (1991). The emergence of adrenocortical circadian function in newborns and infants and its relationship to sleep, feeding and maternal adrenocortical activity. *Early Hum. Dev.* 25 (3), 197–208. doi:10.1016/0378-3782(91)90116-k

Sumova, A., and Cecmanova, V. (2020). Mystery of rhythmic signal emergence within the suprachiasmatic nuclei. *Eur. J. Neurosci.* 51 (1), 300–309. doi:10.1111/ejn.14141

Sumova, A., Sladek, M., Polidarova, L., Novakova, M., and Houdek, P. (2012). Circadian system from conception till adulthood. *Prog. Brain Res.* 199, 83–103. doi:10.1016/B978-0-444-59427-3.00005-8

Sun, K., Yang, K., and Challis, J. R. (1998). Regulation of 11beta-hydroxysteroid dehydrogenase type 2 by progesterone, estrogen, and the cyclic adenosine 5'-monophosphate pathway in cultured human placental and chorionic trophoblasts. *Biol. Reprod.* 58 (6), 1379–1384. doi:10.1095/biolreprod58.6.1379

Supcun, S., Kutz, P., Pielemeier, W., and Roll, C. (2010). Caffeine increases cerebral cortical activity in preterm infants. *J. Pediatr.* 156 (3), 490–491. doi:10.1016/j.jpeds.2009.10.033

Swaab, D. F. (1995). Development of the human hypothalamus. *Neurochem. Res.* 20 (5), 509–519. doi:10.1007/BF01694533

Swaab, D. F., Hofman, M. A., and Honnebier, M. B. (1990). Development of vasopressin neurons in the human suprachiasmatic nucleus in relation to birth. *Brain Res. Dev. Brain Res.* 52 (1-2), 289–293. doi:10.1016/0165-3806(90)90247-v

Swaab, D. F., Zhou, J. N., Ehlhart, T., and Hofman, M. A. (1994). Development of vasoactive intestinal polypeptide neurons in the human suprachiasmatic nucleus in relation to birth and sex. *Brain Res. Dev. Brain Res.* 79 (2), 249–259. doi:10.1016/0165-3806(94)90129-5

Takahashi, J. S. (2004). Finding new clock components: past and future. *J. Biol. Rhythms* 19 (5), 339–347. doi:10.1177/0748730404269151

Takahashi, J. S. (2017). Transcriptional architecture of the mammalian circadian clock. *Nat. Rev. Genet.* 18 (3), 164–179. doi:10.1038/nrg.2016.150

Tenreiro, S., Dowse, H. B., D'Souza, S., Minors, D., Chiswick, M., Simms, D., et al. (1991). The development of ultradian and circadian rhythms in premature babies maintained in constant conditions. *Early Hum. Dev.* 27 (1-2), 33–52. doi:10.1016/0378-3782(91)90026-y

Thoman, E. B., Davis, D. H., Raye, J. R., Philipps, A. F., Rowe, J. C., and Denenberg, V. H. (1985). Theophylline affects sleep-wake state development in premature infants. *Neuropediatrics* 16 (1), 13–18. doi:10.1055/s-2008-1052537

Thomas, K. A. (2001). Biological rhythm development in preterm infants: does health status influence body temperature circadian rhythm? *Res. Nurs. Health* 24 (3), 170–180. doi:10.1002/nur.1020

Thomas, K. A., and Burr, R. (2002). Preterm infant temperature circadian rhythm: possible effect of parental cosleeping. *Biol. Res. Nurs.* 3 (3), 150–159. doi:10.1177/1099800402003003005

Thomas, K. A. (2000). Differential effects of breast- and formula-feeding on preterm infants' sleep-wake patterns. *J. Obstet. Gynecol. Neonatal Nurs.* 29 (2), 145–152. doi:10.1111/j.1552-6909.2000.tb02034.x

Thosar, S. S., and Shea, S. A. (2021). Circadian control of human cardiovascular function. *Curr. Opin. Pharmacol.* 57, 89–97. doi:10.1016/j.coph.2021.01.002

Toorop, A. A., van der Voorn, B., Hollanders, J. J., Dijkstra, L. R., Dolman, K. M., Heijboer, A. C., et al. (2020). Diurnal rhythmicity in breast-milk glucocorticoids, and infant behavior and sleep at age 3 months. *Endocrine* 68 (3), 660–668. doi:10.1007/s12020-020-02273-w

Tourneux, P., Cardot, V., Museux, N., Chardon, K., Léké, A., Telliez, F., et al. (2008). Influence of thermal drive on central sleep apnea in the preterm neonate. *Sleep* 31 (4), 549–556. doi:10.1093/sleep/31.4.549

Uchitel, J., Vanhatalo, S., and Austin, T. (2021). Early development of sleep and brain functional connectivity in term-born and preterm infants. *Pediatr. Res.* 91 (4), 771–786. doi:10.1038/s41390-021-01497-4

Ulanovsky, I., Haleluya, N. S., Blazer, S., and Weissman, A. (2014). The effects of caffeine on heart rate variability in newborns with apnea of prematurity. *J. Perinatol.* 34 (8), 620–623. doi:10.1038/jp.2014.60

Updike, P. A., Accurso, F. J., and Jones, R. H. (1985). Physiologic circadian rhythmicity in preterm infants. *Nurs. Res.* 34 (3), 160–163. doi:10.1097/00006199-198505000-00007

Urry, E., and Landolt, H. P. (2015). Adenosine, caffeine, and performance: from cognitive neuroscience of sleep to sleep pharmacogenetics. *Curr. Top. Behav. Neurosci.* 25, 331–366. doi:10.1007/7854_2014_274

Valenzuela, F. J., Vera, J., Venegas, C., Munoz, S., Oyarce, S., Munoz, K., et al. (2016). Evidences of polymorphism associated with circadian system and risk of pathologies: a review of the literature. *Int. J. Endocrinol.* 2016, 2746909. doi:10.1155/2016/2746909

Valenzuela, F. J., Vera, J., Venegas, C., Pino, F., and Lagunas, C. (2015). Circadian system and melatonin hormone: Risk factors for complications during pregnancy. *Obstet. Gynecol. Int.* 2015, 825802. doi:10.1155/2015/825802

Van Cruchten, S., Vrolyk, V., Perron Lepage, M. F., Baudon, M., Voute, H., Schoofs, S., et al. (2017). Pre- and postnatal development of the eye: A species comparison. *Birth Defects Res.* 109 (19), 1540–1567. doi:10.1002/bdr2.1100

van Dam, R. M., Hu, F. B., and Willett, W. C. (2020). Coffee, caffeine, and health. *N. Engl. J. Med.* 383 (4), 369–378. doi:10.1056/NEJMra1816604

Van den Bergh, B. R. H., van den Heuvel, M. I., Lahti, M., Braeken, M., de Rooij, S. R., Entringer, S., et al. (2020). Prenatal developmental origins of behavior and mental health: The influence of maternal stress in pregnancy. *Neurosci. Biobehav. Rev.* 117, 26–64. doi:10.1016/j.neubiorev.2017.07.003

van der Voorn, B., de Waard, M., van Goudoever, J. B., Rotteveel, J., Heijboer, A. C., and Finken, M. J. (2016). Breast-milk cortisol and cortisone concentrations follow the diurnal rhythm of maternal hypothalamus-pituitary-adrenal Axis Activity. *J. Nutr.* 146 (11), 2174–2179. doi:10.3945/jn.116.236349

van Diepen, H. C., Lucassen, E. A., Yasenkov, R., Groenen, I., Ijzerman, A. P., Meijer, J. H., et al. (2014). Caffeine increases light responsiveness of the mouse circadian pacemaker. *Eur. J. Neurosci.* 40 (10), 3504–3511. doi:10.1111/ejn.12715

Vasquez-Ruiz, S., Maya-Barrios, J. A., Torres-Narvaez, P., Vega-Martinez, B. R., Rojas-Granados, A., Escobar, C., et al. (2014). A light/dark cycle in the NICU accelerates body weight gain and shortens time to discharge in preterm infants. *Early Hum. Dev.* 90 (9), 535–540. doi:10.1016/j.earlhumdev.2014.04.015

Vatish, M., Steer, P. J., Blanks, A. M., Hon, M., and Thornton, S. (2010). Diurnal variation is lost in preterm deliveries before 28 weeks of gestation. *BJOG* 117 (6), 765–767. doi:10.1111/j.1471-0528.2010.02526.x

Verdurmen, K. M., Renckens, J., van Laar, J. O., and Oei, S. G. (2013). The influence of corticosteroids on fetal heart rate variability: a systematic review of the

literature. *Obstet. Gynecol. Surv.* 68 (12), 811–824. doi:10.1097/OGX.0000000000000002

Visscher, M. O., Lacina, L., Casper, T., Dixon, M., Harmeyer, J., Haberman, B., et al. (2015). Conformational positioning improves sleep in premature infants with feeding difficulties. *J. Pediatr.* 166 (1), 44–48. doi:10.1016/j.jpeds.2014.09.012

Vivanco, P., Studholme, K. M., and Morin, L. P. (2013). Drugs that prevent mouse sleep also block light-induced locomotor suppression, circadian rhythm phase shifts and the drop in core temperature. *Neuroscience* 254, 98–109. doi:10.1016/j.neuroscience.2013.09.025

Vogel, J. P., Chawanpaiboon, S., Moller, A. B., Watananirun, K., Bonet, M., and Lumbiganon, P. (2018). The global epidemiology of preterm birth. *Best. Pract. Res. Clin. Obstet. Gynaecol.* 52, 3–12. doi:10.1016/j.bpobgyn.2018.04.003

Vohr, B. (2013). Long-term outcomes of moderately preterm, late preterm, and early term infants. *Clin. Perinatol.* 40 (4), 739–751. doi:10.1016/j.clp.2013.07.006

von Poblotzki, M., Rieger-Fackeldey, E., and Schulze, A. (2003). Effects of theophylline on the pattern of spontaneous breathing in preterm infants less than 1000 g of birth weight. *Early Hum. Dev.* 72 (1), 47–55. doi:10.1016/s0378-3782(03)00010-0

Wachman, E. M., and Lahav, A. (2011). The effects of noise on preterm infants in the NICU. *Arch. Dis. Child. Fetal Neonatal Ed.* 96 (4), F305–F309. doi:10.1136/adc.2009.182014

Waddell, B. J., Wharfe, M. D., Crew, R. C., and Mark, P. J. (2012). A rhythmic placenta? Circadian variation, clock genes and placental function. *Placenta* 33 (7), 533–539. doi:10.1016/j.placenta.2012.03.008

Walani, S. R. (2020). Global burden of preterm birth. *Int. J. Gynaecol. Obstet.* 150 (1), 31–33. doi:10.1002/ijgo.13195

Walther, F. J., Erickson, R., and Sims, M. E. (1990). Cardiovascular effects of caffeine therapy in preterm infants. *Am. J. Dis. Child.* 144 (10), 1164–1166. doi:10.1001/archpedi.1990.02150340110035

Walther, F. J., Sims, M. E., Siassi, B., and Wu, P. Y. (1986). Cardiac output changes secondary to theophylline therapy in preterm infants. *J. Pediatr.* 109 (5), 874–876. doi:10.1016/s0022-3476(86)80717-x

Watanabe, T., Matsuhashi, K., and Takayama, S. (1990). Placental and blood-brain barrier transfer following prenatal and postnatal exposures to neuroactive drugs: relationship with partition coefficient and behavioral teratogenesis. *Toxicol. Appl. Pharmacol.* 105 (1), 66–77. doi:10.1016/0041-008x(90)90359-3

Weibel, J., Lin, Y. S., Landolt, H. P., Berthomier, C., Brandewinder, M., Kistler, J., et al. (2021). Regular caffeine intake delays REM sleep promotion and attenuates sleep quality in healthy men. *J. Biol. Rhythms* 36 (4), 384–394. doi:10.1177/07487304211013995

Weisman, O., Magori-Cohen, R., Louzoun, Y., Eidelman, A. I., and Feldman, R. (2011). Sleep-wake transitions in premature neonates predict early development. *Pediatrics* 128 (4), 706–714. doi:10.1542/peds.2011-0047

Williams, E. E., Hunt, K. A., Jeyakara, J., Subba-Rao, R., Dassios, T., and Greenough, A. (2020). Electrical activity of the diaphragm following a loading dose of caffeine citrate in ventilated preterm infants. *Pediatr. Res.* 87 (4), 740–744. doi:10.1038/s41390-019-0619-x

Williamson, M., Poorun, R., and Hartley, C. (2021). Apnoea of prematurity and neurodevelopmental outcomes: Current understanding and future prospects for research. *Front. Pediatr.* 9, 755677. doi:10.3389/fped.2021.755677

Wong, S. D., Wright, K. P., Jr., Spencer, R. L., Vetter, C., Hicks, L. M., Jenni, O. G., et al. (2022). Development of the circadian system in early life: maternal and environmental factors. *J. Physiol. Anthropol.* 41 (1), 22. doi:10.1186/s40101-022-00294-0

Wright, K. P., Jr., Badia, P., Myers, B. L., Plenzler, S. C., and Hakel, M. (1997). Caffeine and light effects on nighttime melatonin and temperature levels in sleep-deprived humans. *Brain Res.* 747 (1), 78–84. doi:10.1016/s0006-8993(96)01268-1

Wright, K. P., Jr., Myers, B. L., Plenzler, S. C., Drake, C. L., and Badia, P. (2000). Acute effects of bright light and caffeine on nighttime melatonin and temperature levels in women taking and not taking oral contraceptives. *Brain Res.* 873 (2), 310–317. doi:10.1016/s0006-8993(00)02557-9

Xu, T., and Lu, B. (2019). The effects of phytochemicals on circadian rhythm and related diseases. *Crit. Rev. Food Sci. Nutr.* 59 (6), 882–892. doi:10.1080/10408398.2018.1493678

Yang, L., Yu, X., Zhang, Y., Liu, N., Xue, X., and Fu, J. (2021). Encephalopathy in preterm infants: Advances in neuroprotection with caffeine. *Front. Pediatr.* 9, 724161. doi:10.3389/fped.2021.724161

Yue, W., Han, X., Luo, J., Zeng, Z., and Yang, M. (2021). Effect of music therapy on preterm infants in neonatal intensive care unit: Systematic review and meta-analysis of randomized controlled trials. *J. Adv. Nurs.* 77 (2), 635–652. doi:10.1111/jan.14630

Zele, A. J., Feigl, B., Smith, S. S., and Markwell, E. L. (2011). The circadian response of intrinsically photosensitive retinal ganglion cells. *PLoS One* 6 (3), e17860. doi:10.1371/journal.pone.0017860

Zhang, L., and Jain, M. K. (2021). Circadian regulation of cardiac metabolism. *J. Clin. Invest.* 131 (15), e148276. doi:10.1172/JCI148276

Zhu, J. L., Hjollund, N. H., and Olsen, J. (2004). Shift work, duration of pregnancy, and birth weight: the national birth cohort in Denmark. *Am. J. Obstet. Gynecol.* 191 (1), 285–291. doi:10.1016/j.ajog.2003.12.002

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Uterine fibroids — Causes, impact, treatment, and lens to the African perspective

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Leiomyomas, or uterine fibroids as they are commonly known, are mostly seen in women of reproductive age. However, they can go undetected in most women, and approximately 25% of women show clinical symptoms. Although fibroids are a global burden impacting 80% of premenopausal women, they are more prevalent among Black women than among women of other races. Based on clinical diagnosis, the estimated cumulative incidence of fibroids in women ≤50 years old is significantly higher for black (>80%) *versus* white women (~70%). The cause of leiomyomas is not clearly known, but studies have shown evidence of factors that drive the development or exacerbation of the disease. Evidence has linked risk factors such as lifestyle, age, environment, family history of uterine fibroids, and vitamin D deficiencies to an increased risk of uterine fibroids, which impact women of African descent at higher rates. Treatments may be invasive, such as hysterectomy and myomectomy, or non-invasive, such as hormonal or non-hormonal therapies. These treatments are costly and tend to burden women who have the disease. Sub-Saharan Africa is known to have the largest population of black women, yet the majority of uterine fibroid studies do not include populations from the continent. Furthermore, the prevalence of the disease on the continent is not well determined. To effectively treat the disease, its drivers need to be understood, especially with regard to racial preferences. This paper aims to review the existing literature and build a case for conducting future research on African women.

KEYWORDS

fibroid, Africa, causes and treatment, perspective, types and classification, race, lifestyle

Introduction

Uterine fibroids, also known as leiomyomas, are tumors made of smooth muscle and connective tissue from the myometrium or muscular outer layer of the uterus (Nowak, 1993; Fleischer et al., 2008; Suo et al., 2009; Bulun, 2013; Ke et al., 2013). They can be found in premenopausal women and are observed to regress post-menopause (Levy, 2008; Giannubilo et al., 2015; Sarkodie et al., 2016; Ghosh et al., 2018; Giuliani et al., 2020; Ulin et al., 2020; Ali et al., 2021). Uterine fibroids are common in over 70% of women by the onset of menopause and are clinically apparent in 25% of women of reproductive age (Stewart et al., 2017). These tumors are benign neoplasms and are not predicted to lead to cancer (Bulun, 2013; Orellana et al., 2021).

Fibroids can form in various locations around the uterus and can take different forms (Bulun, 2013; Zepiridis et al., 2016). Approximately 20%–50% of women with fibroids show symptoms of heavy menstrual bleeding, which can lead to anemia, bladder dysfunction, and pregnancy complications (Khan et al., 2014; Stewart et al., 2017; Marsh et al., 2018). Notably, most fibroids go undetected by women and may be small and asymptomatic (Laughlin et al., 2011; Zimmerman et al., 2012; Sheng et al., 2020). Although extensive research has been done, it is still inconclusive as to what causes some fibroids to be asymptomatic and others symptomatic (Divakar, 2008; Marsh et al., 2013; Giuliani et al., 2020). It is hypothesized that the size and location of the fibroid may play a role. Fibroids up to the size of a watermelon have been recorded, while some are as small as a tiny stone (Peddada et al., 2008; Fasubaa et al., 2018; Maanongun et al., 2021). Fibroids can be singular tumors or, less commonly, a cluster and are not limited in size (Hodge et al., 2008; Bulun, 2013).

Location

Fibroids can develop within 3 anatomical parts of the uterus and are classified as subserosal, intramural, and submucosal fibroids (Bajekal, 2000; Cook et al., 2010; Zepiridis et al., 2016) (Figures 1–3). Myomas have been found to originate from the plasticity of myometrial cells during tissue development and maintenance; the cells undergo cellular reprogramming and mutations (Longo and Bulun, 2013; Navarro et al., 2021). According to the International Federation of Gynecology and Obstetrics (FIGO), uterine fibroids are categorized into eight different subtypes (Table 1). The FIGO categorization also has a type 8, which includes lesions on extrauterine locations such as the cervix or broad ligament (Gomez et al., 2021). Subtypes are determined by the position of the myoma in relation to the endometrial cavity.

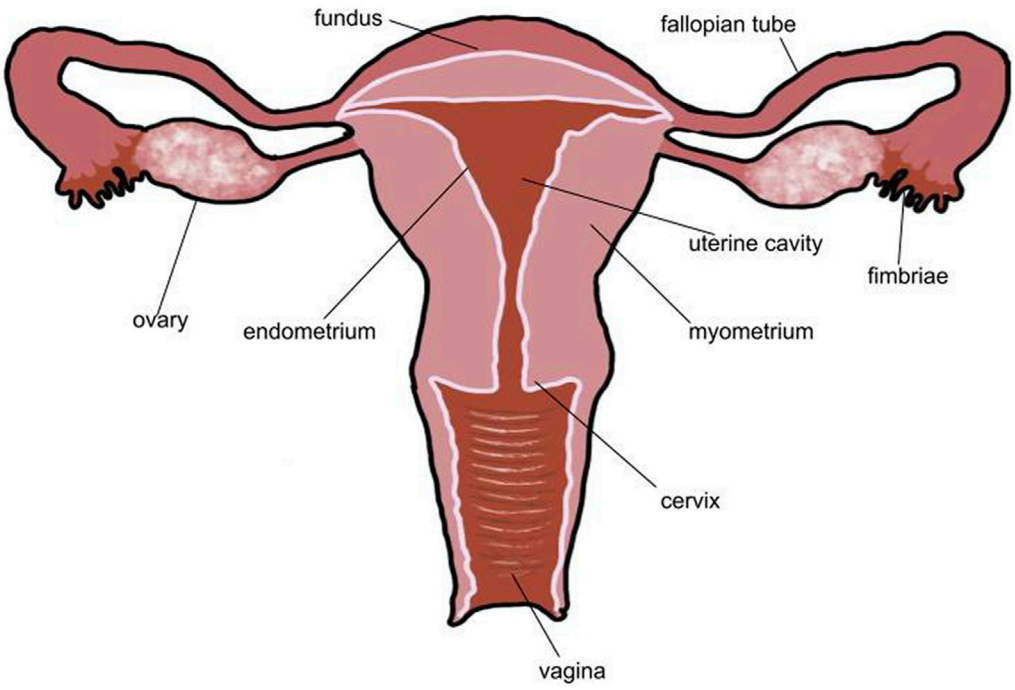

FIGURE 1
Typical healthy uterus. In a healthy uterus, there are no lesions. The endometrium is a thin layer that surrounds the uterine cavity and myometrium. Both fallopian tubes and ovaries are present. The uterine cavity is empty. No part of the uterus is distended or disformed.

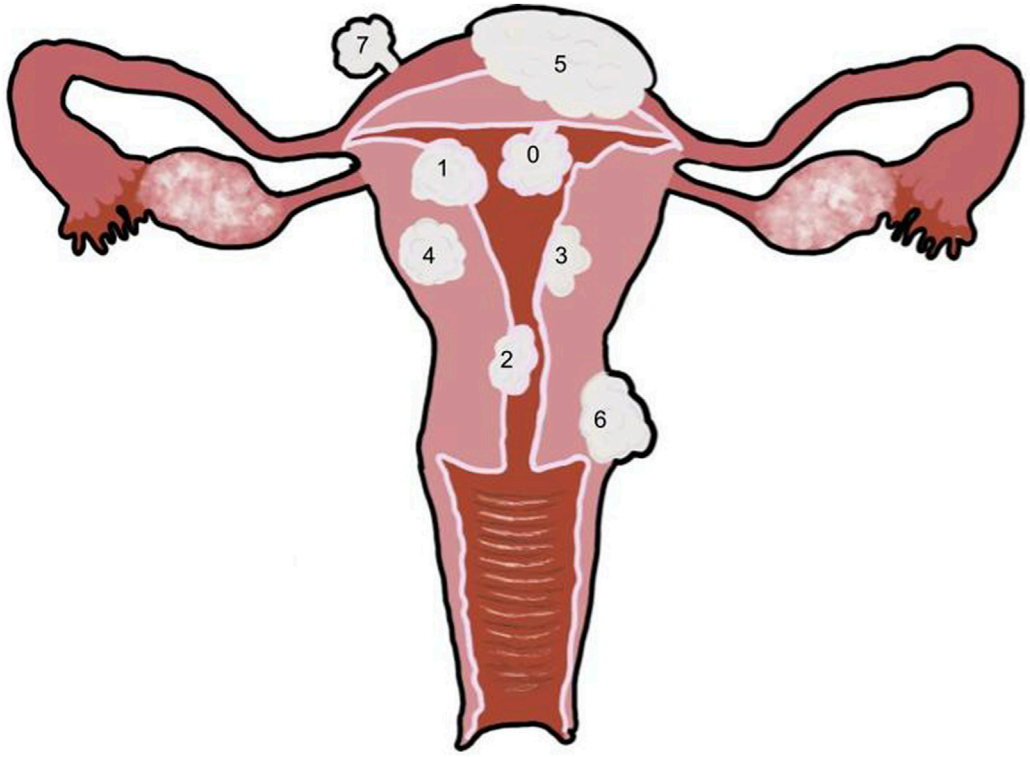

FIGURE 2
Uterus with multiple fibroid types 0: Pedunculated submucosal, 100% of the fibroid is in the uterine cavity. 1: Submucosal, greater than 50% of the fibroid is within the myometrium and the other portion is distorting the endometrium and uterine cavity. 2: Submucosal, less than 50% of the fibroid is within the myometrium and the majority is distorting the endometrium and uterine cavity. 3: Intramural, the fibroid is within the myometrium but touches the endometrium, and it does not distort the uterine cavity. 4: Intramural, the fibroid is completely within the myometrium. 5: Intramural, the fibroid is predominantly within the myometrium with less than 50% extending outside of the myometrium. 6: Subserosal, greater than 50% of the fibroid is located outside of the myometrium. 7: Pedunculated subserosal, 100% of the fibroid is outside of the myometrium. See Table 1 for classification details.

Submucosal

Myomas that cause intramural distortion or reside within the uterine cavity are submucosal fibroids (Puri et al., 2014) (Figures 2, 3). Submucosal fibroids disrupt the endometrial blood supply, which impacts implantation of the embryo (Garcia and Tureck, 1984; Eldar-Geva et al., 1998). In a systematic review completed by Pritts et al., submucosal fibroids were found to lower fertility rates. Submucosal fibroids are also likely to be symptomatic, as they can lead to intermenstrual bleeding and hemorrhage (Divakar, 2008; Wilde and Scott-Barrett, 2009; Bulun, 2013; Puri et al., 2014). Submucosal fibroids can negatively impact the implantation rates of assisted reproductive technology (ART) because the uterine cavity is occupied (Eldar-Geva et al., 1998; Guo and Segars, 2012).

Intramural

Intramural fibroids reside in the myometrium cavity without distorting the endometrial cavity (Wilde & Scott-Barrett, 2009) (Figures 2, 3). Intramural myomas impact the establishment of early pregnancy (Eldar-Geva et al., 1998; Pritts et al., 2009). Intramural fibroids produce significantly lower pregnancy rates, implantation rates, and ongoing pregnancy/live birth rates and even significantly higher rates of spontaneous abortion (Pritts et al., 2009). This effect on implantation is seen even when the fibroid does not reach the uterine cavity (Zepiridis et al., 2016; Farhi et al., 1995; Ramzy et al., 1998; Surrey et al., 2001; Jun et al., 2001). One study found that in women who underwent myomectomy, intramural fibroids were the most common type of fibroid to be removed (Casini et al., 2006).

Subserosal

Subserosal fibroids reside predominantly outside the myometrium (Klatsky et al., 2008) (Figures 2, 3). Subserosal myomas have been found to impact the establishment of early pregnancy (Pritts et al., 2009). However, they have been associated with a very minimal effect on fertility (Zepiridis et al., 2016). Women with subserosal fibroids were found to have no significant differences from those without fibroids (Pritts

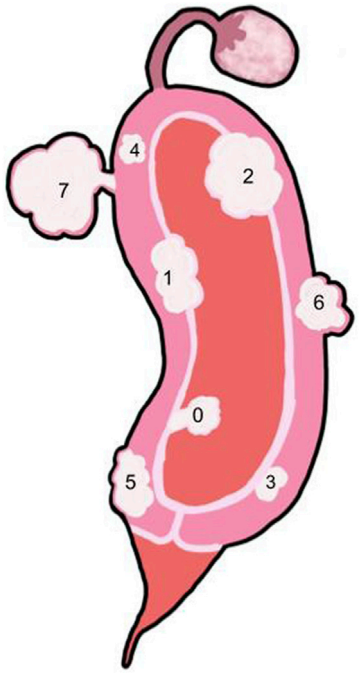

FIGURE 3
Side view of the uterus with multiple fibroid types -Key: See Table 1. 0: Pedunculated submucosal, 100% of the fibroid is in the uterine cavity. 1: Submucosal, greater than 50% of the fibroid is within the myometrium and the other portion is distorting the endometrium and uterine cavity. 2: Submucosal, less than 50% of the fibroid is within the myometrium and the majority is distorting the endometrium and uterine cavity. 3: Intramural, the fibroid is within the myometrium but touches the endometrium and does not distort the uterine cavity. 4: Intramural, the fibroid is completely with the myometrium. 5: Intramural, the fibroid is predominantly within the myometrium with less than 50% extending outside of the myometrium. 6: Subserosal, greater than 50% of the fibroid is located outside of the myometrium. 7: Pedunculated subserosal, 100% of the fibroid is outside of the myometrium. See Table 1 for classification details.

TABLE 1 Classification of uterine fibroids by FIGO.

Classification of uterine fibroids by anatomical positioning		
FIGO	Subtype	Positioning
0	Submucosal - Subtype 0	100% endometrial cavity or intracavity
1	Submucosal - Subtype 1	> 50% intramural
2	Submucosal - Subtype 2	< 50% intramural
3	Intramural	In contact with the endometrium
4	Intramural	100% intramural
5	Intramural	Subserosal >50% intramural
6	Subserosal	Subserosal <50%
7	Subserosal	Pedunculated

et al., 2009). Subserosal fibroids tend to be asymptomatic unless they are large, which can cause substantial pressure or pain (Bulun, 2013; Gomez et al., 2021).

Pedunculated

Fibroids of the final subtype do not reside in a specific location. Pedunculated fibroids can occur both within and outside the uterine cavity (Klatsky et al., 2008), and they are attached to the uterus by a vascular stalk (Gomez et al., 2021) (Figures 2, 3). These fibroids are likely to be asymptomatic unless they are torsioned (Divakar, 2008; Wilde and Scott-Barrett, 2009), but they can also become symptomatic if they grow and begin to push on other masses or detach and become parasitic to the pelvis (Gomez et al., 2021). Parasitic myomas are rare cases where a pedunculated subserosal myoma detaches from the uterus and develops an alternative blood supply from other sources, such as the omental or mesenteric vessels (Cucinella et al., 2011).

Fibroid cell types and architecture

Fibroids have several specific cellular characteristics (Figure 4). A study performed in mice found myometrial proliferation of fusiform smooth muscle cells in the tissue area of the tumor (Romagnolo et al., 1996). The cytoplasm and nuclei of the tumor cells had a normal appearance but displayed high mitotic factors (Romagnolo et al., 1996). There were several fibrous stroma, and within each stroma, spindle cells with high cell proliferation and fibrosis occurred simultaneously. Additionally, it was suggested that fibroids caused narrowing of the lumen in the uterine horn based on their placement (Romagnolo et al., 1996).

Broader slicing of fibroid tissues reveals various patterns in the tissues (Figure 4). These patterns fit into a few main categories. One is the whorled pattern, which is spiraled or twisted; this is the typical pattern expected for fibroids (Khan et al., 2014). In a study that sliced 19 fibroid tissues, approximately 40% of the fibroids examined displayed a whorled pattern (Jayes et al., 2019). Furthermore, approximately 50% displayed a nodular pattern with numerous nodes ranging in size. Another 50% displayed an interweaving trabecular pattern, which is beam-like. Many of the tissues displayed multiple patterns and were classified in both categories (Jayes et al., 2019).

Effects

Uterine fibroids also play a drastic role in various aspects of reproductive health; they cause approximately 5%–10% of

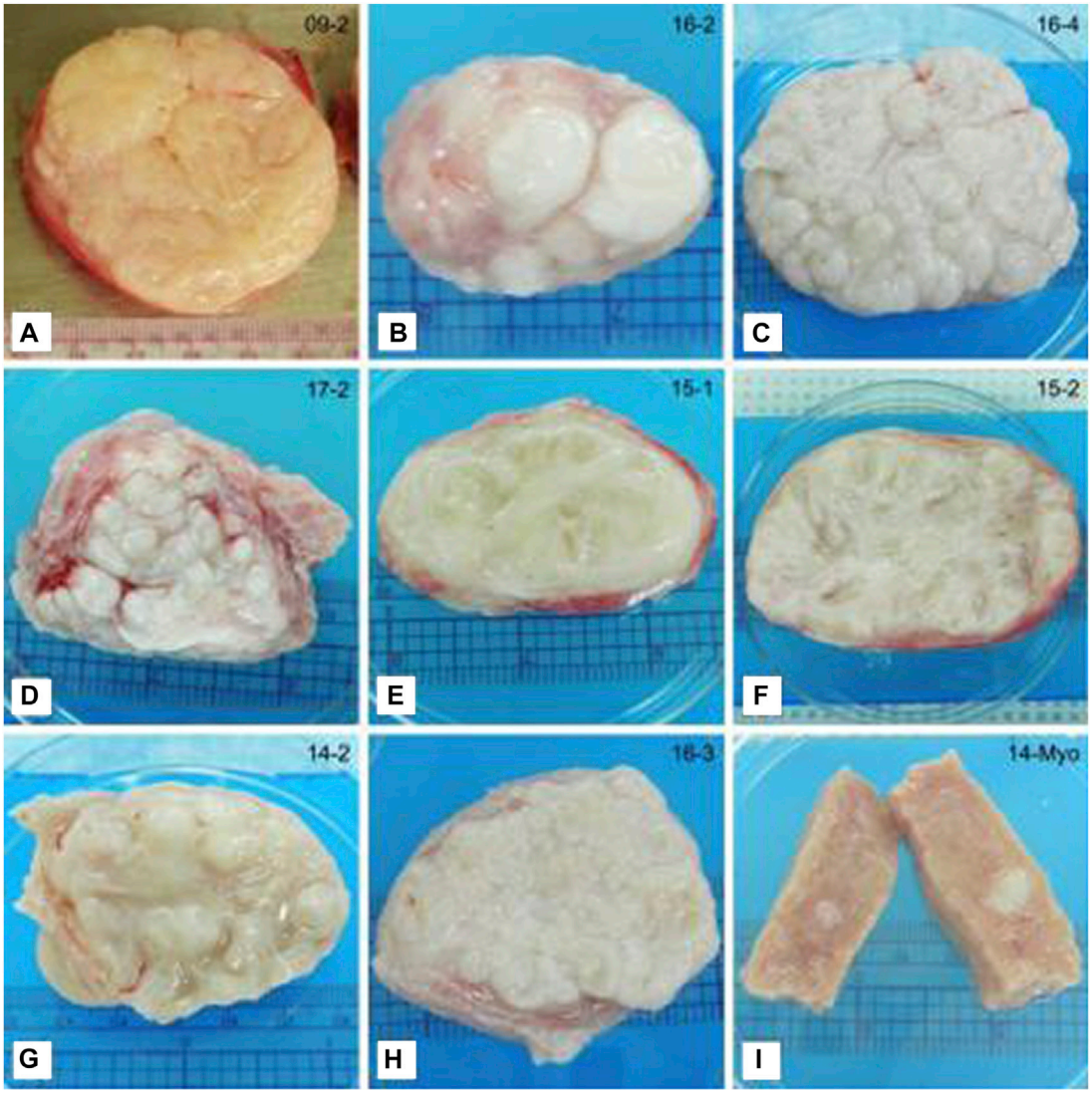

FIGURE 4
Representative photographs of tissue slices showing differences in the gross appearance of fibroids. **(A)** Classical irregular whorled pattern. **(B–D)** Patterns of nodules. **(E,F)** Trabecular structures. **(G)** Characteristics of multiple patterns. This example shows a trabecular/nodular pattern. **(H)** Not categorized. This example shows a tightly gyrated pattern. **(I)** Myometrial tissue shown for comparison. Note the seedling fibroid embedded in the tissue (white). Ruler (cm) shown for size. This figure and description were adapted from Jayes et al., 2019.

infertility cases (Desai & Patel, 2011; Guo and Segars, 2012; Zepiridis et al., 2016). This can be caused by the location of fibroid growth, which can block the fallopian tubes and disable the passage of the gamete. In addition, fibroids can impact the success rate of women using assisted reproductive technologies (ARTs) (Eldar-Geva et al., 1998; Guo and Segars, 2012).

However, due to the increased hormones during pregnancy, many pregnant women tend to experience large growth of their fibroids. Importantly, after giving birth, 70% of *postpartum* women experience shrinkage of their fibroids (Laughlin et al., 2011; Guo and Segars, 2012; Delli Carpini et al., 2019). This shrinkage is hypothesized to be caused by uterine ischemia when the placenta is torn from the uterine wall, causing immense blood loss. To alleviate this blood loss, the uterus experiences clotting, reducing blood flow and also cutting off the blood supply to the myomas, causing them to shrink (Burbank, 2004).

Symptomatic fibroids are associated with two other classes of symptoms, abnormal uterine bleeding and pelvic pressure and pain (Stewart, 2001; Al-Mahrizi ad Tulandi, 2007; Khan et al., 2014; Whitaker and Critchley, 2016; Giuliani et al., 2020). Abnormal bleeding tends to occur during menstruation and is known as menorrhagia or hypomenorrhea. This bleeding pattern is prolonged and excessively heavy (Gupta et al., 2014; Ghosh et al., 2018; Sohn et al., 2018), causing women in some cases to

have to change sanitary products every hour. This symptom is most often seen in submucosal fibroids due to their location, as mentioned previously. Pelvic pressure and pain are caused by enlargement of the uterus. The placement of fibroids can distort the shape of the uterus. Anterior fibroids have been linked to urinary issues and constipation. Rarely, as mentioned above, pedunculated fibroids can cause pain if there is a torsion (Stewart, 2001).

Prevalence

Studies have shown that the prevalence of uterine fibroids is difficult to determine. As a majority of the cases are asymptomatic, methods of prevalence determination can impact the incidence recorded. A study found that using only clinical diagnosis, the prevalence of fibroids is approximately 33%, but when using ultrasound, it rises to approximately 50% of women, and with histological assessment, the incidence rises to approximately 77% of women (Okolo, 2008).

Uterine fibroids have been noted to be present at a high rate, especially in Black women. A study conducted approximately 30 years ago found that fibroids are 3 to 4 times more likely to occur in Black women than white women (Marshall, 1997). This percentage difference has been determined to be statistically significant; it is approximately 3 times more likely for Black women to develop fibroids than white women even when adjusted for age (Baird et al., 2003). Another study that included multiple races supported the significantly higher prevalence for Black women. They found a prevalence rate of 25.5% in Black women, 7.5% in white women, 5.8% in East Asian women, and 5.5% in South Asian women (Chibber et al., 2016). Studies that include Black, white and Asian women are sparse and generally focus on Black and white women. This may be due to the lower prevalence of fibroids in the Asian race indicated in the literature. Recent studies in the United Kingdom have recorded a rate of 70% of white women and approximately 80% of Black women suffering from uterine fibroids (Bulun, 2013; Khan et al., 2014; Florence and Fatehi, 2022). Furthermore, studies have shown that Black women are more likely to have multiple fibroids. In a study performed in 28 hospitals in Maryland, 57% of Black women had seven or more fibroids, whereas 36% of white women had seven or more fibroids (Kjerulff et al., 1996).

In addition to race being an established risk factor, early age at menarche has been associated with an increased risk of uterine fibroids (Dragomir et al., 2010; D'Aloisio et al., 2010; Faerstein, 2001; Wise, 2004; Marshall et al., 1998; Samadi et al., 1996). However, the cumulative incidence of UFs increases as women approach menopause to more than 80% (Cramer, 1990; Baird et al., 2003). This finding could provide further clinical implications for studies.

Causes

The causes of leiomyomas are not well known, and research is still needed to understand their formation. However, some drivers of the disease are discussed below.

Non-hormonal

Genetic modifications

Several studies point to specific genetic mutations that lead to the development of fibroids, specifically the "MED12, HMGA2, COL4A5/COL4A6, FAS or FH genes" (Eggert et al., 2012; Segars et al., 2014). MED12 is one of the more frequently studied genes. MED12, the gene that codes for the mediator subunit 12 protein, is found on chromosome X. Alterations to MED12 have been found in the majority of women with fibroids in whom chromosomal changes have been noticed (Markowski et al., 2012; Bulun, 2013). These alterations can range from clonal chromosomal abnormalities to simple or complex rearrangements or deletions. In addition to MED12, another common alteration that has been found in fibroids with chromosomal changes is HMGA2. HMGA2 codes for the high mobility group AT2 hook proteins. In some cases of fibroid chromosomal rearrangement, the HMGA2 locus has been targeted and upregulated. MED12 and HMGA2 make up approximately 80%–90% of fibroids with chromosomal abnormalities, but these two alterations are mutually exclusive (Markowski et al., 2012; Bulun, 2013).

Additionally, approximately 40% of women with these tumors have chromosomal abnormalities in "trisomy 12, translocation involving chromosomes (t12; 14) (q14–q15; q23–q24), deletions on chromosome 7 (q22q32), 3q and 1p, and rearrangements of 6p21, 10q22 and 13q21–q22" (Hodges et al., 2002) (Table 2). In a study of Japanese women, there was an association between chromosomes 10, 11, and 22 and leiomyomas, and in white women, there was an association with chromosome 17 (Ordulu, 2016). In a UK-based study, the United Kingdom-based biobank that contains Icelandic (Rafnar et al., 2018) and Finnish (Välimäki et al., 2018) data found a variant among loci in chromosomes 16 and 22 in white women. A study on African women and European women also found an association between chromosome six and fibroids (Giri et al., 2017). In Black women, there was a strong link between chromosomes 22 and eight and fibroids (Hellwege et al., 2017). An additional study by Zhang et al., 2015, found an association between chromosome one and fibroids in Black women.

Inflammation

Another factor that has been linked to fibroid development is inflammation. Studies on the association between chronic inflammation and leiomyomas are minimal. One study

TABLE 2 Chromosomal associations by race.

Race	Chromosomes/Genes	Sources
White	17, 6, 16, 22	Ordulu, (2016) Giri et al. (2017) Rafnar et al. (2018) Välimäki et al. (2018)
Asian	10,11,22	Ordulu, (2016)
Black	6, 22, 8, 1	Giri et al. (2017) Hellwege et al. (2017) Zhang et al. (2015)
Population	MED12, HMGA2, COL4A5/COL4A6, FAS or FH genes	Segars et al. (2014) Eggert et al. (2012) Bulun, (2013) Markowski et al. (2012)

conducted by Protic et al. found an abundance of CD68-positive macrophages, which are associated with inflammation, and inflammatory cells in leiomyoma tissues. They found that there were far more CD68 macrophages in leiomyomas and their surrounding tissues than in the distant myometrium. Furthermore, they found an abundance of inflammatory cells in early-stage cellular leiomyomas, thus forming a link between leiomyomas and inflammation (Protic et al., 2016). Fibrotic disorders such as uterine fibroids are associated with altered ECM pathology, which can be a result of excessive wound healing initiated by the inflammatory response (Zannoti et al., 2021). The results of a study by Kabodmehri et al., 2022, which looked at chronic endometriosis and uterine fibroids, displayed different results than hypothesized. Women with fibroids showed a higher rate of chronic endometriosis than those without fibroids, but that difference was not significant. Within the fibroid group, women with submucosal fibroids were more likely to have endometriosis than women with subserosal or intramural fibroids (64% vs. 37%), and this difference was significant (Kabodmehri et al., 2022). The sample size in the Kabodmehri et al. study was small, but it drew attention to the overall role inflammation plays. If inflammation is frequent in women with submucosal fibroids, then it could play a role in the excessive bleeding that these women experience.

Hormonal

Cholesterol-based hormones have been shown to impact tumor growth (Obochi et al., 2009; Chimento et al., 2019). Such hormones include progesterone, estradiol, and vitamin D3 (Table 3). Furthermore, estradiol and progesterone work together to maintain viability for tumor development (Ishikawa et al., 2010; Reis et al., 2016). Progesterone completes the development and proliferation of leiomyomas (Kim et al., 2009; Ishikawa et al., 2010; Reis et al., 2016), and estradiol increases the availability of progesterone receptors on the cells and allows for more sensitivity to progesterone, thus increasing development (Ishikawa et al., 2010; Kim et al., 2013; Reis et al., 2016). These studies do not provide vast racialized data, often only focusing on one or two races.

Estradiol

Cancerous diseases, such as breast cancer, are heavily impacted by hormones such as estradiol and progesterone. Additionally, there are racial differences in hormone levels. Black women had the highest level of estradiol at approximately 166 pg/ml adjusted for BMI, whereas white women had an adjusted level of approximately 142 pg/ml. Asian women were in the middle, with 156 pg/ml (Pinheiro et al., 2005) (Table 3). Several other studies have confirmed this trend. A Haiman et al., 2002, study reported estradiol levels in Black women of 136.1 pg/ml and in white women of 115.9 pg/ml. A study with Asian and white women reported estradiol levels of 547 pg/ml for Asian women and 359 pg/ml for white women, which were again higher for Asian women than for white women (Huddleston et al., 2011). A study with Black and Asian women reported estradiol levels in Black women of 21.4 pg/ml and in Asian women of 16.6 pg/ml (Song et al., 2018). Although these estradiol concentrations were low, they still showed a similar trend as that observed in the Pinheiro study, with Black women having a higher estradiol concentration than Asian women.

Progesterone

Progesterone is vital to the growth of fibroids, as it works to proliferate cells and maintain their rapid growth (Kim et al., 2009; Ishikawa et al., 2010; Reis et al., 2016). Black women had a concentration of 1,321 ng/d adjusted for BMI, white women had an adjusted concentration of 1,289 ng/d, and Asian women had the lowest concentration of 1,205 ng/d (Pinheiro et al., 2005). Although these differences were not statistically significant, there

TABLE 3 BMI and hormonal levels by race.

Hormonal levels by race				
	Black	White	Asian	Sources
Average BMI	32.4 kg/m^2	29.0 kg/m^2	24.7 kg/m^2	Liu et al. (2021)
	33.1 kg/m^2	29.2 kg/m^2	–	Thomas et al. (2013)
	32.2 kg/m^2	–	–	Dodgen and Spence-Almaguer, (2017)
	–	–	25.54 kg/m^2	Zhou et al. (2020)
Estradiol Level	166 pg/ml	142 pg/ml	156 pg/ml	Pinheiro et al. (2005)
	136.1 pg/ml	115.9 pg/ml	–	Haiman et al. (2002)
	225.2 pg/ml	191.5 pg/ml	–	Marsh et al. (2011)
	21.4 pg/ml	–	16.6 pg/ml	Song et al. (2018)
	–	359 pg/ml	547 pg/ml	Huddleston et al. (2011)
	–	–	195.66 pmol/L	Needham et al. (2015)
	–	–	74.1 pmol/L	Ausmanas et al. (2007)
Progesterone Level	1321 ng/d	1289 ng/d	1205 ng/d	Pinheiro et al. (2005)
	15.0 ng/ml	11.0 ng/ml	–	Haiman et al. (2002)
Vitamin D Level (25(OH)D)	20.3 ng/ml	26.7 ng/ml	–	Alzaman et al. (2016)
	18.3 ng/ml	38.0 ng/ml	–	Zhu et al. (2016)
	–	–	19.15 ng/ml	Siddiqee et al. (2021)
	–	–	53.7 nmol/L	Chen et al. (2017)
	–	–	45.1 nmol/L	Wei et al. (2019)

was a difference (Pinheiro et al., 2005). This kind of difference was also seen in the Haiman et al., 2002, study, where Black women again had the highest progesterone levels of 15.0 ng/ml and white women had a level of 11.0 mg/ml (Table 3).

Vitamin D

Another discovered cause of uterine fibroids appears to be a lack of vitamin D. One study found that Black women are severely more likely than white women to be vitamin D deficient, with 42% of Black women being deficient and only 4% of white women being deficient (Kakarala et al., 2007). Using an assay of 25-hydroxyvitamin D (25(OH)D), which is a commonly recognized marker of vitamin D, researchers were able to determine the status of vitamin D in women. The results showed that only 10% of black women and 50% of white women had sufficient vitamin D levels, and women with sufficient vitamin D levels were 32% less likely to have fibroids than women who were deficient (Baird et al., 2013). An optimal level of vitamin D is 25(OH)D at 40–60 ng/ml (Ciebiera et al., 2018).

1,25-Dihydroxyvitamin D3, a biologically active form of vitamin D3, has been shown to decrease tumor proliferation and can induce apoptosis in cancer cells (Halder et al., 2012). Vitamin D is introduced into the body through the skin from UV rays or from 7-dehydrocholesterol or dietary resources in the inactive form. In the liver, it is converted to 25(OH)D and then converted to the active form of 1,25(OH)D in the kidney. It is then carried by vitamin D-binding protein (VDBP) to the skin and different cells (Pike & Christakos, 2017; Ciebiera et al., 2018). VDBP is necessary for maintaining "circulating vitamin D and modulation of the bioavailability, activation, and end-organ responsiveness of the hormone and its metabolites" (Alzaman et al., 2016).

White Americans had a vitamin D level of 26.7 ng/ml in the body, whereas Black Americans had a level of 20.3 ng/ml (Alzaman et al., 2016). Asians may lie in the middle in terms of vitamin D levels, although it is unclear, but they do show a lower level of vitamin D than whites (Siddiqee et al., 2021). However, a population study showed that from 1999 to 2018, white people had a crude cancer rate of 559.1 per 100,000 people, and Black people had a rate of 408.5 per 100,000 people (CDC Wonder). This contradicts the association between VitD levels among the various races, as shown in Table 3.

TABLE 4 Hormonal levels by BMI.

Average BMI				
	$\leq 24\ kg/m^2$	$25-29\ kg/m^2$	$\geq 30\ kg/m^2$	Sources
Estradiol Level	37.7 pg/ml	33.6 pg/ml	30.3 pg/ml	Freeman et al. (2010)
	34.8 pmol/L	43.2 pmol/L	54.9 pmol/L	Key et al. (2003)
	278.9 pg/ml	279.9 pg/ml	258.2 pg/ml	Bellver et al. (2022)
	117.81 pg/ml	149.83 pg/ml	—	Esfahlan et al. (2011)
Progesterone Level	0.19 ng/ml	0.17 ng/ml	0.17 ng/ml	Bellver et al. (2022)
	0.96 ng/ml	1.60 ng/ml	—	Esfahlan et al. (2011)
Vitamin D Level (25(OH)D)	90.4 nmol/L	83.3 nmol/L	77.9 nmol/L	Wei et al. (2019)
	84.9	76.5	73.2 (serum)	Lagunova et al., 2009

Lifestyle

Some studies have associated obesity with uterine fibroids. A literature review performed by Qin et al., 2021, including relevant literature from 1992 to 2020, found a positive correlation between obesity and fibroids. A study on Ghanaian women also showed that there is a greater likelihood of fibroids in obese women (Sarkodie et al., 2016). One study found that obese women accounted for 24% of those undergoing myomectomies and hysterectomies for leiomyomas (Camanni et al., 2010). It is important to note that the correlation has not been completely determined, as some studies have found no association between BMI and fibroids. Possible explanations for obesity increasing the incidence of uterine fibroids include altered sex hormone metabolism, reduced sex hormone binding globulin (SHGB) level, and systematic inflammation (Soave and Marci, 2018).

In terms of altered sex hormone metabolism, it is hypothesized that since adipose tissue is known to affect endocrine tissue, an increase in body fat would increase the amount of estrogen in the body, and estrogen is a driver for uterine fibroids (Soave and Marci, 2018). The reduced SHGB level poses a similar problem, because if there are fewer binding sites, there will be more free estrogen in the bodies of obese women. Finally, systemic inflammation could be a driver, as when fat cells accumulate, there is an increase in inflammatory cytokines in the body that could drive the growth of ECM in fibroids (Soave and Marci, 2018). Various studies show that Black women have the highest average BMI, followed by white women and Asian women, at approximately 32.6, 29.1, and 25.1, respectively (Thomas et al., 2013; Zhou et al., 2020; Liu et al., 2021) (Table 3).

Hormonal levels seemed to fluctuate between the BMI categories, but some overall trends were observed for estradiol and vitamin D (Table 4). In terms of estradiol, some of the findings contradict what is hypothesized about the correlation between estradiol and BMI. In a study by Freeman et al., it was observed that as BMI increases, estradiol level decreases. This refutes the previous hypothesis that estradiol increases as BMI increases. Although a study done by Key et al., 2003, supports the hypothesis of estradiol increasing as BMI increases, they found an estradiol level of 34.8 pmol/L for women with a BMI $\leq 24\ kg/m^2$, 43.2 pmol/L for women with a BMI of $25-29\ kg/m^2$, and 54.9 pmol/L for women with a BMI $\geq 30\ kg/m^2$ (Table 4).

There is also an unclear association between progesterone levels and BMI. One study found a decrease in progesterone levels as BMI increased, from 0.19 ng/ml in women with a BMI $\leq 24\ kg/m^2$ to 0.17 ng/ml in women with a BMI $25-29\ kg/m^2$ and $\geq 30\ kg/m^2$ (Bellver et al., 2022). Another study found that progesterone levels increased as BMI increased, from 0.96 ng/ml in women with a BMI $\leq 24\ kg/m^2$ to 1.60 ng/ml in women with a BMI of $25-29\ kg/m^2$ (Esfahlan et al., 2011).

In terms of vitamin D, there was a trend of vitamin D levels decreasing as BMI increased in a Wei et al., 2019, study that reviewed the United Kingdom population. Individuals with a BMI $\leq 24\ kg/m^2$ had an average level of 90.4 nmol/L, individuals with a BMI of $25-29\ kg/m^2$ had a level of 83.3 nmol/L, and individuals with a BMI $\geq 30\ kg/m^2$ had a level of 77.9 nmol/L.

Treatments

UFs have been associated with fertility complications, and depending on the location of the fibroids, they can contribute to recurrent pregnancy loss (Freytag et al., 2021). Fibroid categorizations are dependent on the location of the fibroid in

TABLE 5 Uterine fibroid treatments.

Treatment type	Procedure type	Description	References/Source
Surgical	Endometrial ablation	Endometrium thickness reduction	Florence and Fatehi, (2022) Giuliani et al. (2020)
	Uterine artery embolization	Blood flow reduction	Florence and Fatehi, (2022) Yerezhepbayeva et al. (2022) Giuliani et al. (2020)
	High-frequency magnetic resonance-guided focused ultrasound surgery	Fibroid size reduction	Yerezhepbayeva et al. (2022) Khan et al. (2014) Keserci et al. (2020) Duc et al. (2018)
	Myomectomy	Fibroid removal	Florence and Fatehi, (2022) De La Cruz & Buchanan, (2017) Giuliani et al. (2020)
	Hysterectomy	Uterus removal	Stewart et al. (2016) Bala et al., 2015 Giuliani et al. (2020)
Hormonal	Mifepristone, Prollex, asoprismil, ulipristal acetate	SPRM	Farris et al. (2019) Donnez et al. (2019) Doherty et al. (2014)
	Combined oral contraceptives	Control menstrual bleeding	Florence and Fatehi, (2022) Kashani et al. (2016) Khan et al. (2014) Giuliani et al. (2020)
	Leuprolide acetate, centrorelix, gamirelix, elagolix, relugolix, linzagolix	GnRH antagonists	Kashani et al. (2016) De La Cruz & Buchanan, (2017) Bulun, (2013) Giuliani et al. (2020) Schlaff et al. (2020) Al-Hendy et al. (2021) Donnez and Donnez (2020)
	Vitamin D	Fibroid size reduction	Baird et al. (2013) Khan et al. (2014)
Non-hormonal	Tranexamic acid	Menstrual bleeding reduction	Kashani et al. (2016) Khan et al. (2014)
	Non-steroidal anti-inflammatory drugs	Menstrual bleeding reduction	Kashani et al. (2016)
	Epigallocatechin gallate	Fibroid size reduction	Grandi et al. (2022) Al-Hendy et al. (2021) Khan et al. (2014)

the uterus, and treatment is determined with consideration of fertility preservation. Currently, the removal of UFs ranges from invasive (hysterectomy, myomectomy) to minimally invasive (uterine artery embolization, high-frequency magnetic resonance-guided focused ultrasound surgery) to non-invasive pharmaceuticals (Table 5). Pharmaceutical therapies are classified by their mechanism: 1) therapies aimed at controlling the symptoms of UFs, such as progestins, oral contraceptives, and antifibrinolytics, and 2) therapies aimed at reducing the size of fibroids, such as gonadotropin-releasing hormone agonists and antagonists. However, these therapies are not curative (Soliman et al., 2015).

Surgical

Treatment for fibroids is often surgical, as it has proven to be the most effective method (Ishikawa et al., 2010; Sheng et al., 2020; Florence and Fatehi, 2022). Surgical options depend on the severity of the case (Table 5). One non-invasive option is endometrial ablation, which removes the thickness of the endometrium but requires the use of permanent contraception post-surgery. Another option is uterine artery embolization, which reduces the blood flow to specific fibroids to alleviate symptoms (Levy, 2008; Khan et al., 2014; Giuliani et al., 2020; Florence and Fatehi, 2022). High-frequency magnetic resonance-guided focused ultrasound surgery is another non-invasive

option that destroys the fibroid with high-frequency ultrasound (Levy, 2008; Khan et al., 2014; De La Cruz and Buchanan, 2017; Yerezhepbayeva et al., 2022).

A more invasive option is a myomectomy, which will remove the fibroids themselves, although many women require multiple myomectomies for recurrent fibroids (Levy, 2008; Florence and Fatehi, 2022). The most invasive treatment is a hysterectomy, in which the uterus is removed (Clayton, 2006; Levy, 2008; Stewart et al., 2016; Faustino et al., 2017). Unfortunately, approximately ⅓ of hysterectomies performed are due to uterine fibroids (Stewart et al., 2016). An analysis of the hysterectomy trends in India found that fibroids were the cause of 40% of the hysterectomies performed, followed by chronic cervicitis at 13.6% and dysfunctional uterine bleeding at 12%. The study found that there was a wide array of diseases that could require a hysterectomy, but fibroids were the most prominent (Bala et al., 2015). A full hysterectomy is not always needed; if the risk of bleeding out is low or the fibroids are smaller in size, doctors may opt for more conservative treatment in hopes of saving the uterus. As time progresses, there have been more advances in less invasive techniques to attempt and treat fibroids, with hopes that non-surgical treatments will be effective.

Hormonal

Progesterone and estrogen modulators along with other hormonal interventions have shown an ability to slow and reduce fibroid growth (Table 5) (Farris et al., 2019). Progesterone modulators include mifepristone, which is a selective progesterone receptor modulator (SPRM) that works to decrease the size of leiomyomas (Farris et al., 2019; Giuliani et al., 2020). Other utilized forms of SPRMs include but are not limited to Proellex and asoprisnil (Farris et al., 2019). Additionally, combined forms of oral contraceptives to regulate both progesterone and estrogen levels in the body have been shown to treat fibroids (Khan et al., 2014; Florence and Fatehi, 2022). Combined oral contraceptive pills are prescribed to women with or without fibroids to control heavy menstrual bleeding. In women with fibroids, oral contraceptives are not expected to shrink the tumor. They work to suppress endometrial proliferation and thus reduce menstrual bleeding (Kashani et al., 2016).

A common treatment to shrink fibroids involves GnRH agonists. These agonists were accepted by the FDA in 1999 in the form of leuprolide acetate for short-term use prior to surgery. GnRH agonists are the synthetic model of the gonadotropin-releasing hormone GnRH. These agonists have been shown to have greater binding affinity and longer half-lives (Kashani et al., 2016). GnRH agonists work to bind to and downregulate the GnRH receptors. This downregulation decreases the production of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and leads to a hypoestrogenic state, which causes the tumors to shrink. GnRH agonists have been shown to decrease fibroid size by 30%–65% (Kashani et al., 2016). The hypoestrogenic state caused by agonists is not sustainable, and a study showed that women using agonists needed some add-back therapy to reverse some of the symptoms of the hypoestrogenic state (Kashani et al., 2016).

GnRH antagonists have also been used to treat symptomatic fibroids (Levy, 2008; Khan et al., 2014; Kashani et al., 2016). These antagonists work similarly to agonists, but antagonists have an amino acid substitution from the original GnRH and competes with it for the binding sites (Kashani et al., 2016). Antagonists have been shown to decrease the volume and symptoms of the fibroid. A study showed that when used for 19 days, there was a 41% decrease in the volume of the fibroids. A downside of both antagonists and agonists is that they can cause many adverse side effects because of the hypoestrogenic state and cannot be used for an extended period of time. In the United Kingdom, the injectable GnRH antagonists cetrorelix and ganirelix are rarely used, as they have only been part of observational studies (Kashani et al., 2016). One study with nearly 400 women from across the world determined relugolix to be a GnRH antagonist that is suitable for everyday use and has been proven to reduce menstrual bleeding (Al-Hendy et al., 2021). Another GnRH antagonist that has been shown to decrease heavy menstrual bleeding is elagolix (Schlaff et al., 2020).

Finally, an emerging therapy to treat uterine fibroids is vitamin D. In Eker rats, the active metabolite in vitamin D has been observed to stop the proliferation and production of fibroid cells and their extracellular matrix, thus reducing their volume (Baird et al., 2013; Khan et al., 2014; Sheng et al., 2020).

Non-hormonal

One non-hormonal treatment is tranexamic acid, which is a lysine derivative that prevents fibrin degradation and stabilizes clot formation (Table 5) (Florence and Fatehi, 2022). Fibrin is necessary to form clots and stop bleeding (Litvinov and Weisel, 2016). Heavy menstrual bleeding is a prevalent symptom for those suffering from uterine fibroids, which affects the coagulation and homeostatic factors of platelets. Thus, tranexamic acid is used to inhibit the activation of plasminogen to plasmin. This inhibition decreases fibrinolysis, clot breakdowns, menstrual flow and blood loss (Khan et al., 2014; Kashani et al., 2016). Tranexamic acid was FDA approved in 2009 and is given to women both with fibroids and without fibroids to treat heavy menstrual bleeding (Kashani et al., 2016).

Another non-hormonal treatment is non-steroidal anti-inflammatory drugs (NSAIDs), which are used to control uterine bleeding. NSAIDs reduce prostaglandin synthesis by inhibiting the cyclooxygenase enzyme. Endometrial prostaglandin receptors are known to promote the growth of new vasculature in tumors, which can lead to abnormal bleeding. Thus, reducing the synthesis of prostaglandin with NSAIDs reduces the amount of menstrual bleeding (Kashani et al., 2016).

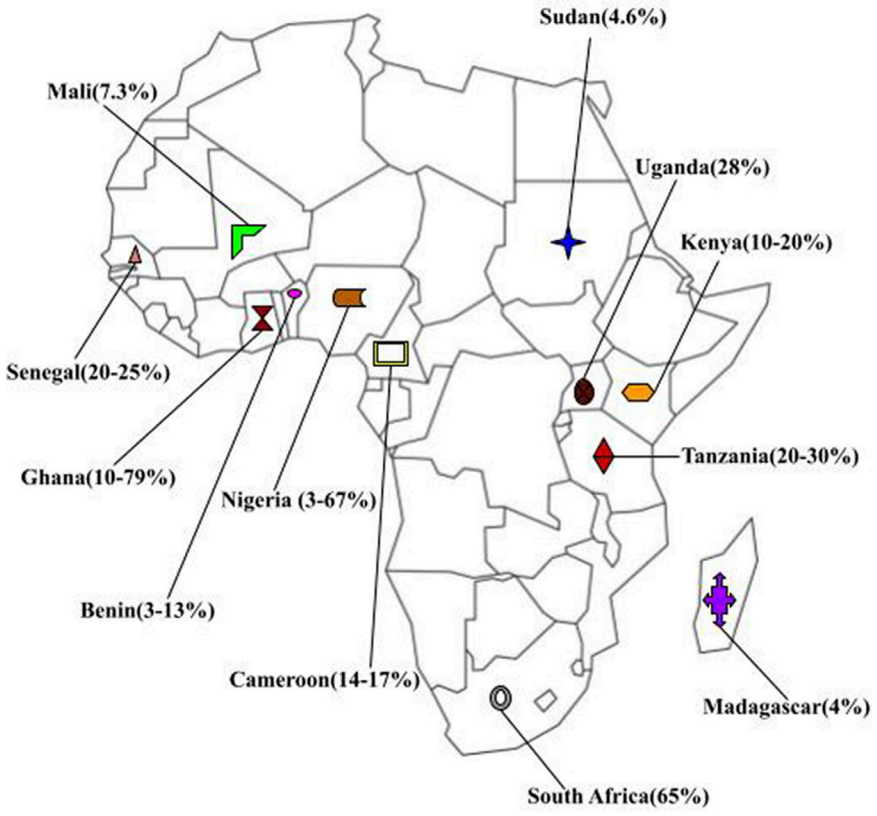

FIGURE 5
Map of Fibroid prevalence across africa.

Additionally, the green tea extract epigallocatechin gallate (EGCG) has been shown to decrease the size of uterine fibroids both *in vivo* and *in vitro* (Khan et al., 2014; Grandi et al., 2022). EGCG has been shown to provide anti-inflammatory, antiproliferative, antioxidant and anticancer effects (Al-Hendy et al., 2021). These effects help shrink fibroids. One study found a 17.8% uterine fibroid size reduction (Grandi et al., 2022); another study found a 32% size reduction after four months of use (Al-Hendy et al., 2021).

African perspective

Prevalence across africa

On the continent of Africa, other challenges arise for women suffering from uterine fibroids. Upon reviewing several countries on the continent, published documentations of prevalence were difficult to obtain (Figure 5). Notably, there are publications about uterine fibroids by authors across the African continent, but many of them do not include prevalence data (Morhason-Bello and Adebamowo, 2022). This may be due to the lack of medical record digitization until recently.

Furthermore, the rates of uterine fibroid prevalence varied greatly among African countries (Figure 5). Even within countries, the occurrence rates differed vastly. The smallest prevalence was found to be 3.1% in a 5-year study conducted in a teaching hospital in Kano, Nigeria (Muhammad et al., 2013). Over 12,000 women were screened in this study, and 386 of them had leiomyomas. Another study performed in Nigeria with 4500 women found an incidence of 67% (Elugwaraonu et al., 2013). The highest occurrence was found in Ghana, in a study at a teaching hospital in Kumasi where histology showed that 79% of the women had uterine fibroids (Titiloye et al., 2018).

The values found in many countries on the African continent do not match those from the global perspective (Figure 5), particularly those accepted as the prevalence values for Black women. The majority of the studies revealed fibroid occurrences of less than 30% (Mutai et al., 2015; Ernest et al., 2016; Bineta et al., 2018; Hortence et al., 2021; Adawe et al., 2022). There may be severe undercoverage, as some of the available studies do not have access to many regions and are usually conducted in one hospital, resulting in a sampling bias.

Diagnosis

Typically, to diagnose a fibroid, a woman must come to a hospital or health facility equipped with an ultrasound system (Sarkodie et al., 2016). The combined use of a physical examination and ultrasound helps physicians identify the presence of fibroids (Sarkodie et al., 2016; Igboeli et al., 2019). The determination can also be made using patient history and laboratory investigation. Further testing to detect specific fibroids, such as hysterosalpingography and hysterosonography, can be performed.

There can be challenges in obtaining a diagnosis, some economic and some behavioral. Sociocultural stigma and perceptions and financial handicaps of UF create a barrier to seeking treatment and management of this chronic disease. In a study employed to understand the delay in treatment and diagnosis of disease, many attributed it to perceiving symptoms as "normal" or stating "life must continue" or "bills need to be paid" (Ghant et al., 2016). Furthermore, this delay could be caused by women not having the time, transportation, or money to see a professional (Igboeli et al., 2019). Avoidance-based coping, altered perception of normalcy, limited knowledge of the disease, and lack of financial means deter women from seeking care (Dominic et al., 2019). Thus, women will wait to seek diagnosis until their situation becomes highly symptomatic. Often, they will use orthodox options as a last resort (Okon et al., 2020).

Treatment

After diagnosis of leiomyomas, treatment is administered. Some women may opt to try traditional practices before an orthodox method (Igboeli et al., 2019; Okon et al., 2020). Orthodox treatments in Africa include "expectant management, surgery, uterine artery embolization, ablative techniques, and medical management" (Akinola et al., 2003; Okon et al., 2020). Of these treatments, surgery is utilized most often. In one study with 656 women seeking gynecological treatment at Korle Bu Teaching Hospital, of those who had fibroids, 79% underwent surgical treatment (Ofori-Dankwa et al., 2019). Furthermore, of the surgical treatments, myomectomy is the most common, with one study reporting that 85% of fibroid treatments were myomectomies and the other 15% were hysterectomies (Okon et al., 2020). Hysterectomy is performed at a lower rate than myomectomy because it eliminates the possibility of further pregnancy. The high usage of surgery as a treatment for uterine fibroids in Africa could be due to numerous reasons. One is that, as discussed previously, women tend to wait to seek a diagnosis, and in those cases, the fibroids tend to be highly symptomatic or large and reduce the possibility of using other treatments. Additionally, as mentioned in the discussion of overall treatments, surgery is the standard of uterine fibroid treatment worldwide, as it has proven to be effective (Florence and Fatehi, 2022).

The non-surgical treatment methods that are being used in other countries, such as progesterone modulators, are too expensive for African women to afford (Igboeli et al., 2019). One study assessed the costs of uterine fibroid treatments in US dollars, and it was found that women who chose surgical options, as opposed to hormonal or non-hormonal options, overall incurred the lowest costs. This was due to fewer missed days of work and less repeated treatment and overall procedure cost (Carls et al., 2008). Additionally, none of these non-surgical treatments offer as permanent an option as myomectomies and hysterectomies (Igboeli et al., 2019). Thus, the women would have to continue ongoing treatment, which could take away time and resources. In terms of treatments such as vitamin D, which can be supplemented, there has not been adequate research to formulate a standard treatment or widespread acceptance of it as a treatment. As research continues, this may become a helpful treatment used worldwide.

Future directions

Several issues exist surrounding our understanding of and determination of appropriate treatment options for fibroids. First, there is a common understanding that Black women are at a higher risk of developing fibroids than women of any other race. It is therefore necessary to assess the prevalence of the disease on the African continent through a systematic review of medical charts. The data generated can support or refute current understandings and provide options for fibroid prevention and treatment. A medical chart review will provide a clearer picture of the prevailing burden and trend of the disease across generations.

This article suggests that nearly half of uterine fibroids are caused by chromosomal abnormalities. Further research into genetic drivers of the disease, such as chromosomal aberrations, and their stratification by race will shed more light on why the burden is higher for Black women than for white or Asian women. Additionally, by understanding the chromosomal abnormalities that occur, there may be emerging technologies to assess fibroid causes and prevention.

As mentioned previously, the present data covering the association between BMI and fibroids vary. Some studies found no association, while others found significant results showing that fibroids increase as BMI increases. These studies need to be continued with large populations and include data from all races. Such findings are critical now, as average BMI continues to increase across races. Lifestyle is a driver that

patients and physicians can actively correct, whether by diet or exercise. A woman with a history of fibroids in her family needs to understand the role that lifestyle can play in the occurrence of the disease. This understanding is especially important for Black women, as they are known to have the highest BMI among other races as well as the highest prevalence of leiomyomas. Incorporating African women into these studies can help determine if the correlation between fibroids and BMI also exists in African women living outside of Western cultures, where their diet and environment are different.

Similarly, a driver that requires further investigation is inflammation. Inflammatory cells are present in the fibroid tissue and extracellular matrix. This may be due to its endogenous origins along the reproductive tract. Effort should be directed at evaluating women with prior inflammatory conditions and assessing whether treating such inflammation can lead to a decrease in fibroid volume or symptoms. Larger population studies will aid in understanding the correlation between fibroids and inflammation and help researchers design better research interventions.

A key research area is understanding the fibroid internal cellular architecture and the various patterns displayed in the tissues. By creating personalized 3D fibroid organelles in the laboratory using tissue samples from women with fibroids, researchers across the African continent can 1) create a fibroid biobank, 2) develop molecular tools to study the drivers of the disease and 3) test both conventional pharmaceutical and herbal drugs. This can help us to study the role of locations in fibroid patterns. Additionally, beyond the patterns identified, these 3D cultures will provide an understanding of the fibroid tissue layers and their ECM. The organelles will continue to produce important biomarkers, which will aid in the establishment of new treatments for the disease and mechanisms to inhibit their proliferation. Furthermore, the establishment of an immortal cell line from African fibroid tissues will be crucial in the sequencing of fibroids and analysis between races. Additionally, by understanding the signaling pathways that fibroid tissue undergoes as it grows in a culture medium, researchers can provide clearer answers to timelines women can expect for their fibroids, especially if specific drivers are more likely to cause fibroids to grow.

Additionally, it has been demonstrated that histological assessments provide the widest scope to accurately identify the prevalence of uterine fibroids. Researchers should aim to develop better diagnostic tools to identify emerging fibroids at an early stage or as the condition changes from asymptomatic to symptomatic.

Non-surgical treatments have proven to be effective in many cases but unfortunately cause severe side effects and often require patients to endure long-term therapy. These treatments tend to be more costly than surgery. Further research should be conducted to help mitigate the side effects of hormonal treatments, provide women with options outside of surgery, and find cost-effective treatment for women. Fibroids have been shown to be highly hormone dependent, which means that women suffering from them could benefit from seeing an endocrinologist. During the annual physical examination, hormone levels should be assessed for high-risk women as a means to identify fibroids, particularly as they evolve from asymptomatic to symptomatic.

The current standard of care, which involves myomectomy and hysterectomy, has evolved with precision medicine. In many advanced countries, myomectomies and hysterectomies can take place laparoscopically, which can lower recovery times and overall costs for women. This is not usually the case in sub-Saharan Africa. As technologies advance, the proper equipment to perform these procedures needs to be expanded worldwide.

Author contributions

NS and AAA conceived, wrote and revised the paper. SN, LP, EK, MA, AN, HT, and AAA wrote, edited and revised the paper.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abdelmtalab, M. A. A., Tahir, O., Hussein, K., and Badawi, K. (2020). Anatomical locations of uterine fibroids in Sudanese women. *Anat. J. Afr.* 9 (1), 1701–1706. doi:10.4314/aja.v9i1.6

Adawe, M., Sezalio, M., Kanyesigye, H., Kajabwangu, R., Okello, S., Bajunirwe, F., et al. (2022). Prevalence, clinical presentation and factors associated with Uterine fibroids among women attending the Gynecology Outpatient Department at a large Referral Hospital in Southwestern Uganda. *East Afr. Sci.* 4 (1), 38–53. doi:10.24248/easci.v4i1.58

Adisso, S., Hounsossou, H., Alle, I. R., Adisso, E. L., Takpara, L., and Alihonou, E. (2014). Quelle issue pour la grossesse jeun dans un uterus myomateux. *JBC* 21, 13

Akinola, O. I., Ottun, T. A., Fabamwo, A. O., and Akinniyi, A. O. (2003). Bilateral uterine artery ligation: An effective low-technology option in the management of symptomatic uterine fibroids. *Trop. J. Obstetrics Gynaecol.* 20 (1), 4–6. doi:10.4314/tjog.v20i1.14389

Al-Hendy, A., Lukes, A. S., Poindexter, A. N., 3rd, Venturella, R., Villarroel, C., Critchley, H. O. D., et al. (2021). Treatment of uterine fibroid symptoms with relugolix combination therapy. *Obstetrical Gynecol. Surv.* 76 (6), 334–336. doi:10.1097/01.ogx.0000753012.23858.46

Al-Mahrizi, S., and Tulandi, T. (2007). Treatment of uterine fibroids for abnormal uterine bleeding: Myomectomy and uterine artery embolization. *Best Pract. Res. Clin. Obstetrics Gynaecol.* 21 (6), 995–1005. doi:10.1016/j.bpobgyn.2007.03.017

Ali, M., Sara, A. R., and Al Hendy, A. (2021). Elagolix in the treatment of heavy menstrual bleeding associated with uterine fibroids in premenopausal women. *Expert Rev. Clin. Pharmacol.* 14 (4), 427–437. doi:10.1080/17512433.2021.1900726

Alzaman, N. S., Dawson-Hughes, B., Nelson, J., D'Alessio, D., and Pittas, A. G. (2016). Vitamin D status of black and white Americans and changes in vitamin D metabolites after varied doses of vitamin D supplementation. *Am. J. Clin. Nutr.* 104 (1), 205–214. doi:10.3945/ajcn.115.129478

Ausmanas, M. K., Tan, D. A., Jaisamrarn, U., Tian, X. W., and Holinka, C. F. (2007). Estradiol, FSH and LH profiles in nine ethnic groups of postmenopausal asian women: The pan-asia menopause (PAM) study. *Climacteric* 10 (5), 427–437. doi:10.1080/13697130701610780

Bala, R., Devi, K. P., and Singh, C. M. (2015). Trend of hysterectomy: A retrospective analysis in Regional Institute of Medical Sciences (RIMS). *Journal of Medical Society*. 29 (1), 4. doi:10.4103/0972-4958.158917

Baird, D. D., Dunson, D. B., Hill, M. C., Cousins, D., and Schectman, J. M. (2003). High cumulative incidence of uterine leiomyoma in black and white women: Ultrasound evidence. *Am. J. obstetrics Gynecol.* 188 (1), 100–107. doi:10.1067/mob.2003.99

Baird, D. D., Hill, M. C., Schectman, J. M., and Hollis, B. W. (2013). Vitamin d and the risk of uterine fibroids. *Epidemiol. Camb. Mass.)* 24 (3), 447–453. doi:10.1097/EDE.0b013e31828acca0

Bajekal, N. (2000). Fibroids, infertility and pregnancy wastage. *Hum. Reprod. Update* 6 (6), 614–620. doi:10.1093/humupd/6.6.614

Bellver, J., Rodríguez-Varela, C., Brandão, P., and Labarta, E. (2022). *Serum progesterone concentrations are reduced in obese women on the day of embryo transfer*. Netherlands, Amsterdam: Elsevier's Obstetrics & Gynecology Journals, S1472.

Bineta, K., Ciss, D., Ka, S., Mbaye, F., Dem, A., and Sembene, M. (2018). Uterine fibroids in Senegal: Polymorphism of MED12 gene and correlation with epidemiological factors. *Am. J. cancer Res. Rev.* 2, 4. doi:10.28933/ajocrr-2017-12-2601

Bulun, S. E. (2013). Uterine fibroids. *N. Engl. J. Med.* 369 (14), 1344–1355. doi:10.1056/NEJMra1209993

Burbank, F. (2004). Childbirth and Myoma treatment by uterine artery occlusion: Do they share a common biology? *J. Am. Assoc. Gynecol. Laparoscopists* 11 (2), 138–152. doi:10.1016/s1074-3804(05)60189-2

Camanni, M., Bonino, L., Delpiano, E. M., Migliaretti, G., Berchialla, P., and Deltetto, F. (2010). Laparoscopy and body mass index: Feasibility and outcome in obese patients treated for gynecologic diseases. *J. Minim. invasive Gynecol.* 17 (5), 576–582. doi:10.1016/j.jmig.2010.04.002

Carls, G. S., Lee, D. W., Ozminkowski, R. J., Wang, S., Gibson, T. B., and Stewart, E. (2008). What are the total costs of surgical treatment for uterine fibroids? *J. women's health* 17 (7), 1119–1132. doi:10.1089/jwh.2008.0456

Casini, M. L., Rossi, F., Agostini, R., and Unfer, V. (2006). Effects of the position of fibroids on fertility. *Gynecol. Endocrinol.* 22 (2), 106–109. doi:10.1080/09513590600604673

Chen, J., Yun, C., He, Y., Piao, J., Yang, L., and Yang, X. (2017). Vitamin D status among the elderly Chinese population: A cross-sectional analysis of the 2010–2013 China national nutrition and health survey (CNNHS). *Nutr. J.* 16 (1), 3–8. doi:10.1186/s12937-016-0224-3

Chibber, S., Mendoza, G., Cohen, L., and Marsh, E. E. (2016). Racial and ethnic differences in uterine fibroid prevalence in a diverse cohort of young asymptomatic women (18-30 yo). *Fertil. Steril.* 106 (3), e97. doi:10.1016/j.fertnstert.2016.07.281

Chimento, A., Casaburi, I., Avena, P., Trotta, F., De Luca, A., Rago, V., et al. (2019). Cholesterol and its metabolites in tumor growth: Therapeutic potential of statins in cancer treatment. *Front. Endocrinol.* 9, 807. doi:10.3389/fendo.2018.00807

Ciebiera, M., Włodarczyk, M., Ciebiera, M., Zaręba, K., Łukaszuk, K., and Jakiel, G. (2018). Vitamin D and uterine fibroids—Review of the literature and novel concepts. *Int. J. Mol. Sci.* 19 (7), 2051. doi:10.3390/ijms19072051

Clayton, R. D. (2006). Hysterectomy. *Best Pract. Res. Clin. Obstetrics Gynaecol.* 20 (1), 73–87. doi:10.1016/j.bpobgyn.2005.09.007

Cook, H., Ezzati, M., Segars, J. H., and McCarthy, D. (2010). The impact of uterine leiomyomas on reproductive outcomes. *Minerva Ginecol.* 62 (3), 225–236.

Cucinella, G., Granese, R., Calagna, G., Somigliana, E., and Perino, A. (2011). Parasitic myomas after laparoscopic surgery: An emerging complication in the use of morcellator? Description of four cases. *Fertil. Steril.* 96 (2), e90–e96. doi:10.1016/j.fertnstert.2011.05.095

D'Aloisio, A. A., Baird, D. D., DeRoo, L. A., and Sandler, D. P. (2010). Association of intrauterine and early-life exposures with diagnosis of uterine leiomyomata by 35 years of age in the Sister Study. *Environ. health Perspect.* 118 (3), 375–381. doi:10.1289/ehp.0901423

De La Cruz, M. S. D., and Buchanan, E. M. (2017). Uterine fibroids: Diagnosis and treatment. *Am. Fam. physician* 95 (2), 100–107.

Delli Carpini, G., Morini, S., Papiccio, M., Serri, M., Damiani, V., Grelloni, C., et al. (2019). The association between childbirth, breastfeeding, and uterine fibroids: An observational study. *Sci. Rep.* 9 (1), 10117–10118. doi:10.1038/s41598-019-46513-0

Desai, P., and Patel, P. (2011). Fibroids, infertility and laparoscopic myomectomy. *J. Gynecol. Endosc. Surg.* 2 (1), 36–42. doi:10.4103/0974-1216.85280

Divakar, H. (2008). Asymptomatic uterine fibroids. *Best Pract. Res. Clin. Obstetrics Gynaecol.* 22 (4), 643–654. doi:10.1016/j.bpobgyn.2008.01.007

Dodgen, L., and Spence-Almaguer, E. (2017). Beyond body mass index: Are weight-loss programs the best way to improve the health of African American women? *Prev. Chronic Dis.* 14, E48. doi:10.5888/pcd14.160573

Doherty, L., Mutlu, L., Sinclair, D., and Taylor, H. (2014). Uterine fibroids: Clinical manifestations and contemporary management. *Reprod. Sci.* 21 (9), 1067–1092. doi:10.1177/1933719114533728

Dominic, A., Ogundipe, A., and Ogundipe, O. (2019). Determinants of women access to healthcare services in Sub-Saharan Africa. *Open Public Health J.* 12 (1), 504–514. doi:10.2174/1874944501912010504

Donnez, J., Courtoy, G. E., and Dolmans, M. M. (2019). Fibroid management in premenopausal women. *Climacteric* 22 (1), 27–33. doi:10.1080/13697137.2018.1549216

Donnez, O., and Donnez, J. (2020). Gonadotropin-releasing hormone antagonist (linzagolix): A new therapy for uterine adenomyosis. *Fertil. Steril.* 114 (3), 640–645. doi:10.1016/j.fertnstert.2020.04.017

Dragomir, A. D., Schroeder, J. C., Connolly, A., Kupper, L. L., Hill, M. C., Olshan, A. F., et al. (2010). Potential risk factors associated with subtypes of uterine leiomyomata. *Reprod. Sci.* 17 (11), 1029–1035. doi:10.1177/1933719110376979

Duc, N. M., and Huy, H. Q. (2018). Effect of magnetic resonance imaging characteristics on uterine fibroid treatment. *Reports in Medical Imaging.* 11, 1–8. doi:10.2147/RMI.S162910

Eduwem, D. U., Akintomide, A. O., Bassey, D. E., and Ekott, M. I. (2016). Hysterosalpingographic patterns and relevance in the management of infertility in a Nigerian tertiary health institution. *Asian J. Med. Sci.* 7 (5), 70–74. doi:10.3126/ajms.v7i5.15169

Egbe, T. O., Badjang, T. G., Tchounzou, R., Egbe, E. N., and Ngowe, M. N. (2018). Uterine fibroids in pregnancy: Prevalence, clinical presentation, associated factors and outcomes at the limbe and buea regional hospitals, Cameroon: A cross-sectional study. *BMC Res. notes* 11 (1), 889–896. doi:10.1186/s13104-018-4007-0

Eggert, S. L., Huyck, K. L., Somasundaram, P., Kavalla, R., Stewart, E. A., Lu, A. T., et al. (2012). Genome-wide linkage and association analyses implicate FASN in predisposition to uterine leiomyomata. *Am. J. Hum. Genet.* 91 (4), 621–628. doi:10.1016/j.ajhg.2012.08.009

Eldar-Geva, T., Meagher, S., Healy, D. L., MacLachlan, V., Breheny, S., and Wood, C. (1998). Effect of intramural, subserosal, and submucosal uterine fibroids on the

outcome of assisted reproductive technology treatment. *Fertil. Steril.* 70 (4), 687–691. doi:10.1016/s0015-0282(98)00265-9

Elugwaraonu, O., Okojie, A. I., Okhia, O., and Oyadoghan, G. P. (2013). The incidence of uterine fibroid among reproductive age women: A five year review of cases at isth, irrua, edo, Nigeria. *Int. J. Basic, Appl. Innovative Res.* 2 (3), 55

Ernest, A., Mwakalebela, A., and Mpondo, B. C. (2016). Uterine Leiomyoma in a 19-year-old girl: Case report and literature review. *Malawi Med. J.* 28 (1), 31–33. doi:10.4314/mmj.v28i1.8

Esfahlan, R. J., Zarghami, N., Esfahlan, A. J., Mollazadeh, M., Nejati, K., and Nasiri, M. (2011). The possible impact of obesity on androgen, progesterone and estrogen receptors (ERα and ERβ) gene expression in breast cancer patients. *Breast Cancer Basic Clin. Res.* 5, 227–237. BCBCR-S7707. doi:10.4137/BCBCR.S7707

Eze, C. U., Odumeru, E. A., Ochie, K., Nwadike, U. I., and Agwuna, K. K. (2013). Sonographic assessment of pregnancy co-existing with uterine leiomyoma in Owerri, Nigeria. *Afr. health Sci.* 13 (2), 453–460. doi:10.4314/ahs.v13i2.36

Faerstein, E., Szklo, M., and Rosenshein, N. (2001). Risk factors for uterine leiomyoma: A practice-based case-control study. I. African-American heritage, reproductive history, body size, and smoking. *Am. J. Epidemiol.* 153 (1), 1–10. doi:10.1093/aje/153.1.1

Farhi, J., Ashkenazi, J., Feldberg, D., Dicker, D., Orvieto, R., and Ben Rafael, Z. (1995). Effect of uterine leiomyomata on the results of *in-vitro* fertilization treatment. *Hum. Reprod.* 10 (10), 2576–2578. doi:10.1093/oxfordjournals.humrep.a135748

Farris, M., Bastianelli, C., Rosato, E., Brosens, I., and Benagiano, G. (2019). Uterine fibroids: An update on current and emerging medical treatment options. *Ther. Clin. risk Manag.* 15, 157–178. doi:10.2147/TCRM.S147318

Fasubaa, O. B., Sowemimo, O. O., Ayegbusi, O. E., Abdur-Rahim, Z. F., Idowu, B. S., Ayobami, O., et al. (2018). Contributions of uterine fibroids to infertility at Ile-Ife, South-Western Nigeria. *Trop. J. Obstetrics Gynaecol.* 35 (3), 266–270. doi:10.4103/tjog.tjog_71_18

Faustino, F., Martinho, M., Reis, J., and Águas, F. (2017). Update on medical treatment of uterine fibroids. *Eur. J. Obstetrics Gynecol. Reproductive Biol.* 216, 61–68. doi:10.1016/j.ejogrb.2017.06.047

Fleischer, R., Weston, G. C., Vollenhoven, B. J., and Rogers, P. A. (2008). Pathophysiology of fibroid disease: Angiogenesis and regulation of smooth muscle proliferation. *Best Pract. Res. Clin. Obstetrics Gynaecol.* 22 (4), 603–614. doi:10.1016/j.bpobgyn.2008.01.005

Florence, A. M., and Fatehi, M. (2022). "Leiomyoma," in *StatPearls. Treasure island* (FL: StatPearls Publishing).

Freeman, E. W., Sammel, M. D., Lin, H., and Gracia, C. R. (2010). Obesity and reproductive hormone levels in the transition to menopause. *Menopause* 17, 678–679. doi:10.1097/gme.0b013e3181e3a10a

Freytag, D., Günther, V., Maass, N., and Alkatout, I. (2021). Uterine fibroids and infertility. *Diagnostics* 11 (8), 1455. doi:10.3390/diagnostics11081455

Garcia, C. R., and Tureck, R. W. (1984). Submucosal leiomyomas and infertility. *Fertil. Steril.* 42 (1), 16–19. doi:10.1016/s0015-0282(16)47951-3

Ghant, M. S., Sengoba, K. S., Vogelzang, R., Lawson, A. K., and Marsh, E. E. (2016). An altered perception of normal: Understanding causes for treatment delay in women with symptomatic uterine fibroids. *J. Women's Health* 25 (8), 846–852. doi:10.1089/jwh.2015.5531

Ghosh, S., Naftalin, J., Imrie, R., and Hoo, W. L. (2018). Natural history of uterine fibroids: A radiological perspective. *Curr. obstetrics Gynecol. Rep.* 7 (3), 117–121. doi:10.1007/s13669-018-0243-5

Giannubilo, S. R., Ciavattini, A., Petraglia, F., Castellucci, M., and Ciarmela, P. (2015). Management of fibroids in perimenopausal women. *Curr. Opin. Obstetrics Gynecol.* 27 (6), 416–421. doi:10.1097/GCO.0000000000000219

Giri, A., Edwards, T. L., Hartmann, K. E., Torstenson, E. S., Wellons, M., Schreiner, P. J., et al. (2017). African genetic ancestry interacts with body mass index to modify risk for uterine fibroids. *PLoS Genet.* 13 (7), e1006871. doi:10.1371/journal.pgen.1006871

Giuliani, E., As-Sanie, S., and Marsh, E. E. (2020). Epidemiology and management of uterine fibroids. *Int. J. Gynecol. Obstetrics* 149 (1), 3–9. doi:10.1002/ijgo.13102

Gomez, E., Nguyen, M. L. T., Fursevich, D., Macura, K., and Gupta, A. (2021). MRI-based pictorial review of the FIGO classification system for uterine fibroids. *Abdom. Radiol.* 46 (5), 2146–2155. doi:10.1007/s00261-020-02882-z

Grandi, G., Del Savio, M. C., Melotti, C., Feliciello, L., and Facchinetti, F. (2022). Vitamin D and green tea extracts for the treatment of uterine fibroids in late reproductive life: A pilot, prospective, daily-diary based study. *Gynecol. Endocrinol.* 38 (1), 63–67. doi:10.1080/09513590.2021.1991909

Guo, X. C., and Segars, J. H. (2012). The impact and management of fibroids for fertility: An evidence-based approach. *Obstetrics Gynecol. Clin. N. Am.* 39 (4), 521–533. doi:10.1016/j.ogc.2012.09.005

Gupta, J. K., Sinha, A., Lumsden, M. A., and Hickey, M. (2014). Uterine artery embolization for symptomatic uterine fibroids. *Cochrane Database Syst. Rev.* 26 (12), 5073. doi:10.1002/14651858.cd005073.pub4

Haiman, C. A., Pike, M. C., Bernstein, L., Jaque, S. V., Stanczyk, F. Z., Afghani, A., et al. (2002). Ethnic differences in ovulatory function in nulliparous women. *Br. J. Cancer* 86 (3), 367–371. doi:10.1038/sj.bjc.6600098

Halder, S. K., Sharan, C., and Al-Hendy, A. (2012). 1, 25-dihydroxyvitamin D3 treatment shrinks uterine leiomyoma tumors in the Eker rat model. *Biol. Reproduction* 86 (4), 116. doi:10.1095/biolreprod.111.098145

Hellwege, J. N., Jeff, J. M., Wise, L. A., Gallagher, C. S., Wellons, M., Hartmann, K. E., et al. (2017). A multi-stage genome-wide association study of uterine fibroids in African Americans. *Hum. Genet.* 136 (10), 1363–1373. doi:10.1007/s00439-017-1836-1

Hodge, J. C., Quade, B. J., Rubin, M. A., Stewart, E. A., Dal Cin, P., and Morton, C. C. (2008). Molecular and cytogenetic characterization of plexiform leiomyomata provide further evidence for genetic heterogeneity underlying uterine fibroids. *Am. J. pathology* 172 (5), 1403–1410. doi:10.2353/ajpath.2008.071102

Hodges, L. C., Houston, K. D., Hunter, D. S., Fuchs-Young, R., Zhang, Z., Wineker, R. C., et al. (2002). Transdominant suppression of estrogen receptor signaling by progesterone receptor ligands in uterine leiomyoma cells. *Mol. Cell. Endocrinol.* 196 (1-2), 11–20. doi:10.1016/s0303-7207(02)00230-7

Hortence, F. J., Florent, F. Y., Brigitte, W., and Enow, M. R. (2021). Uterine fibroids in the yaoundé central hospital: Epidemiological, clinical and therapeutic aspects. *J. Obstetrics Gynecol. Problems* 2021, 100021.

Huddleston, H. G., Rosen, M. P., Gibson, M., Cedars, M. I., and Fujimoto, V. Y. (2011). Ethnic variation in estradiol metabolism in reproductive age Asian and white women treated with transdermal estradiol. *Fertil. Steril.* 96 (3), 797–799. doi:10.1016/j.fertnstert.2011.06.023

Igboeli, P., Walker, W., McHugh, A., Sultan, A., and Al-Hendy, A. (2019). Burden of uterine fibroids: An african perspective, A call for action and opportunity for intervention. *Curr. Opin. Gynecol. obstetrics* 2 (1), 287–294. doi:10.18314/cogo.v2i1.1701

Ishikawa, H., Ishi, K., Serna, V. A., Kakazu, R., Bulun, S. E., and Kurita, T. (2010). Progesterone is essential for maintenance and growth of uterine leiomyoma. *Endocrinology* 151 (6), 2433–2442. doi:10.1210/en.2009-1225

Jayes, F. L., Liu, B., Feng, L., Aviles-Espinoza, N., Leikin, S., and Leppert, P. C. (2019). Evidence of biomechanical and collagen heterogeneity in uterine fibroids. *PLoS One* 14 (4), e0215646. doi:10.1371/journal.pone.0215646

Kabodmehri, R., Etezadi, A., Sharami, S. H., Ghanaei, M. M., Hosseinzadeh, F., Heirati, S. F. D., et al. (2022). The association between chronic endometritis and uterine fibroids. *J. Fam. Med. Prim. Care* 11 (2), 653–659. doi:10.4103/jfmpc.jfmpc_1470_21

Kakarala, R. R., Chandana, S. R., Harris, S. S., Kocharla, L. P., and Dvorin, E. (2007). Prevalence of vitamin D deficiency in uninsured women. *J. general Intern. Med.* 22 (8), 1180–1183. doi:10.1007/s11606-007-0245-x

Kashani, B. N., Centini, G., Morelli, S. S., Weiss, G., and Petraglia, F. (2016). Role of medical management for uterine leiomyomas. *Best Pract. Res. Clin. Obstetrics Gynaecol.* 34, 85–103. doi:10.1016/j.bpobgyn.2015.11.016

Ke, X., Dou, F., Cheng, Z., Dai, H., Zhang, W., Qu, X., et al. (2013). High expression of cyclooxygenase-2 in uterine fibroids and its correlation with cell proliferation. *Eur. J. Obstetrics Gynecol. Reproductive Biol.* 168 (2), 199–203. doi:10.1016/j.ejogrb.2013.01.006

Keserci, B., Duc, N. M., Nadarajan, C., Huy, H. Q., Saizan, A., Wan Ahmed, W. A., et al. (2020). Volumetric MRI-guided, high-intensity focused ultrasound ablation of uterine leiomyomas: ASEAN preliminary experience. *Diagn. Interv. Radiol.* 26 (3), 207–215. doi:10.5152/dir.2019.19157

Key, T. J., Appleby, P. N., Reeves, G. K., Roddam, A., Dorgan, J. F., Longcope, C., et al. (2003). Endogenous Hormones Breast Cancer Collaborative GroupBody mass index, serum sex hormones, and breast cancer risk in postmenopausal women. *J. Natl. Cancer Inst.* 95 (16), 1218–1226. doi:10.1093/jnci/djg022

Khan, A., Shehmar, M., and Gupta, J. (2014). *Uterine fibroids: Current perspectives.* Birmingham, United Kingdom: International Journal of Women's Health, 95.

Kim, J. J., Kurita, T., and Bulun, S. E. (2013). Progesterone action in endometrial cancer, endometriosis, uterine fibroids, and breast cancer. *Endocr. Rev.* 34 (1), 130–162. doi:10.1210/er.2012-1043

Kim, J. J., Sefton, E. C., and Bulun, S. E. (2009). Progesterone receptor action in leiomyoma and endometrial cancer. *Prog. Mol. Biol. Transl. Sci.* 87, 53–85. doi:10.1016/S1877-1173(09)87002-6

Kjerulff, K. H., Langenberg, P., Seidman, J. D., Stolley, P. D., and Guzinski, G. M. (1996). Uterine leiomyomas. Racial differences in severity, symptoms and age at diagnosis. *J. reproductive Med.* 41 (7), 483

Klatsky, P. C., Tran, N. D., Caughey, A. B., and Fujimoto, V. Y. (2008). Fibroids and reproductive outcomes: A systematic literature review from conception to delivery. *Am. J. obstetrics Gynecol.* 198 (4), 357–366. doi:10.1016/j.ajog.2007.12.039

Laughlin, S. K., Hartmann, K. E., and Baird, D. D. (2011). Postpartum factors and natural fibroid regression. *Am. J. Obstetrics Gynecol.* 204 (6), e1–e6. doi:10.1016/j.ajog.2011.02.018

Levy, B. S. (2008). Modern management of uterine fibroids. *Acta Obstetricia Gynecol. Scand.* 87 (8), 812–823. doi:10.1080/00016340802146912

Litvinov, R., and Weisel, J. (2016). What is the biological and clinical relevance of Fibrin? *Seminars Thrombosis Hemostasis* 42 (04), 333–343. doi:10.1055/s-0036-1571342

Liu, B., Du, Y., Wu, Y., Snetselaar, L. G., Wallace, R. B., and Bao, W. (2021). Trends in obesity and adiposity measures by race or ethnicity among adults in the United States 2011-18: Population based study. *Bmj* 372, 365. doi:10.1136/bmj.n365

Longo, D. L., and Bulun, S. E. (2013). Mechanisms of disease: Uterine fibroids. *N. Engl. J. Med.* 369 (14), 1344–1355. doi:10.1056/nejmra1209993

Maanongun, M. T., Ornguze, A. A., and Ojo, B. (2021). Giant uterine fibroid: A case report of a young nulliparous woman and literature review. *Asian Res. J. Gynaecol. Obstetrics* 5 (2), 1–6.

Markowski, D. N., Bartnitzke, S., Löning, T., Drieschner, N., Helmke, B. M., and Bullerdiek, J. (2012). MED12 mutations in uterine fibroids—Their relationship to cytogenetic subgroups. *Int. J. cancer* 131 (7), 1528–1536. doi:10.1002/ijc.27424

Marsh, E. E., Al-Hendy, A., Kappus, D., Galitsky, A., Stewart, E. A., and Kerolous, M. (2018). Burden, prevalence, and treatment of uterine fibroids: A survey of US women. *J. women's health* 27 (11), 1359–1367. doi:10.1089/jwh.2018.7076

Marsh, E. E., Ekpo, G. E., Cardozo, E. R., Brocks, M., Dune, T., and Cohen, L. S. (2013). Racial differences in fibroid prevalence and ultrasound findings in asymptomatic young women (18–30 years old): A pilot study. *Fertil. Steril.* 99 (7), 1951–1957. doi:10.1016/j.fertnstert.2013.02.017

Marsh, E. E., Shaw, N. D., Klingman, K. M., Tiamfook-Morgan, T. O., Yialamas, M. A., Sluss, P. M., et al. (2011). Estrogen levels are higher across the menstrual cycle in African-American women compared with Caucasian women. *J. Clin. Endocrinol. Metabolism* 96 (10), 3199–3206. doi:10.1210/jc.2011-1314

Marshall, L. M., Spiegelman, D., Goldman, M. B., Manson, J. E., Colditz, G. A., Barbieri, R. L., et al. (1998). A prospective study of reproductive factors and oral contraceptive use in relation to the risk of uterine leiomyomata. *Fertil. Steril.* 70 (3), 432–439. doi:10.1016/s0015-0282(98)00208-8

Marshall, L., Spiegelman, D., Barbieri, R. L., Goldman, M. B., Manson, J. E., Colditz, G. A., et al. (1997). Variation in the incidence of uterine leiomyoma among premenopausal women by age and race. *Obstetrics Gynecol.* 90 (6), 967–973. doi:10.1016/s0029-7844(97)00534-6

Morhason-Bello, I. O., and Adebamowo, C. A. (2022). Epidemiology of uterine fibroid in black african women: A systematic scoping review. *BMJ Open* 12 (8), e052053. doi:10.1136/bmjopen-2021-052053

Muhammad, Z., Yakasai, I. A., and Abdulrahman, A. (2013). Surgical management of uterine fibroids at aminu Kano teaching hospital, Kano, Nigeria: A 5 year review. *Trop. J. Obstetrics Gynaecol.* 30 (2), 113

Mutai, J. K., Vinayak, S., Stones, W., Hacking, N., and Mariara, C. (2015). Uterine fibroid embolization for symptomatic fibroids: Study at a teaching hospital in Kenya. *J. Clin. Imaging Sci.* 5, 18. doi:10.4103/2156-7514.154351

Navarro, A., Bariani, M. V., Yang, Q., and Al-Hendy, A. (2021). Understanding the impact of uterine fibroids on human endometrium function. *Front. Cell. Dev. Biol.* 9, 633180. doi:10.3389/fcell.2021.633180

Needham, B. L., Kim, C., Mukherjee, B., Bagchi, P., Stanczyk, F. Z., and Kanaya, A. M. (2015). Endogenous sex steroid hormones and glucose in a south-asian population without diabetes: The metabolic syndrome and atherosclerosis in South-asians living in America pilot study. *Diabet. Med.* 32 (9), 1193–1200. doi:10.1111/dme.12642

Nowak, R. A., Rein, M. S., Heffner, L. J., Friedman, A. J., and Tashjian, A. H. (1993). Production of prolactin by smooth muscle cells cultured from human uterine fibroid tumors. *J. Clin. Endocrinol. Metabolism* 76 (5), 1308–1313. doi:10.1210/jcem.76.5.8496322

Obochi, G. O., Malu, S. P., Obi-Abang, M., Alozie, Y., and Iyam, M. A. (2009). Effect of garlic extracts on monosodium glutamate (MSG) induced fibroid in Wistar rats. *Pak. J. Nutr.* 8 (7), 970–976. doi:10.3923/pjn.2009.970.976

Ofori-Dankwa, Z., Ibine, B., and Ganyaglo, G. Y. (2019). The uterine fibroid disease burden in a tertiary care setting in Ghana: Prevalence, cost, and policy implications [7C]. *Obstetrics Gynecol.* 133 (1), 33S. doi:10.1097/01.aog.0000559428.84633.12

Okolo, S. (2008). Incidence, aetiology and epidemiology of uterine fibroids. *Best Pract. Res. Clin. Obstetrics Gynaecol.* 22 (4), 571–588. doi:10.1016/j.bpobgyn.2008.04.002

Okon, O. A., Ago, B. U., and Eyong, E. (2020). Presentation and surgical treatment outcomes of patients with uterine fibroids in a tertiary centre, south-south Nigeria. *Afr. J. Health Sci.* 33 (5), 41

Oluwole, A. A., Owie, E., Babah, O. A., Afolabi, B. B., and Oye-Adeniran, B. A. (2015). Epidemiology of uterine leiomyomata at the lagos university teaching hospital, idi-araba, lagos. *Niger. Hosp. Pract.* 15 (1-3), 14

Omu, A., Ihejerika, I., and Tabowei, G. (1984). Management of uterine fibroids at the university of Benin teaching hospital. *Trop. Dr.* 14 (2), 82–85. doi:10.1177/004947558401400213

Ordulu, Z. (2016). Fibroids: Genotype and phenotype. *Clin. Obstetrics Gynecol.* 59 (1), 25–29. doi:10.1097/GRF.0000000000000177

Orellana, M., Riggan, K. A., Dsouza, K., Stewart, E. A., Venable, S., Balls-Berry, J. E., et al. (2022). Perceptions of ethnoracial factors in the management and treatment of uterine fibroids. *J. Racial Ethn. Health Disparities* 9 (4), 1184–1191. doi:10.1007/s40615-021-01059-8

Peddada, S. D., Laughlin, S. K., Miner, K., Guyon, J. P., Haneke, K., Vahdat, H. L., et al. (2008). Growth of uterine leiomyomata among premenopausal black and white women. *Proc. Natl. Acad. Sci.* 105 (50), 19887–19892. doi:10.1073/pnas.0808188105

Pike, J. W., and Christakos, S. (2017). Biology and mechanisms of action of the vitamin D hormone. *Endocrinol. Metabolism Clin. N. Am.* 46 (4), 815–843. doi:10.1016/j.ecl.2017.07.001

Pinheiro, S. P., Holmes, M. D., Pollak, M. N., Barbieri, R. L., and Hankinson, S. E. (2005). Racial differences in premenopausal endogenous hormones. *Cancer Epidemiol. Biomarkers Prev.* 14 (9), 2147–2153. doi:10.1158/1055-9965.EPI-04-0944

Pritts, E. A., Parker, W. H., and Olive, D. L. (2009). Fibroids and infertility: An updated systematic review of the evidence. *Fertil. Steril.* 91 (4), 1215–1223. doi:10.1016/j.fertnstert.2008.01.051

Protic, O., Toti, P., Islam, M. S., Occhini, R., Giannubilo, S. R., Catherino, W. H., et al. (2016). Possible involvement of inflammatory/reparative processes in the development of uterine fibroids. *Cell. tissue Res.* 364 (2), 415–427. doi:10.1007/s00441-015-2324-3

Puri, K., Famuyide, A. O., Erwin, P. J., Stewart, E. A., and Laughlin-Tommaso, S. K. (2014). Submucosal fibroids and the relation to heavy menstrual bleeding and anemia. *Am. J. obstetrics Gynecol.* 210 (1), e1–e7. doi:10.1016/j.ajog.2013.09.038

Qin, H., Lin, Z., Vásquez, E., Luan, X., Guo, F., and Xu, L. (2021). Association between obesity and the risk of uterine fibroids: A systematic review and meta-analysis. *J. Epidemiol. Community Health* 75 (2), 197–204. doi:10.1136/jech-2019-213364

Rafnar, T., Gunnarsson, B., Stefansson, O. A., Sulem, P., Ingason, A., Frigge, M. L., et al. (2018). Variants associating with uterine leiomyoma highlight genetic background shared by various cancers and hormone-related traits. *Nat. Commun.* 9 (1), 3636–3639. doi:10.1038/s41467-018-05428-6

Rainibarijaona, L. N. A., Randriamahavonjy, R., Ibrahim, H., Solange, R. B., Andrianampanalalinarivo, H. R., et al. (2018). Epidemiological, clinical and therapeutic profile of uterine fibroids at the Befelatanana University hospital centre of obstetric gynecology of Antananarivo, Madagascar. *Int. J. Reproduction, Contracept. Obstetrics Gynecol.* 7 (11), 4349–4355. doi:10.18203/2320-1770.ijrcog20184476

Ramzy, A. M., Sattar, M., Amin, Y., Mansour, R. T., Serour, G. I., and Aboulghar, M. A. (1998). Uterine myomata and outcome of assisted reproduction. *Hum. Reprod. Oxf. Engl.* 13 (1), 198–202. doi:10.1093/humrep/13.1.198

Reis, F. M., Bloise, E., and Ortiga-Carvalho, T. M. (2016). Hormones and pathogenesis of uterine fibroids. *Best Pract. Res. Clin. Obstetrics Gynaecol.* 34, 13–24. doi:10.1016/j.bpobgyn.2015.11.015

Romagnolo, B., Molina, T., Leroy, G., Blin, C., Porteux, A., Thomasset, M., et al. (1996). Estradiol-dependent uterine leiomyomas in transgenic mice. *J. Clin. investigation* 98 (3), 777–784. doi:10.1172/JCI118850

Samadi, A. R., Lee, N. C., Flanders, W. D., Boring, J. R., 3rd, and Parris, E. B. (1996). Risk factors for self-reported uterine fibroids: A case-control study. *Am. J. public health* 86 (6), 858–862. doi:10.2105/ajph.86.6.858

Sarkodie, B. D., Botwe, B. O., Adjei, D. N., and Ofori, E. (2016). Factors associated with uterine fibroid in Ghanaian women undergoing pelvic scans with suspected uterine fibroid. *Fertil. Res. Pract.* 2 (1), 9–7. doi:10.1186/s40738-016-0022-9

Schlaff, W. D., Ackerman, R. T., Al-Hendy, A., Archer, D. F., Barnhart, K. T., Bradley, L. D., et al. (2020). Elagolix for heavy menstrual bleeding in women with uterine fibroids. *N. Engl. J. Med.* 382, 328–340. doi:10.1056/nejmoa1904351

Seffah, J. D., and Adanu, R. M. K. (2006). Hysterectomy for uterine fibroids in Nullipara at korle bu teaching hospital, Ghana. *Trop. J. Obstetrics Gynaecol.* 22 (2), 14510. doi:10.4314/tjog.v22i2.14510

Segars, J. H., Parrott, E. C., Nagel, J. D., Guo, X. C., Gao, X., Birnbaum, L. S., et al. (2014). Proceedings from the third national institutes of health international congress on advances in uterine leiomyoma research: Comprehensive review, conference summary and future recommendations. *Hum. Reprod. update* 20 (3), 309–333. doi:10.1093/humupd/dmt058

Sheng, B., Song, Y., Liu, Y., Jiang, C., and Zhu, X. (2020). Association between vitamin D and uterine fibroids: A study protocol of an open-label, randomised controlled trial. *BMJ open* 10 (11), e038709. doi:10.1136/bmjopen-2020-038709

Siddiqee, M. H., Bhattacharjee, B., Siddiqi, U. R., and MeshbahurRahman, M. (2021). High prevalence of vitamin D deficiency among the South Asian adults: A systematic review and meta-analysis. *BMC public health* 21 (1), 1–18. doi:10.1186/s12889-021-11888-1

Soave, I., and Marci, R. (2018). From obesity to uterine fibroids: An intricate network. *Curr. Med. Res. Opin.* 34 (11), 1877–1879. doi:10.1080/03007995.2018.1505606

Sohn, G. S., Cho, S., Kim, Y. M., Cho, C. H., Kim, M. R., Lee, S. R., et al. (2018). Current medical treatment of uterine fibroids. *Obstetrics Gynecol. Sci.* 61 (2), 192–201. doi:10.5468/ogs.2018.61.2.192

Soliman, A. M., Yang, H., Du, E. X., Kelkar, S. S., and Winkel, C. (2015). The direct and indirect costs of uterine fibroid tumors: A systematic review of the literature between 2000 and 2013. *Am. J. obstetrics Gynecol.* 213 (2), 141–160. doi:10.1016/j.ajog.2015.03.019

Song, Y., Cho, M., Brennan, K. M., Chen, B. H., Song, Y., Manson, J. E., et al. (2018). Relationships of sex hormone levels with leukocyte telomere length in Black, Hispanic, and Asian/Pacific Islander postmenopausal women. *J. diabetes* 10 (6), 502–511. doi:10.1111/1753-0407.12577

Stewart, E. A., Cookson, C. L., Gandolfo, R. A., and Schulze-Rath, R. (2017). Epidemiology of uterine fibroids: A systematic review. *BJOG Int. J. Obstetrics Gynaecol.* 124 (10), 1501–1512. doi:10.1111/1471-0528.14640

Stewart, E. A., Laughlin-Tommaso, S. K., Catherino, W. H., Lalitkumar, S., Gupta, D., and Vollenhoven, B. (2016). Uterine fibroids. *Nat. Rev. Dis. Prim.* 2 (1), 1513. doi:10.1038/nrdp.2016.43

Stewart, E. A. (2001). Uterine fibroids. *Lancet* 357 (9252), 293–298. doi:10.1016/S0140-6736(00)03622-9

Suo, G., Jiang, Y., Cowan, B., and Wang, J. Y. (2009). Platelet-derived growth factor C is upregulated in human uterine fibroids and regulates uterine smooth muscle cell growth. *Biol. reproduction* 81 (4), 749–758. doi:10.1095/biolreprod.109.076869

Surrey, E. S., Lietz, A. K., and Schoolcraft, W. B. (2001). Impact of intramural leiomyomata in patients with a normal endometrial cavity on *in vitro* fertilization–embryo transfer cycle outcome. *Fertil. Steril.* 75 (2), 405–410. doi:10.1016/s0015-0282(00)01714-3

Téguété, I., Traore, Y., Abdoulaye, S., Djire, M., Mounkoro, N., Traoré, M., et al. (2012). W430 epidemiology of uterine fibroids at gabriel toure teaching hospital, bamako, Mali (west africa). *Int. J. Gynecol. Obstetrics* 119, S844. doi:10.1016/s0020-7292(12)62151-1

Thomas, S., Ness, R. B., Thurston, R. C., Matthews, K., Chang, C. C., and Hess, R. (2013). Racial differences in perception of healthy body weight in midlife women: Results from the do stage transitions result in detectable effects study. *Menopause* 20 (3), 269–273. doi:10.1097/GME.0b013e31826e7574

Tiltman, A. J. (1998). Leiomyomas of the uterine cervix: A study of frequency. *Int. J. Gynecol. pathology official J. Int. Soc. Gynecol. Pathologists* 17 (3), 231–234. doi:10.1097/00004347-199807000-00006

Titiloye, N. A., Duduyemi, B. M., Asiamah, E. A., Okai, I., Ossei, P. P. S., Konney, T. O., et al. (2018). Total abdominal hysterectomy in a tertiary hospital in Kumasi: Indication, histopathological findings and clinicopathological correlation. *J. Med. Biomed. Sci.* 7 (1), 22–28. doi:10.4314/jmbs.v7i1.3

Ukaonu, C. B. (2017). *Prevalence and sonographic patterns of uterine fibroid among women of reproductive age in jos, plateau state, Nigeria*. JOS, Plateau State, Nigeria: Radiology.

Ulin, M., Ali, M., Chaudhry, Z. T., Al-Hendy, A., and Yang, Q. (2020). Uterine fibroids in menopause and perimenopause. *Menopause* 27, 238–242. doi:10.1097/GME.0000000000001438

Välimäki, N., Kuisma, H., Pasanen, A., Heikinheimo, O., Sjöberg, J., Bützow, R., et al. (2018). Genetic predisposition to uterine leiomyoma is determined by loci for genitourinary development and genome stability. *Elife* 7, e37110. doi:10.7554/eLife.37110

Wei, J., Zhu, A., and Ji, J. S. (2019). A comparison study of vitamin D deficiency among older adults in China and the United States. *Sci. Rep.* 9 (1), 19713. doi:10.1038/s41598-019-56297-y

Whitaker, L., and Critchley, H. O. D. (2016). Abnormal uterine bleeding. *Best Pract. Res. Clin. Obstetrics Gynaecol.* 34, 54–65. doi:10.1016/j.bpobgyn.2015.11.012

Wilde, S., and Scott-Barrett, S. (2009). Radiological appearances of uterine fibroids. *Indian J. Radiology Imaging* 19 (03), 222–231. doi:10.4103/0971-3026.54887

Wise, L. A., Palmer, J. R., Harlow, B. L., Spiegelman, D., Stewart, E. A., Adams-Campbell, L. L., et al. (2004). Reproductive factors, hormonal contraception, and risk of uterine leiomyomata in african-American women: A prospective study. *Am. J. Epidemiol.* 159 (2), 113–123. doi:10.1093/aje/kwh016

Yerezhepbayeva, M., Terzic, M., Aimagambetova, G., and Crape, B. (2022). Comparison of two invasive non-surgical treatment options for uterine myomas: Uterine artery embolization and magnetic resonance guided high intensity focused ultrasound—systematic review. *BMC Women's Health* 22 (1), 55–11. doi:10.1186/s12905-022-01627-y

Zannotti, A., Greco, S., Pellegrino, P., Giantomassi, F., Delli Carpini, G., Goteri, G., et al. (2021). Macrophages and immune responses in uterine fibroids. *Cells* 10 (5), 982. doi:10.3390/cells10050982

Zepiridis, L. I., Grimbizis, G. F., and Tarlatzis, B. C. (2016). Infertility and uterine fibroids. *Best Pract. Res. Clin. Obstetrics Gynaecol.* 34, 66–73. doi:10.1016/j.bpobgyn.2015.12.001

Zhang, K., Wiener, H., and Aissani, B. (2015). Admixture mapping of genetic variants for uterine fibroids. *J. Hum. Genet.* 60 (9), 533–538. doi:10.1038/jhg.2015.60

Zhou, H., Zhang, D., Luo, Z., Yang, A., Cui, N., Hao, G., et al. (2020). *Association between body mass index and reproductive outcome in women with polycystic ovary syndrome receiving IVF/ICSI-ET*. (Shijiazhuang, Hebei, China: Research Samples and electronic records: The Second Clinical Hospital, Hebei Medical University.

Zhu, H., Bhagatwala, J., Huang, Y., Pollock, N. K., Parikh, S., Raed, A., et al. (2016). Race/ethnicity-specific association of vitamin D and global DNA methylation: Cross-sectional and interventional findings. *PloS one* 11 (4), e0152849. doi:10.1371/journal.pone.0152849

Zimmermann, A., Bernuit, D., Gerlinger, C., Schaefers, M., and Geppert, K. (2012). Prevalence, symptoms and management of uterine fibroids: An international internet-based survey of 21, 746 women. *BMC women's health* 12 (1), 6–11. doi:10.1186/1472-6874-12-6

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The current trend of exosome in epithelial ovarian cancer studies: A bibliometric review

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Background: Epithelial ovarian cancer (EOC) is the most common type of ovarian cancer. About 90% of ovary tumors are epithelial. The current treatment for EOC involves surgical debulking of the tumors followed by a combination of chemotherapy. While most patients achieve complete remission, many EOCs will recur and develop chemoresistance. The cancer cells can adapt to several stress stimuli, becoming resistant. Therefore, new ways to fight resistant cells during the disease are being studied. Recently, exosomes, which reflect cell behavior in normal and pathological conditions such as epithelial ovarian cancer, are of academic interest as new biomarkers for diagnosis and therapy. Consequently, the current study aimed to investigate the research output of exosomes in EOC.

Method: A bibliometric method was used for analyzing publications on exosome and epithelial ovarian cancer from the beginning to 15 October 2022 by searching keywords in Scopus, PubMed and Google scholar. Annual scientific publications, authors, citations, journals, co-authorships, and keywords co-occurrence were analyzed and plotted using Microsoft Office Excel and VOS viewer. 39 original journal articles and 3 reviews have been published since 2015 up to 15 October 2022.

Results: The findings showed that China is the top country in research output, international collaborations, organization, author, and sponsorship. The top journals were the Journal of Ovarian Research, Oncotarget, and Tumor Biology, all in the United States. The top institution was Shanghai Jiao Tong University in China. The top author was Xipeng Wang. Co-occurrence analysis showed that academics' interest is toward:1) 1) Exosomes as prognostic biomarkers of EOC as well as their role in the proliferation and migration of cells. 2) The role of exosomes in metastasis through different mechanisms; 3) The role of exosomes in epithelial-mesenchymal transition of ovarian cancer cells; 4) The diagnostic role of EVs in EOC; and 5) Conferring chemoresistance in EOC through the exosomal transfer of miRNAs.

Conclusion: Research on the exosome and EOC has an increasing trend, and China is much more involved than other countries in research, financial support, and international cooperation. These findings could aid researcher in understanding novel ideas and subjects interested by sponsors in this field.

KEYWORDS

MicroRNAs, female, cancer, ovarian, extracellular vesicle

1 Introduction

Despite decades of efforts for improving the effectiveness of treatment approaches, epithelial ovarian cancer (EOC) is still the most lethal of all gynecological malignancies (Obermair et al., 2021). From a clinical point of view, the standard approach of EOC management is still debulking surgery and chemotherapy (Gadducci et al., 2022; Lodewijk et al., 2022). However, innovative surgical and medical developments were associated with only marginal survival improvements. The negative prognosis of patients suffering from OCE is normal because of late-stage diagnosis and the chemoresistance development during the illness period (Ma et al., 2014; Akter et al., 2022; Wanyama et al., 2022). Overdiagnosed, EOC remains an asymptomatic disease until the development of diffuse peritoneal carcinomatosis, including abdominal disease (van Baal et al., 2018). In addition, the transvaginal ultrasound combined with tumor markers carbohydrate antigen 125 (CA-125) serum level dosage as a promising screening method attracted attention, but yet had limitations in identifying EOC as an early-stage disease (Fishman et al., 2005; Olivier et al., 2006). Accordingly, 70% of patients diagnosed with EOC are frequently at the end stage of the disease with a 5-year survival rate of less than 40% (Meng et al., 2016a), so the necessity of developing non-invasive tools with highly sensitive for ensuring an early-stage diagnosis is one of the main challenges in biomarker investigate. Drug resistance development is another significant point in EOC investigations (Liu et al., 2018; Bukowski et al., 2020). In this regard, new advanced methods have been presented to date, precise approaches for detecting and treating different cancers (Bartosh et al., 2016; Baghban et al., 2022; Miri et al., 2022). One of the most newly known biomarkers of diseases is extracellular nano-vesicles, called exosomes (Hubert et al., 2015; Nawaz et al., 2016; Yousafzai et al., 2018; Ghafourian et al., 2022). Exosomes are produced by cells and carry several genetic materials and proteins playing key roles in the signaling and crosstalk of cells (Mashouri et al., 2019; Afshar et al., 2021; Bayat et al., 2021; Zhankina et al., 2021; Akbar et al., 2022). It has been reported that exosomes and their cargoes are an appropriate tool for maintaining the homeostasis of cancer tissues as they are able to mediate intercellular communication (Salido-Guadarrama et al., 2014; Caruso Bavisotto et al., 2019). Moreover, researchers propose that a panel of exosome-derived circulating miRNAs may aid to come for diagnosing early-onset of cancer and monitoring disease over cancer therapy (Wang and Chen, 2014). In this regard, increasing attention has been concentrated on the function of exosomes and their molecular cargo in EOC (Li and Wang, 2017).

In light of the above-mentioned, the present bibliometrics study focuses on analyzing and examining the scientific productivity developed specifically on exosomes in EOC up to 15 October 2022 to observe its evolution. In this context, the scientific productivity by year, countries, subject areas, organizations, sponsors, authors, citations, keyword co-occurrence, and co-authorships mapping related to exosomes and EOC were extracted from the titles and keywords of the included documents and analyzed. According to the best of our knowledge, there is no similar study on the subject of the exosome in EOC. The findings and statistics attained are of great value for academics working on this topic or beginning their initial steps.

2 Methods

The current research focuses on bibliometric analysis, which applies methods and software that allow identification, documentation and combination of diverse properties of the knowledge area (Donthu et al., 2021). Scientific mapping is performed according to the obtained data and different networks are resulted that define their relations between diverse factors. The proper expression of these networks will determine the upcoming lines of strengthening the scientific production of this field. Documents are tracked and selected according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Figure 1) (Moher et al., 2009). The data for this study, dating from the beginning to 2022 (15 October 2022), will be extracted using the PoP software (version 8) from Scopus, PubMed and Google scholar using the following search terms in title and keywords: “epithelial ovarian cancer” and “exosomes” or “exosome” or “exosomal” or “extracellular vesicles” or “extracellular vesicle”. Then, those articles investigating ovarian cancer in general (which also includes epithelial ones) or microvesicles and extracellular vesicles (which also contain exosomes) were excluded as this study aims to investigate the studies focusing on only EOC and only exosomes. All the information including metadata on citation information and abstract, among other information will be exported in CSV format to the Microsoft Office Excel software for data analysis after merging the obtained files. Additionally, the VOSviewer software bibliometric analysis software, which enabled to build diverse illustrations of scientific mapping, will be employed to generate the collaboration and word co-occurrence networks.

3 Results

3.1 Scientific productivity by years and subject areas

The primary research yielded 111 documents in Scopus, PubMed and Google scholar. Only articles published in English were included. After the exclusion of duplicate, irrelevant journal articles and non-journal articles, 39 articles on exosome and EOC were selected for bibliometric analysis; 36 of which were original articles (Zhang et al., 2016a; Meng et al., 2016b; Zhang et al., 2016b; Labani-Motlagh et al., 2016; Ying et al., 2016; Chen et al., 2017; Hu et al., 2017; Li et al., 2017; Wu et al., 2017; Chen et al., 2018; Qiu et al., 2018; Zhang et al., 2018; Zhou et al., 2018; Zhang et al., 2019a; Keserű et al., 2019; Tang et al., 2019; Zhu et al., 2019; Cheng et al., 2020; Li et al., 2020; Lu et al., 2020; Luo and Gui, 2020; Maeda et al., 2020; Masoumi-Dehghi et al., 2020; Alharbi et al., 2021; Xiong et al., 2021a; Cai et al., 2021; Gao et al., 2021; Li et al., 2021; Liu et al., 2021; Ma et al., 2021; Zhu et al., 2022a; Chen et al., 2022; Jeon et al., 2022; Lai et al., 2022; Yang et al., 2022), and the rest of them were review articles (Li and Wang, 2017; Lucidi et al., 2020; Shiao et al., 2021). The first English article is “Characterization of exosomes derived

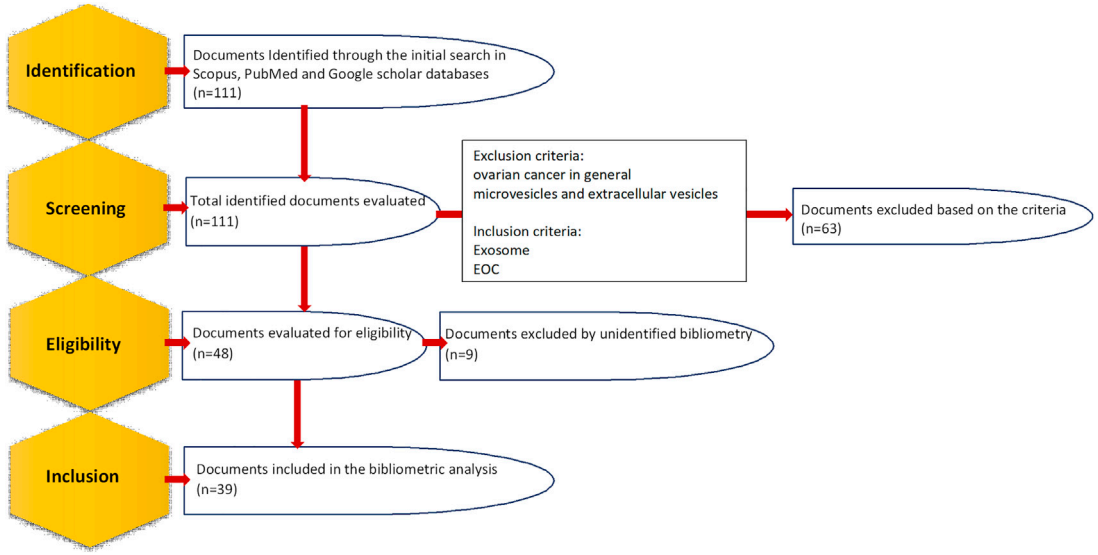


FIGURE 1
Steps of identifying and selecting documents according to preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines.

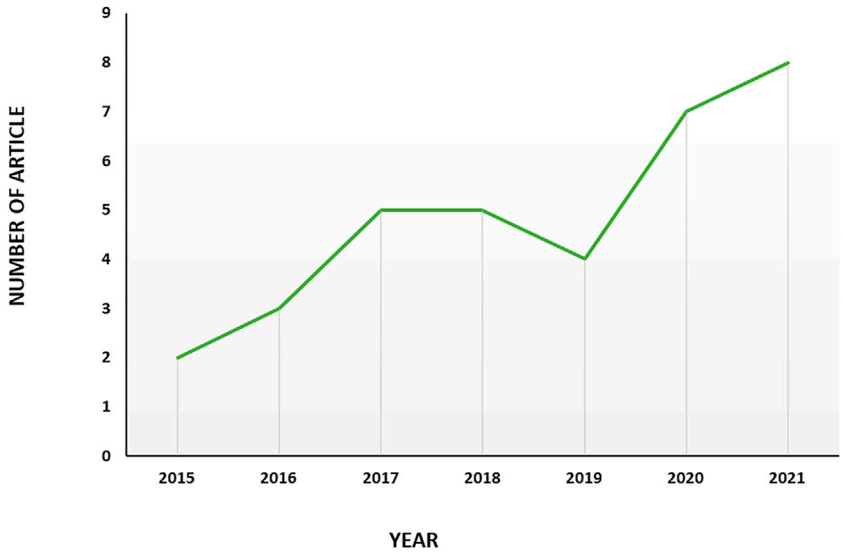


FIGURE 2
Trend of productivity by years in terms of number of publications in the field of exosome and EOC.

from ovarian cancer cells and normal ovarian epithelial cells by nanoparticle tracking analysis" in 2015 (Zhang et al., 2016a). The scientific production evolution has been illustrated in Figure 2. 2021 is the year with the greatest scientific production with 8 articles and a 4-fold increase in publication compared to the first year, 2015. In the case of 2022, as the year is not completed it is soon to judge about.

Selected articles on exosome and OEC has been published in the context of 11 areas based on area categories of the Dimension database. "Biomedical and clinical sciences" has the maximum publication share (37.36%), followed by "oncology and carcinogenesis" (34.06%), "biological sciences" (6.50%), "biochemistry and cell biology" (4.58%), "multidisciplinary" (3.30%) "multidisciplinary" (3.30%) "multidisciplinary" (7.69%), "biochemistry and cell biology" and "immunology" (5.49%), "chemical sciences", "microbiology" and "medicinal and biomolecular chemistry" (2.20%) and "medical biotechnology", "clinical sciences" and "reproductive medicine" (1.10%) (Figure 3).

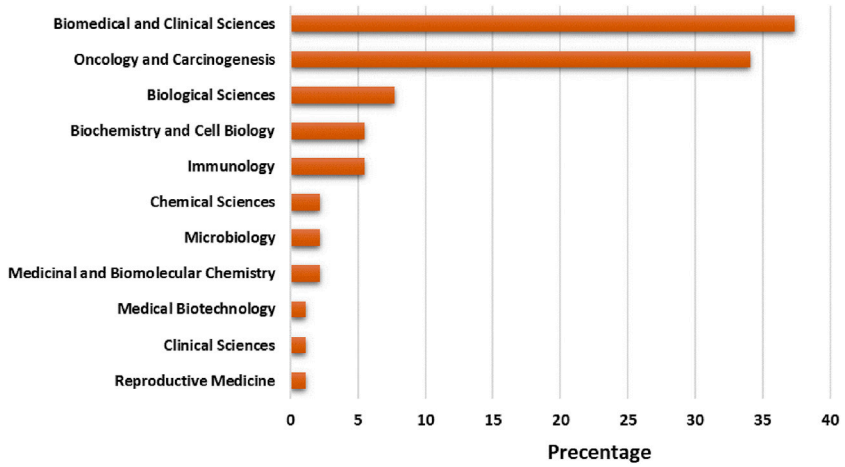
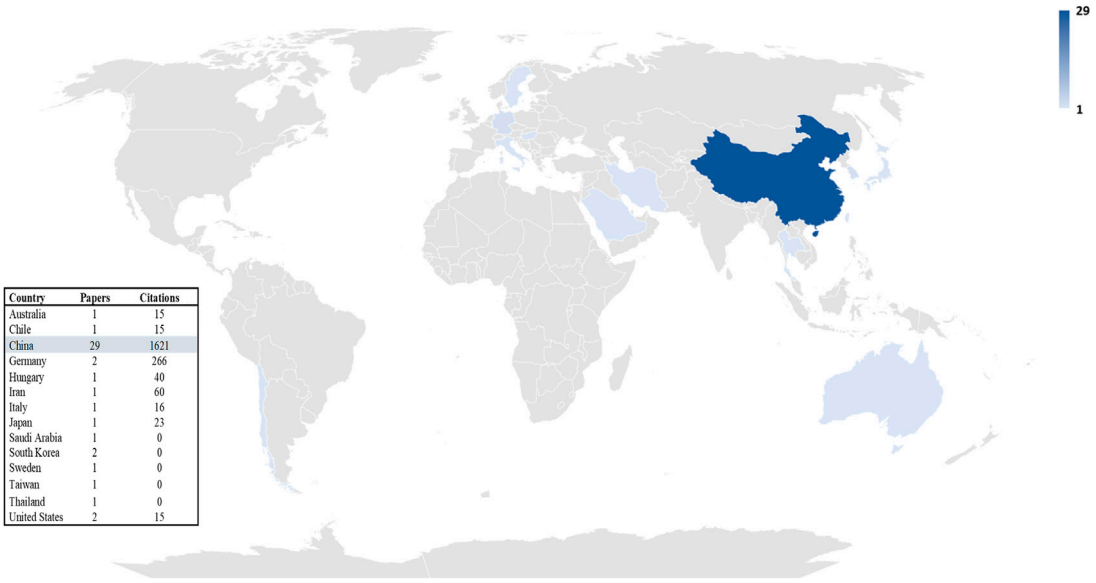


FIGURE 3
Trend of productivity by subject areas in terms of number of publications in the field of exosome and EOC.



Country	Papers	Citations
Australia	1	15
Chile	1	15
China	29	1621
Germany	2	266
Hungary	1	40
Iran	1	60
Italy	1	16
Japan	1	23
Saudi Arabia	1	0
South Korea	2	0
Sweden	1	0
Taiwan	1	0
Thailand	1	0
United States	2	15

FIGURE 4
Trend of productivity by countries in terms of number of publications in the field of exosome and EOC.

3.2 Countries

A total of 14 countries including China, Germany, Sweden, Hungary, Japan, the United States, Italy, Iran, Thailand, South Korea, Taiwan, Australia, Saudi Arabia, and Chile had published at least one article related to the exosome and EOC from 2015 to 2022. As observed in Figure 4, China with 29 is the top country.

In order to analyze country co-authorship or international collaboration, all 14 countries were included in the analysis. This analysis resulted in 9 clusters that 3 of which contains only two or more country. These three clusters contain 8 countries and 2 out of these 3 clusters are linked together. These results have been illustrated in Figure 5. It shows that authors in eight countries have international collaboration. Among these countries, China has the best international collaboration.

3.3 Organizations

A total of 63 organizations including 25 hospitals and 38 universities and colleges published at least one journal article from 2015 up to 15 October 2022. Among these organizations, the Shanghai Jiao Tong University in China with 6 articles is ranked as

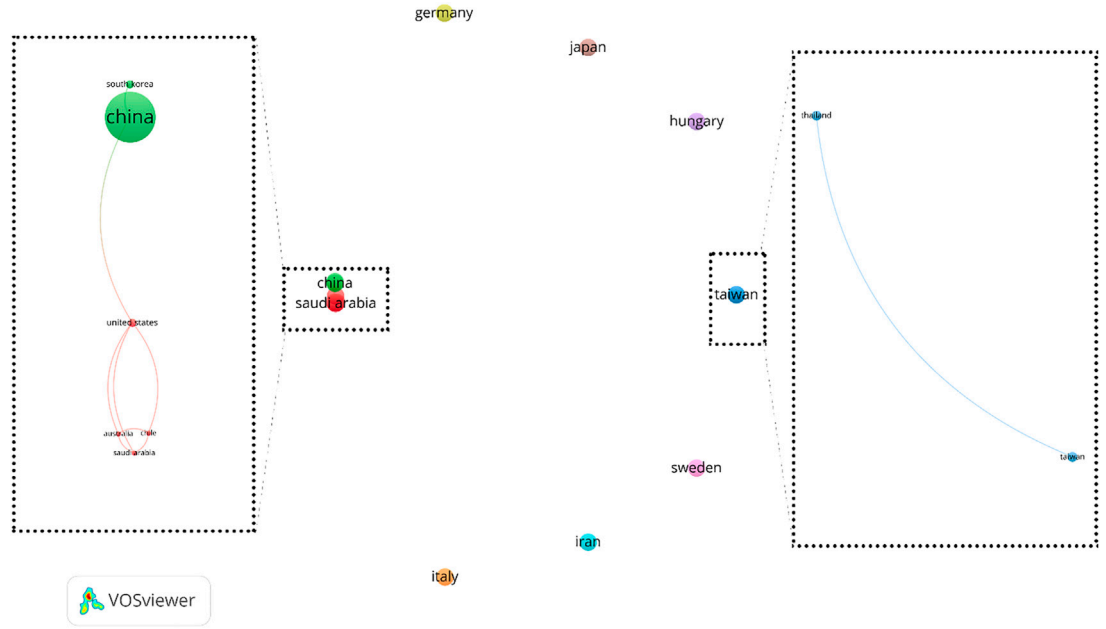


FIGURE 5
Country co-authorship networks (criteria: countries with at least 1 article in the field of exosome and OEC).

TABLE 1 Trend of productivity by institutions.

Organization	Country	Papers	Citations
Shanghai jiao tong university	China	6	599
Tongji university	China	5	671
Renji hospital	China	4	436
Xinhua hospital	China	4	434
University medical center hamburg-eppendorf	Germany	2	266
Affiliated hospital of jiangsu university	China	2	186
Jiangsu university	China	2	186
Fudan university	China	2	118
Huazhong university of science and technology	China	2	101
Tongji hospital	China	2	101
Chinese academy of medical sciences and peking union medical college	China	2	79
Peking union medical college hospital	China	2	79
Fudan university shanghai cancer center	China	2	78

the first producer university and the Tongji University in China with 5 articles is ranked as the second one. Table 1 shows the ranking of organizations with more than one journal article in this field between 2015 to 15 October 2022.

To analyze the organizations' collaboration, the organizations' co-authorship network was obtained using VOSviewer software (Figure 6). All 63 identified organizations were included in the primary analysis and it resulted in 26 clusters that 9 of them has no collaboration with other organizations. The largest cluster contains 8 organizations including, which has been shown in detail in Figure 6.

3.4 Authors

245 authors are identified in 39 articles implying a productivity index of 0.16 articles per author. Authors with more than 2 articles

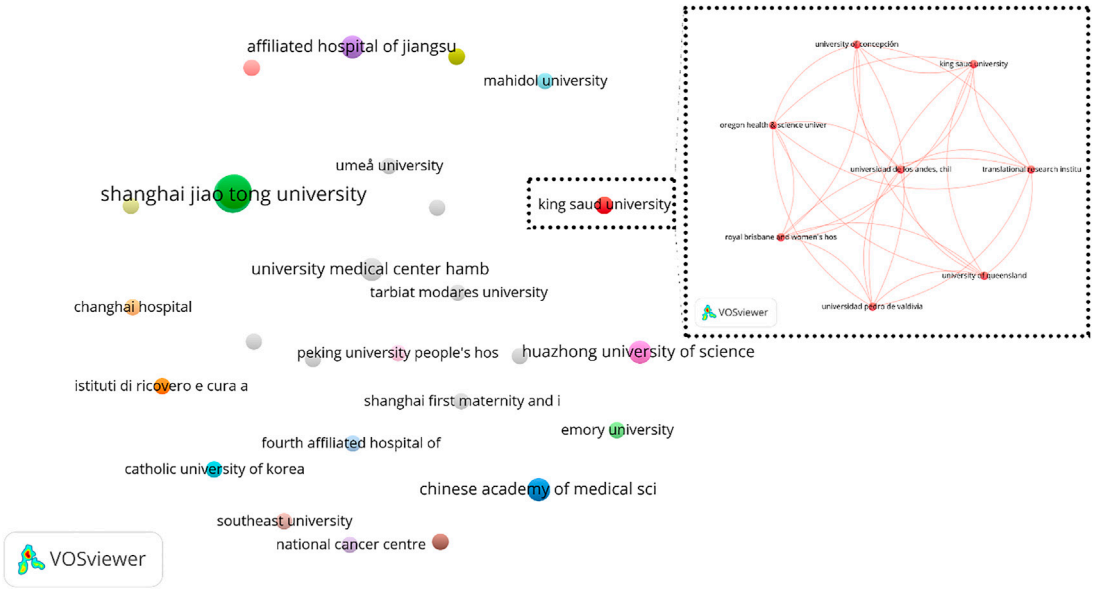


FIGURE 6
Organization co-authorship network (criteria: organizations with at least 1 article in the field of exosome and OEC).

TABLE 2 Most productive authors in terms of number of publications in the field of exosome and EOC.

Author	Papers	Citations
xipeng wang	7	887
xinjing wang	6	811
qinyi zhu	5	651
xin chen	4	601
xiaoduan li	4	446
xiaoli wu	4	627
yingying lin	3	370
xiang ying	3	441
wei zhang	3	143

in the field of exosome and EOC have been presented in Table 2. Xipeng Wang (Ying et al., 2016; Chen et al., 2017; Li and Wang, 2017; Wu et al., 2017; Chen et al., 2018; Zhou et al., 2018; Li et al., 2020), Xinjing Wang (Ying et al., 2016; Chen et al., 2017; Wu et al., 2017; Chen et al., 2018; Zhou et al., 2018; Li et al., 2020), and Qinyi Zhu (Ying et al., 2016; Chen et al., 2017; Wu et al., 2017; Zhou et al., 2018; Li et al., 2020) are the most productive author with 7, 6, and 5 articles in the field of exosome and EOC. Most citations are also related to the same authors with values of 887, 811, and 651, respectively. It is worth notable that these authors are in the same group.

Regarding the author collaboration trend in producing journal articles, there is no single authorship document and all articles display a contribution of two or more authors that authors in 25 of which have different affiliations. The participation of several authors with diverse affiliations in one manuscript displays thematic maturity (Berelson, 1952; López López, 1996).

For authors' co-authorship analysis, only the authors with at least 2 articles were included in this analysis; so, 25 of 246 authors were entered into the network analysis. As it was demonstrated in Figures 6, 7 groups were resulted by clustering the co-authorships among these 25 authors. Only 2 clusters link together and the rest of them have no link with each other. In this network, a total of 70 links were found between authors.

3.5 Citations

A primary analysis of citations showed that the selected articles had been cited 2,101 times, and the mean citation to each article was 53.87 times. 2018 is the year with the maximum number of citations, with 549 citations (26.13%). For articles with the maximum citations, three original articles by Ying et al. (Ying et al., 2016) with 272 citations, Zhou et al. (Zhou et al., 2018) with 230 citations and Meng et al. (Meng et al., 2016b) with 220 citations were first, second, and third, respectively. Table 3 indicates ten articles with the maximum citations in detail. As observed 7 out of these 10 articles focused on the delivery of microRNAs (miRNAs) by exosomes.

On the other hand, the authors' co-citation analysis (ACA) indicates how often authors of the former articles are co-cited by authors of subsequent articles. These groups cause clusters with central nodes whose size shows the co-citation trend reached by each author. Figure 8 demonstrates a scientific mapping by ACA. The structure contains 10,504 authors, 15 of which meet the threshold recognized in fifteen citations, resulting in 3 clusters. The authors with the maximum citations were: Xipeng Wang (33 co-citations), Klaus Pantel (28 co-citations), and Heidi Schwarzenbach (27 co-citations).

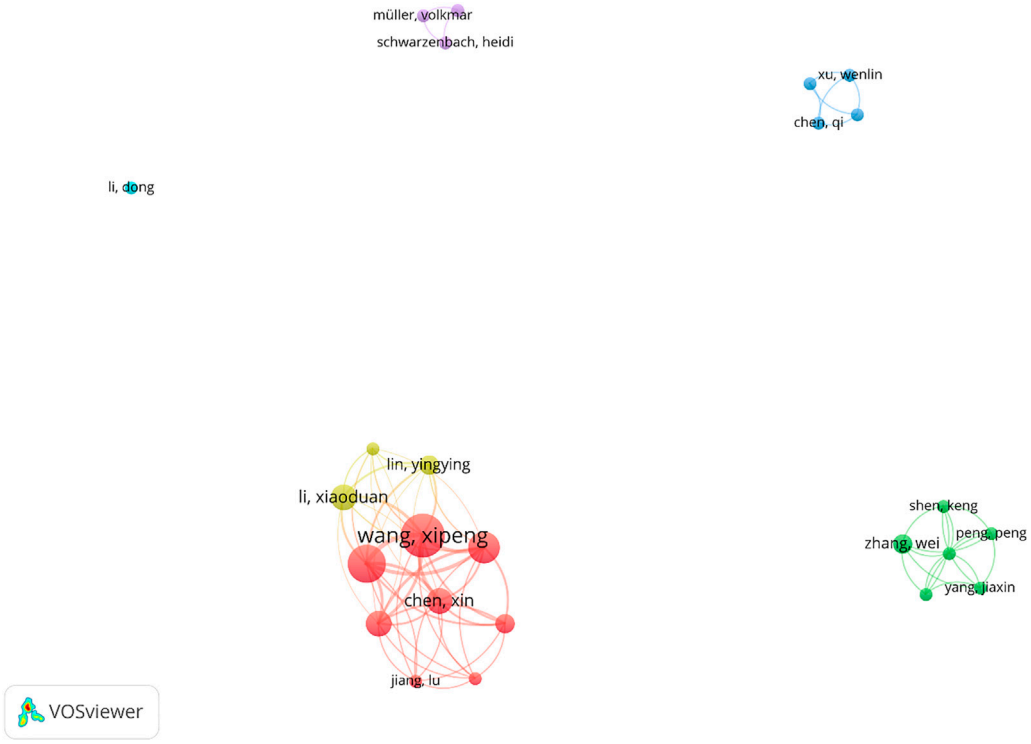


FIGURE 7
Author co-authorship networks (criteria: authors with at least 2 articles in the field of exosome and OEC).

TABLE 3 The most cited articles in the field of exosome and EOC.

Title	Journal	Year	Citation	References
Epithelial ovarian cancer-secreted exosomal miR-222-3p induces polarization of tumor-associated macrophages	Oncotarget	2016	225	Ying et al. (2016)
Exosomes released from tumor-associated macrophages transfer miRNAs that induce a Treg/Th17 cell imbalance in epithelial ovarian cancer	Cancer immunology research	2018	186	Zhou et al. (2018)
Diagnostic and prognostic relevance of circulating exosomal miR-373, miR-200a, miR-200b and miR-200c in patients with epithelial ovarian cancer	Oncotarget	2016	182	Meng et al. (2016b)
Macrophages derived exosomes deliver miR-223 to epithelial ovarian cancer cells to elicit a chemoresistant phenotype	Journal of experimental and clinical cancer research	2019	181	Zhu et al. (2019)
Exosomes derived from hypoxic epithelial ovarian cancer cells deliver microRNAs to macrophages and elicit a tumor-promoted phenotype	Cancer letters	2018	160	Chen et al. (2018)
Exosomes derived from hypoxic epithelial ovarian cancer deliver microRNA-940 to induce macrophage M2 polarization	Oncology reports	2017	152	Chen et al. (2017)
Exosomal metastasis-associated lung adenocarcinoma transcript 1 promotes angiogenesis and predicts poor prognosis in epithelial ovarian cancer	International journal of biological sciences	2018	99	Qiu et al. (2018)
TGFβ1 in fibroblasts-derived exosomes promotes epithelial-mesenchymal transition of ovarian cancer cells	Oncotarget	2017	85	Li et al. (2017)
Exosomal microRNAs as tumor markers in epithelial ovarian cancer	Molecular oncology	2018	84	Pan et al. (2018)
The emerging roles and therapeutic potential of exosomes in epithelial ovarian cancer	Molecular cancer	2017	76	Li and Wang (2017)

In fact, each cluster in Figure 8 forms a "school of thought" (Zupic and Čater, 2015), which allows us to observe the methods shared between the authors. Cluster 1, which is red, is composed of 7 academics including Xin Chen (15 co-citations), Ahmedin Jemal (24 co-citations), Rebecca l. Siegel (18 co-citations), Xinjing Wang (19 co-citations), Xipeng Wang (33 co-

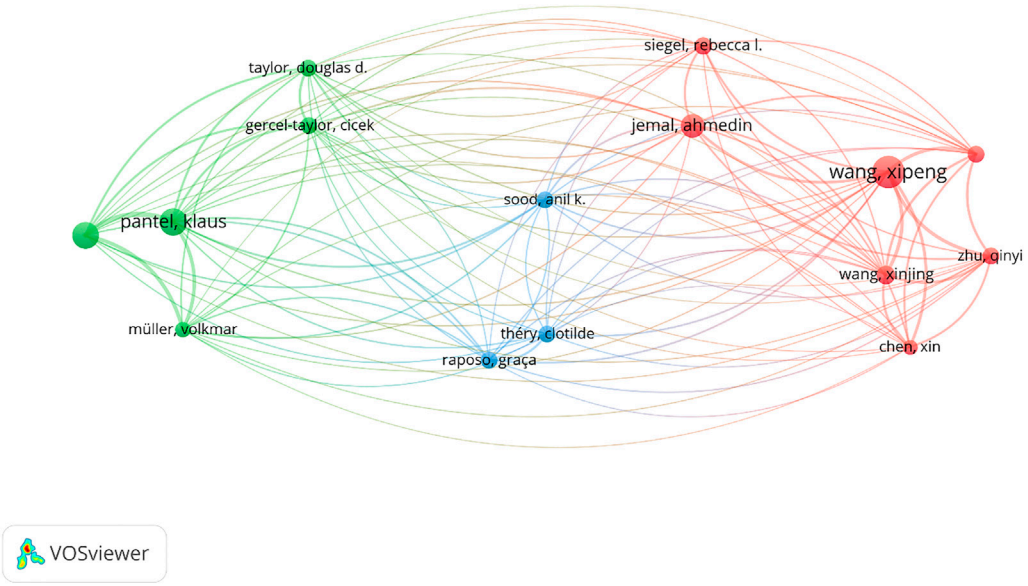


FIGURE 8
Author co-citation analysis (ACA) (criteria: at least fifteen citations).

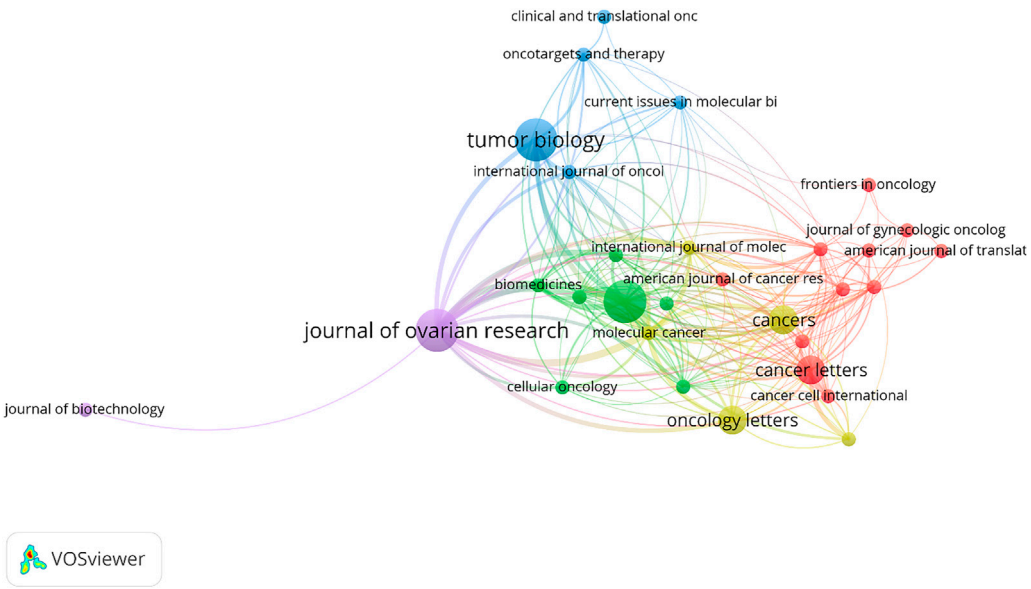


FIGURE 9
Bibliographic coupling of journals (criteria: journals with at least one article in the field of exosome and OEC).

citations), Xiaoli Wu (17 co-citations), and Qinyi Zhu (17 co-citations). This school of thought focuses on different accepts of cancer research (Ying et al., 2016; Siegel et al., 2019). The green cluster 2 is made up of five authors, who focus on cancer biomarkers: Cicek Gercel-Taylor (17 co-citations), Klaus Pantel (28 co-citations), Volkmar Müller (16 co-citations), Heidi Schwarzenbach (27 co-citations), and Douglas D. Taylor (17 co-citations). Among the main lines of this school are tumor-derived exosomes as diagnostic biomarkers of ovarian cancer (Taylor and Gercel-Taylor, 2008) and exosomal microRNAs as EOC markers (Pan et al., 2018). Finally, cluster 3, which is blue, concentrates on 3 researchers: (17 co-citations), Anil K. Sood (17 co-citations), and Clotilde Théry (17 co-citations). These authors address the functions of extracellular vesicles in cancer therapy (Zhao et al., 2022a; Singh et al., 2022).

TABLE 4 Journals with at least one article in the field of exosome and EOC.

Journal	Papers	Citations	Country	H Index	Q
Journal of ovarian research	3	68	United States	49	1
Oncotarget	3	492	United States	148	1
Tumor biology	3	137	United States	92	1
Cancer letters	2	226	Ireland	192	1
Cancers	2	15	Switzerland	92	2
Oncology letters	2	23	Greec	63	2
American journal of cancer research	1	5	United States	—	—
American journal of translational research	1	14	United States	66	-
Biomedicines	1	2	Switzerland	38	2
Cancer cell international	1	64	UK	62	1
Cancer immunology research	1	186	United States	101	1
Cell death and disease	1	16	United States	128	1
Cellular oncology	1	24	Netherland	47	1
Clinical and translational oncology	1	0	Italy	23	1
Current issues in molecular biology	1	0	Switzerland	54	3
Frontiers in oncology	1	1	Switzerland	102	2
Future oncology	1	0	UK	72	2
International journal of biological sciences	1	99	Australia	98	1
International journal of molecular sciences	1	16	Switzerland	198	1
International journal of oncology	1	64	Greece	128	1
Journal of biotechnology	1	40	Netherlands	164	2
Journal of cell communication and signaling	1	60	Netherlands	47	1
Journal of cellular and molecular medicine	1	7	UK	138	2
Journal of experimental and clinical cancer research	1	181	UK	95	1
Journal of gynecologic oncology	1	29	South Korea	42	1
Molecular cancer	1	76	United States	146	1
Molecular oncology	1	84	Netherlands	97	1
Oncology reports	1	152	Greec	101	1
Oncotargets and therapy	1	19	New Zealand	66	1
Journal of gene medicine	1	1	United States	94	2

3.6 Journals

According to the bibliographic coupling of resources, a total of 30 journals with five clusters were identified (Figure 9). The most productive ones are the Journal of ovarian research, Oncotarget, and Tumor biology, with three articles each. The Oncotarget has the maximum number of citations received by accumulating 492 (Table 4). A total number of 25 journals only published one article in the field of exosome and OEC. 9 out of which (15 out of 39 documents) were published mostly in the United States. 19 out of 25 journals are Q1 suggesting the high quality of the research in this field.

3.7 Funding sponsors

The main financial sponsors for "exosome and EOC" research was presented in Table 5. The obtained results showed that the National Natural Science Foundation of China was the top funding sponsor with sponsoring 20 projects on this topic.

TABLE 5 Sponsors of research in the field of exosome and EOC.

Funder	No of articles
China scholarship council	2
Else kröner-fresenius-stiftung	1
European research council	1
National natural science foundation of China	20
Shanghai municipal commission of health and family planning	2
Ministry of science and technology of the People's Republic of China	2
Wilhelm sander stiftung	1
Science and technology commission of Shanghai municipality	1
Shanghai hospital development center	1
Shanghai municipal education commission	1
National cancer institute	1
Japan society for the promotion of science	1
Cystic fibrosis foundation	1
Iran National Science Foundation	1
National research foundation of Korea	2
Agencia nacional de investigación y desarrollo	1
National health and medical research council	1

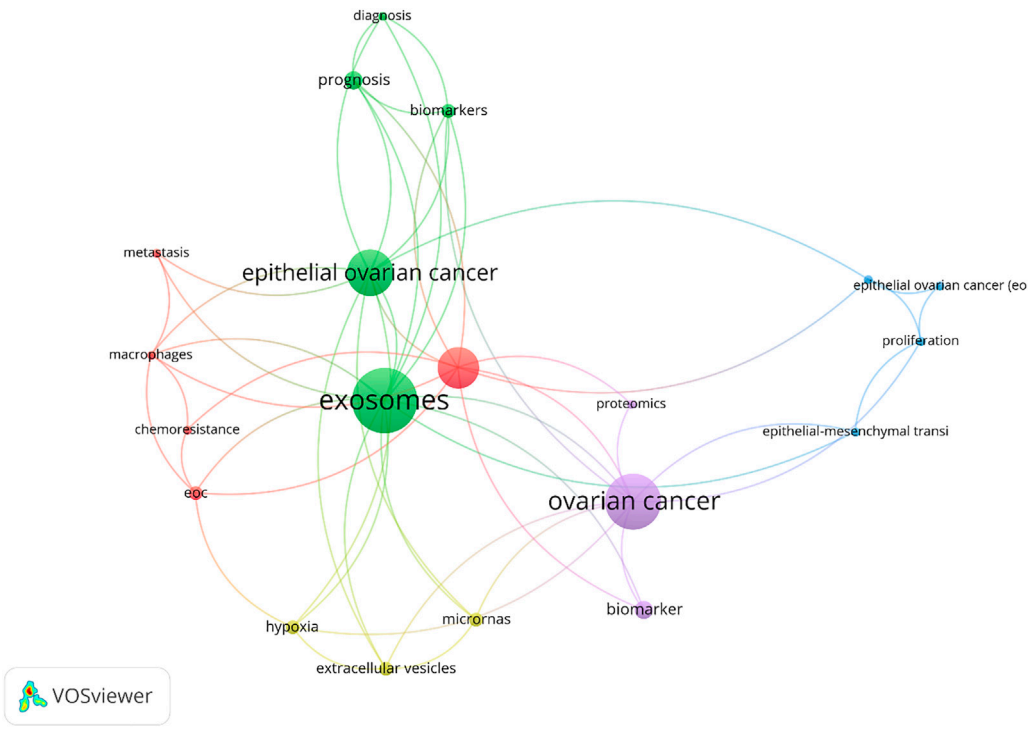


FIGURE 10
Co-occurrence author keyword (criteria: at least 2 author keyword co-occurrences).

TABLE 6 List of articles in the filed of miRNA, exosome and ovarian cancer.

Title	Country	Year	Citation	Ref
Exosomal miRNA confers chemo resistance *via* targeting Cav1/p-gp/M2-type macrophage axis in ovarian cancer	United States	2018	91	Kanlikilicer et al. (2018)
Exosome-Derived microRNA: Efficacy in cancer	United States	2021	5	Padda et al. (2021)
microRNAs as biomarkers of ovarian cancer	China	2020	10	Zhang and Lu (2020)
Expression of CD24 in plasma, exosome and ovarian tissue samples of serous ovarian cancer patients	Hungary	2019	11	Soltész et al. (2019)
Detection of plasma exosomal miRNA-205 as a biomarker for early diagnosis and an adjuvant indicator of ovarian cancer staging	China	2022	7	Zhu et al. (2022b)
Ovarian cancer cell invasiveness is associated with discordant exosomal sequestration of Let-7 miRNA and miR-200	Australia	2014	99	Kobayashi et al. (2014)
miR-139 Controls Viability of Ovarian Cancer Cells Through Apoptosis Induction and Exosome Shedding Inhibition By Targeting ATP7A	China	2019	5	Xiao et al. (2019)
Serum exosomal miRNA-145 and miRNA-200c as promising biomarkers for preoperative diagnosis of ovarian carcinomas	Korea	2019	34	Kim et al. (2019)
Targeted delivery of exosomal miR-484 reprograms tumor vasculature for chemotherapy sensitization	China	2022	7	Zhao et al. (2022b)
MiR-200b is upregulated in plasma-derived exosomes and functions as an oncogene by promoting macrophage M2 polarization in ovarian cancer	China	2021	13	Xiong et al. (2021b)
Quantitative and stoichiometric analysis of the microRNA content of exosomes	United States	2014	478	Chevillet et al. (2014)
Exosomal MicroRNA as Biomarkers for Diagnosing or Monitoring the Progression of Ovarian Clear Cell Carcinoma: A Pilot Study	Japan	2022	3	Horie et al. (2022)
MicroRNA profiling of plasma exosomes from patients with ovarian cancer using high-throughput sequencing	China	2019	8	Zhang et al. (2019b)
Ubiquitous release of exosomal tumor suppressor miR-6126 from ovarian cancer cells	China	2016	31	Kanlikilicer et al. (2016)
Unbiased RNA-Seq-driven identification and validation of reference genes for quantitative RT-PCR analyses of pooled cancer exosomes	United States	2021	8	Dai et al. (2021)
Upregulated expression of serum exosomal miR-375 and miR-1307 enhance the diagnostic power of CA125 for ovarian cancer	China	2019	38	Su et al. (2019)
Exosomes as a potential tool for a specific delivery of functional molecules	Germany	2013	28	Nazarenko et al. (2013)
Exploring the potential of engineered exosomes as delivery systems for tumor-suppressor microRNA replacement therapy in ovarian cancer	United States	2020	37	Kobayashi et al. (2020)
Human CAP cells represent a novel source for functional, miRNA-loaded exosome production	Germany	2019	-	Zeh et al. (2019)
The passenger strand, miR-21-3p, plays a role in mediating cisplatin resistance in ovarian cancer cells	UK	2015	85	Pink et al. (2015)

3.8 Keywords

Despite the current relevance of using keywords in some analyses, 3 articles without the keyword section were recognized after reviewing selected articles. In the rest of the articles, the co-occurrence analysis of author keywords is used. For this purpose, at least 2 author keyword co-occurrences were considered as the criteria. 20 out of 114 diverse keywords met the inclusion criteria. As revealed in Figure 10, the analysis of involved keywords resulted in 5 clusters. The keywords with the maximum number of co-occurrences in each group are as follows: Exosomes (14 co-citations), ovarian cancer (12 co-citations), epithelial ovarian cancer (10 co-citations), exosome (9 co-citations), biomarker (4 co-citations), prognosis (4 co-citations), EOC (3 co-citations), biomarkers (3 co-citations), extracellular vesicles (3 co-citations), hypoxia (3 co-citations), microRNAs (3 co-citations), metastasis (2 co-citations), macrophages (2 co-citations), epithelial-mesenchymal transition (2 co-citations), epithelial ovarian cancer (EOC) (2 co-citations), diagnosis (2 co-citations), chemoresistance (2 co-citations), migration (2 co-citations), proliferation (2 co-citations), proteomics (2 co-citations).

4 Discussion

The present research mainly purposed to study the international research activities in the field of exosome and EOC from the start of data published in this field up to 15 October 2022. It is emphasized that this research included studies focused on EOC, not ovarian cancer in general and exosomes alone, not microvesicles and extracellular vesicles.

There are several bibliometric reviews on different aspects of ovarian cancer (Wang et al., 2016; El Bairi et al., 2021; Gupta et al., 2021; Khedkar et al., 2021; Liu et al., 2022; Xu and Li, 2022) but there is no bibliometric review on EOC and applications of exosomes in EOC. The analysis of productivity by year showed that despite the growth of research interest in exosomes in different fields, research on exosomes and EOC has opened up in recent decades, and it is in the early stages. According to the findings of the current study, China is ranked the first publisher of journal articles in this field. This can be contributed to a large number of highly ranked institutions in publication and more funding sponsors in this region. However, Wang B et al., in 2019 and Wang Y et al., in 2017 stated that the United States ranked as the first producer in the field of exosomes and China ranked second at that time (Wang et al., 2017; Wang et al., 2019). Moreover, Yuanxia Liu et al. reported that the United States and UK are the first and second article producer from inception to 2021 (Liu et al., 2022). When it comes to funding sponsors of the research, Chinese sponsors especially the National Natural Science Foundation of China are impressive. This is since China efforts to impact high-tech technologies and promote their research and development sections and businesses.

Regarding the journal that publishes the articles, the Journal of Ovarian Research is the top publisher of journal articles in the field of exosome and EOC based on our analysis. Wang Y et al. and Wang B et al. stated that Plos One, Journal of Biological Chemistry, and Scientific Reports are the top publishers of articles in the exosome field (Wang et al., 2017; Wang et al., 2019). Furthermore, Bernardo Pereira Cabral et al. reported that Oncotarget led published most cancer-related articles between 2012–2017 (Cabral et al., 2018). All of these journals are among high-ranking journals showing the field of study is novel and high-tech.

Citation analysis showed that 7 out of these 10 most cited articles focused on the delivery of microRNAs (miRNAs) by exosomes. The maximum co-occurrence author keywords showed the research flashpoints in the studied area over the study period. According to this analysis, the academics are interested to work on: 1) Exosomes as prognostic biomarkers of EOC and their role in the proliferation and migration of cells. 2) The role of exosomes in metastasis through different mechanisms; 3) The role of exosomes in epithelial-mesenchymal transition of ovarian cancer cells; 4) The diagnostic role of EVs in EOC; and 5) Conferring chemoresistance in EOC through Exosomal transfer of miRNAs.

5 Study limitations and sampling bias

The sampling bias is one of the key limitations of this study. As mentioned, this study focuses on research investigating specifically EOC and exosome. Therefore, sampling criteria excluded the published reports on extracellular vesicles, microvesicles and ovarian cancer, while exosomes are a subclass of extracellular vesicles and microvesicles. Moreover, EOC is one type of ovarian cancer. Therefore, considering exosome and EOC alone cannot make a general conclusion for highly cited or most productive authors in this field of ovarian cancer.

To reduce this limitation, according to citation analysis results which showed most cited articles focused on the delivery of miRNAs by exosomes, an extra search was conducted using the PubMed database on studies in the field of ovarian cancer and exosomal miRNA. 20 other articles other than those found above were found in this field. These articles are related to different countries including the United States, China, Hungary, Australia, Korea, Japan, Germany and the UK. The most cited article in this area is related to the United States with 487 citations, which is considerable. The information related to these articles has been listed in Table 6.

6 Conclusion

The present research findings showed the increasing trend of research activities in the field of exosomes and EOC. The scientific production was made up of 39 journal articles found in the international databases of Scopus, PubMed and Google scholar. China is the main contributor to the publications, institutions, funding, and international collaborations on the studied topic. The United States ranked first in terms of journals published articles on the studied topic. Moreover, results show that the scientists working in this area are mostly interested into apply microRNA delivery using exosomes in EOC contexts. However, it showed be noted that these results only related to studies in the field of EOC, not ovarian cancer in general or nanoscale extracellular vesicles, not all scales extracellular vesicles. These findings could be helpful for academics working in the field of exosomes and EOC to develop their studies and find new pipelines along the way.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

Conceptualization, NB; methodology, NB; software, NB; validation, NB; formal analysis, NB; investigation, NB; resources, NB, MU, and IN; data curation, NB; writing—original draft preparation, NB; writing—review and editing, MU and IN; visualization, NB and MU; supervision, NB; project administration, NB All authors have read and agreed to the published version of the manuscript.

Conflict of interest

MU was employed by the company Department of Cancer Immunology, Genentech Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Afshar, A., Zare, M., Farrar, Z., Hashemi, A., Baghban, N., Khoradmehr, A., et al. (2021). Exosomes of mesenchymal stem cells as nano-cargos for anti-SARS-CoV-2 asRNAs. *Mod. Med. Laboratory J.* 4, 11–18.

Akbar, A., Malekian, F., Baghban, N., Kodam, S. P., and Ullah, M. (2022). Methodologies to isolate and purify clinical grade extracellular vesicles for medical applications. *Cells* 11, 186. doi:10.3390/cells11020186

Akter, S., Rahman, M. A., Hasan, M. N., Akhter, H., Noor, P., Islam, R., et al. (2022). Recent advances in ovarian cancer: Therapeutic strategies, potential biomarkers, and technological improvements. *Cells* 11, 650. doi:10.3390/cells11040650

Alharbi, M., Lai, A., Sharma, S., Kalita-de Croft, P., Godbole, N., Campos, A., et al. (2021). Extracellular vesicle transmission of chemoresistance to ovarian cancer cells is associated with hypoxia-induced expression of glycolytic pathway proteins, and prediction of epithelial ovarian cancer disease recurrence. *Cancers* 13, 3388. doi:10.3390/cancers13143388

Baghban, N., Khoradmehr, A., Nabipour, I., Tamadon, A., and Ullah, M. (2022). The potential of marine-based gold nanomaterials in cancer therapy: A mini-review. *Gold Bull.* 55, 53–63. doi:10.1007/s13404-021-00304-6

Bayat, F., Afshar, A., and Baghban, N. (2021). Algal cells-derived extracellular vesicles: A review with special emphasis on their antimicrobial effects. *Front. Microbiol.* 12, 785716. doi:10.3389/fmicb.2021.785716

Berelson, B. (1952). *Content analysis in communication research*. Washington, DC: American Psychological Association

Bukowski, K., Kciuk, M., and Kontek, R. (2020). Mechanisms of multidrug resistance in cancer chemotherapy. *Int. J. Mol. Sci.* 21, 3233. doi:10.3390/ijms21093233

Cabral, B. P., da Graça Derengowski Fonseca, M., and Mota, F. B. (2018). The recent landscape of cancer research worldwide: A bibliometric and network analysis. *Oncotarget* 9, 30474–30484. doi:10.18632/oncotarget.25730

Cai, J., Gong, L., Li, G., Guo, J., Yi, X., and Wang, Z. (2021). Exosomes in ovarian cancer ascites promote epithelial–mesenchymal transition of ovarian cancer cells by delivery of miR-6780b-5p. *Cell death Dis.* 12, 210–217. doi:10.1038/s41419-021-03490-5

Caruso Bavisotto, C., Scalia, F., Marino Gammazza, A., Carlisi, D., Bucchieri, F., Conway de Macario, E., et al. (2019). Extracellular vesicle-mediated cell–cell communication in the nervous system: Focus on neurological diseases. *Int. J. Mol. Sci.* 20, 434. doi:10.3390/ijms20020434

Chen, L., Wang, K., Li, L., Zheng, B., Zhang, Q., Zhang, F., et al. (2022). Plasma exosomal miR-1260a, miR-7977 and miR-192-5p as diagnostic biomarkers in epithelial ovarian cancer. *Future Oncol.* 18, 2919–2931. doi:10.2217/fon-2022-0321

Chen, X., Ying, X., Wang, X., Wu, X., Zhu, Q., and Wang, X. (2017). Exosomes derived from hypoxic epithelial ovarian cancer deliver microRNA-940 to induce macrophage M2 polarization. *Oncol. Rep.* 38, 522–528. doi:10.3892/or.2017.5697

Chen, X., Zhou, J., Li, X., Wang, X., Lin, Y., and Wang, X. (2018). Exosomes derived from hypoxic epithelial ovarian cancer cells deliver microRNAs to macrophages and elicit a tumor-promoted phenotype. *Cancer Lett.* 435, 80–91. doi:10.1016/j.canlet.2018.08.001

Cheng, L., Zhang, K., Qing, Y., Li, D., Cui, M., Jin, P., et al. (2020). Proteomic and lipidomic analysis of exosomes derived from ovarian cancer cells and ovarian surface epithelial cells. *J. ovarian Res.* 13, 9–13. doi:10.1186/s13048-020-0609-y

Chevillet, J. R., Kang, Q., Ruf, I. K., Briggs, H. A., Vojtech, L. N., Hughes, S. M., et al. (2014). Quantitative and stoichiometric analysis of the microRNA content of exosomes. *Proc. Natl. Acad. Sci. U. S. A.* 111, 14888–14893. doi:10.1073/pnas.1408301111

Dai, Y., Cao, Y., Köhler, J., Lu, A., Xu, S., and Wang, H. (2021). Unbiased RNA-Seq-driven identification and validation of reference genes for quantitative RT-PCR analyses of pooled cancer exosomes. *BMC genomics* 22, 27. doi:10.1186/s12864-020-07318-y

Donthu, N., Kumar, S., Mukherjee, D., Pandey, N., and Lim, W. M. (2021). How to conduct a bibliometric analysis: An overview and guidelines. *J. Bus. Res.* 133, 285–296. doi:10.1016/j.jbusres.2021.04.070

El Bairi, K., Al Jarroudi, O., and Afqir, S. (2021). Tracing ovarian cancer research in Morocco: A bibliometric analysis. *Gynecol. Oncol. Rep.* 37, 100777. doi:10.1016/j.gore.2021.100777

Fishman, D. A., Cohen, L., Blank, S. V., Shulman, L., Singh, D., Bozorgi, K., et al. (2005). The role of ultrasound evaluation in the detection of early-stage epithelial ovarian cancer. *Am. J. Obstetrics Gynecol.* 192, 1214–1221. doi:10.1016/j.ajog.2005.01.041

Gadducci, A., Cosio, S., and Lippolis, P. V. (2022). Hyperthermic intraperitoneal chemotherapy in the management of primary epithelial ovarian cancer: A debated issue for gynecologic oncologists. *Anticancer Res.* 42, 4659–4665. doi:10.21873/anticanres.15970

Gao, L., Nie, X., Gou, R., Hu, Y., Dong, H., Li, X., et al. (2021). Exosomal ANXA2 derived from ovarian cancer cells regulates epithelial-mesenchymal plasticity of human peritoneal mesothelial cells. *J. Cell. Mol. Med.* 25, 10916–10929. doi:10.1111/jcmm.16983

Ghafourian, M., Mahdavi, R., Akbari Jonoush, Z., Sadeghi, M., Ghadiri, N., Farzaneh, M., et al. (2022). The implications of exosomes in pregnancy: Emerging as new diagnostic markers and therapeutics targets. *Cell Commun. Signal.* 20, 51–19. doi:10.1186/s12964-022-00853-z

Gupta, A., Agrawal, M. A., Jaggi, K., and Goswami, A. D. (2021). *Bibliometric analysis of emerging technologies in the field of computer science helping in ovarian cancer research*.

Horie, K., Nanashima, N., Yokoyama, Y., Yoshioka, H., and Watanabe, J. (2022). Exosomal MicroRNA as biomarkers for diagnosing or monitoring the progression of ovarian clear cell carcinoma: A pilot study. *Molecules* 27, 3953. doi:10.3390/molecules27123953

Hu, Y., Li, D., Wu, A., Qiu, X., Di, W., Huang, L., et al. (2017). TWEAK-stimulated macrophages inhibit metastasis of epithelial ovarian cancer via exosomal shuttling of microRNA. *Cancer Lett.* 393, 60–67. doi:10.1016/j.canlet.2017.02.009

Hubert, A., Subra, C., Jenabian, M.-A., Labrecque, P.-F. T., Tremblay, C., Laffont, B., et al. (2015). Elevated abundance, size, and MicroRNA content of plasma extracellular vesicles in viremic HIV-1+ patients: Correlations with known markers of disease progression. *J. Acquir. immune Defic. syndromes* 70, 219–227. doi:10.1097/QAI.0000000000000756

Jeon, H., Seo, S. M., Kim, T. W., Ryu, J., Kong, H., Jang, S. H., et al. (2022). Circulating exosomal miR-1290 for diagnosis of epithelial ovarian cancer. *Curr. Issues Mol. Biol.* 44, 288–300. doi:10.3390/cimb44010021

Kanlikilicer, P., Bayraktar, R., Denizli, M., Rashed, M. H., Ivan, C., Aslan, B., et al. (2018). Exosomal miRNA confers chemo resistance via targeting Cav1/p-gp/M2-type macrophage axis in ovarian cancer. *EBioMedicine* 38, 100–112. doi:10.1016/j.ebiom.2018.11.004

Kanlikilicer, P., Rashed, M. H., Bayraktar, R., Mitra, R., Ivan, C., Aslan, B., et al. (2016). Ubiquitous release of exosomal tumor suppressor miR-6126 from ovarian cancer cells. *Cancer Res.* 76, 7194–7207. doi:10.1158/0008-5472.CAN-16-0714

Keserű, J. S., Soltész, B., Lukács, J., Márton, É., Szilágyi-Bónizs, M., Penyige, A., et al. (2019). Detection of cell-free, exosomal and whole blood mitochondrial DNA copy number in plasma or whole blood of patients with serous epithelial ovarian cancer. *J. Biotechnol.* 298, 76–81. doi:10.1016/j.jbiotec.2019.04.015

Khedkar, V., Fernandes, C., Desai, D., Mansi, R., Chavan, G., Tidke, S. K., et al. (2021). *Bibliometric analysis of named entity recognition for chemoinformatics and biomedical information extraction of ovarian cancer*. Lincoln: Library Philosophy and Practice, University of Nebraska, 1–10.

Kim, S., Choi, M. C., Jeong, J. Y., Hwang, S., Jung, S. G., Joo, W. D., et al. (2019). Serum exosomal miRNA-145 and miRNA-200c as promising biomarkers for preoperative diagnosis of ovarian carcinomas. *J. Cancer* 10, 1958–1967. doi:10.7150/jca.30231

Kobayashi, M., Salomon, C., Tapia, J., Illanes, S. E., Mitchell, M. D., and Rice, G. E. (2014). Ovarian cancer cell invasiveness is associated with discordant exosomal sequestration of Let-7 miRNA and miR-200. *J. Transl. Med.* 12, 4. doi:10.1186/1479-5876-12-4

Kobayashi, M., Sawada, K., Miyamoto, M., Shimizu, A., Yamamoto, M., Kinose, Y., et al. (2020). Exploring the potential of engineered exosomes as delivery systems for tumor-suppressor microRNA replacement therapy in ovarian cancer. *Biochem. biophysical Res. Commun.* 527, 153–161. doi:10.1016/j.bbrc.2020.04.076

Labani-Motlagh, A., Israelsson, P., Ottander, U., Lundin, E., Nagaev, I., Nagaeva, O., et al. (2016). Differential expression of ligands for NKG2D and DNAM-1 receptors by epithelial ovarian cancer-derived exosomes and its influence on NK cell cytotoxicity. *Tumor Biol.* 37, 5455–5466. doi:10.1007/s13277-015-4313-2

Lai, H., Guo, Y., Tian, L., Wu, L., Li, X., Yang, Z., et al. (2022). Protein panel of serum-derived small extracellular vesicles for the screening and diagnosis of epithelial ovarian cancer. *Cancers* 14, 3719. doi:10.3390/cancers14153719

Li, T., Lin, L., Liu, Q., Gao, W., Chen, L., Sha, C., et al. (2021). Exosomal transfer of miR-429 confers chemoresistance in epithelial ovarian cancer. *Am. J. cancer Res.* 11, 2124–2141.

Li, W., Zhang, X., Wang, J., Li, M., Cao, C., Tan, J., et al. (2017). TGFβ1 in fibroblasts-derived exosomes promotes epithelial-mesenchymal transition of ovarian cancer cells. *Oncotarget* 8, 96035–96047. doi:10.18632/oncotarget.21635

Li, X., Tang, M., Zhu, Q., Wang, X., Lin, Y., and Wang, X. (2020). The exosomal integrin α5β1/AEP complex derived from epithelial ovarian cancer cells promotes peritoneal metastasis through regulating mesothelial cell proliferation and migration. *Cell. Oncol.* 43, 263–277. doi:10.1007/s13402-019-00486-4

Li, X., and Wang, X. (2017). The emerging roles and therapeutic potential of exosomes in epithelial ovarian cancer. *Mol. cancer* 16, 92–10. doi:10.1186/s12943-017-0659-y

Liu, J., Yoo, J., Ho, J. Y., Jung, Y., Lee, S., Hur, S. Y., et al. (2021). Plasma-derived exosomal miR-4732-5p is a promising noninvasive diagnostic biomarker for epithelial ovarian cancer. *J. ovarian Res.* 14, 59–14. doi:10.1186/s13048-021-00814-z

Liu, Y., Liu, Q., and Jiang, X. (2022). Bibliometric analysis of hotspots and frontiers in cancer-related fatigue among ovarian cancer survivors. *PloS one* 17, e0274802. doi:10.1371/journal.pone.0274802

Liu, Y., Tang, J., Liu, D., Zhang, L., He, Y., Li, J., et al. (2018). Increased autophagy in EOC re-ascites cells can inhibit cell death and promote drug resistance. *Cell Death Dis.* 9, 419. doi:10.1038/s41419-018-0449-5

Lodewijk, I., Bernardini, A., Suárez-Cabrera, C., Bernal, E., Sánchez, R., Garcia, J., et al. (2022). Genomic landscape and immune-related gene expression profiling of epithelial ovarian cancer after neoadjuvant chemotherapy. *NPJ Precis. Oncol.* 6, 7–11. doi:10.1038/s41698-021-00247-3

López López, P. (1996). *La investigación bibliométrica, Introducción a la bibliometría*. Valencia, España: Promolibro, 43–63.

Lu, S., Liu, W., Shi, H., and Zhou, H. (2020). Exosomal miR-34b inhibits proliferation and the epithelial-mesenchymal transition by targeting Notch2 in ovarian cancer. *Oncol. Lett.* 20, 2721–2728. doi:10.3892/ol.2020.11837

Lucidi, A., Buca, D., Ronsini, C., Tinari, S., Bologna, G., Buca, D., et al. (2020). Role of extracellular vesicles in epithelial ovarian cancer: A systematic review. *Int. J. Mol. Sci.* 21, 8762. doi:10.3390/ijms21228762

Luo, Y., and Gui, R. (2020). Circulating exosomal circFoxp1 confers cisplatin resistance in epithelial ovarian cancer cells. *J. Gynecol. Oncol.* 31, e75. doi:10.3802/jgo.2020.31.e75

Ma, R., Ye, X., Cheng, H., Cui, H., and Chang, X. (2021). Tumor-derived exosomal circRNA051239 promotes proliferation and migration of epithelial ovarian cancer. *Am. J. Transl. Res.* 13, 1125–1139.

Ma, X., Wang, Y., Sheng, H., Tian, W., Qi, Z., Teng, F., et al. (2014). Prognostic significance of thrombocytosis, platelet parameters and aggregation rates in epithelial ovarian cancer. *J. Obstetrics Gynaecol. Res.* 40, 178–183. doi:10.1111/jog.12151

Maeda, K., Sasaki, H., Ueda, S., Miyamoto, S., Terada, S., Konishi, H., et al. (2020). Serum exosomal microRNA-34a as a potential biomarker in epithelial ovarian cancer. *J. ovarian Res.* 13, 47–49. doi:10.1186/s13048-020-00648-1

Mashouri, L., Yousefi, H., Aref, A. R., Molaei, F., and Alahari, S. K. (2019). Exosomes: Composition, biogenesis, and mechanisms in cancer metastasis and drug resistance. *Mol. cancer* 18, 75–14. doi:10.1186/s12943-019-0991-5

Masoumi-Dehghi, S., Babashah, S., and Sadeghizadeh, M. (2020). microRNA-141-3p-containing small extracellular vesicles derived from epithelial ovarian cancer cells promote endothelial cell angiogenesis through activating the JAK/STAT3 and NF-κB signaling pathways. *J. Cell Commun. Signal.* 14, 233–244. doi:10.1007/s12079-020-00548-5

Meng, X., Müller, V., Milde-Langosch, K., Trillsch, F., Pantel, K., and Schwarzenbach, H. (2016). "Circulating cell-free miR-373, miR-200a, miR-200b and miR-200c in patients with epithelial ovarian cancer," in *Circulating nucleic acids in serum and plasma–CNAPS IX* (Springer), 3–8.

Meng, X., Müller, V., Milde-Langosch, K., Trillsch, F., Pantel, K., and Schwarzenbach, H. (2016). Diagnostic and prognostic relevance of circulating exosomal miR-373, miR-200a, miR-200b and miR-200c in patients with epithelial ovarian cancer. *Oncotarget* 7, 16923–16935. doi:10.18632/oncotarget.7850

Miri, M. R., Zare, A., Saberzadeh, J., Baghban, N., Nabipour, I., and Tamadon, A. (2022). Anti-lung cancer marine compounds: A review. *Ther. Innovation Regul. Sci.* 56, 191–205. doi:10.1007/s43441-022-00375-3

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., and Group, P. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *Ann. Intern. Med.* 151, 264–269. doi:10.7326/0003-4819-151-4-200908180-00135

Nawaz, M., Fatima, F., Nazarenko, I., Ekström, K., Murtaza, I., Anees, M., et al. (2016). Extracellular vesicles in ovarian cancer: Applications to tumor biology, immunotherapy and biomarker discovery. *Expert Rev. proteomics* 13, 395–409. doi:10.1586/14789450.2016.1165613

Nazarenko, I., Rupp, A. K., and Altevogt, P. (2013). Exosomes as a potential tool for a specific delivery of functional molecules. *Methods Mol. Biol. Clift. N.J.)* 1049, 495–511. doi:10.1007/978-1-62703-547-7_37

Obermair, A., Beale, P., Scott, C. L., Beshay, V., Kichenadasse, G., Simcock, B., et al. (2021). Insights into ovarian cancer care: Report from the ANZGOG ovarian cancer webinar series 2020. *J. Gynecol. Oncol.* 32, e95. doi:10.3802/jgo.2021.32.e95

Olivier, R. I., Lubsen-Brandsma, M. A., Verhoef, S., and van Beurden, M. (2006). CA125 and transvaginal ultrasound monitoring in high-risk women cannot prevent the diagnosis of advanced ovarian cancer. *Gynecol. Oncol.* 100, 20–26. doi:10.1016/j.ygyno.2005.08.038

Padda, J., Khalid, K., Khedr, A., Patel, V., Al-Ewaidat, O. A., Tasnim, F., et al. (2021). Exosome-derived microRNA: Efficacy in cancer. *Cureus* 13, e17441. doi:10.7759/cureus.17441

Pan, C., Stevic, I., Müller, V., Ni, Q., Oliveira-Ferrer, L., Pantel, K., et al. (2018). Exosomal microRNAs as tumor markers in epithelial ovarian cancer. *Mol. Oncol.* 12, 1935–1948. doi:10.1002/1878-0261.12371

Pink, R. C., Samuel, P., Massa, D., Caley, D. P., Brooks, S. A., and Carter, D. R. (2015). The passenger strand, miR-21-3p, plays a role in mediating cisplatin resistance in ovarian cancer cells. *Gynecol. Oncol.* 137, 143–151. doi:10.1016/j.ygyno.2014.12.042

Qiu, J.-J., Lin, X.-J., Tang, X.-Y., Zheng, T.-T., Lin, Y.-Y., and Hua, K.-Q. (2018). Exosomal metastasis-associated lung adenocarcinoma transcript 1 promotes angiogenesis and predicts poor prognosis in epithelial ovarian cancer. *Int. J. Biol. Sci.* 14, 1960–1973. doi:10.7150/ijbs.28048

Salido-Guadarrama, I., Romero-Cordoba, S., Peralta-Zaragoza, O., Hidalgo-Miranda, A., and Rodriguez-Dorantes, M. (2014). MicroRNAs transported by exosomes in body fluids as mediators of intercellular communication in cancer. *OncoTargets Ther.* 7, 1327–1338. doi:10.2147/OTT.S61562

Shiao, M.-S., Chang, J.-M., Lertkhachonsuk, A.-A., Rermluk, N., and Jinawath, N. (2021). Circulating exosomal miRNAs as biomarkers in epithelial ovarian cancer. *Biomedicines* 9, 1433. doi:10.3390/biomedicines9101433

Siegel, R. L., Miller, K. D., and Jemal, A. (2019). Cancer statistics. *CA a cancer J. Clin.* 69, 7–34. doi:10.3322/caac.21551

Singh, S., Goyal, D., Karthikeyan, R., Kumar, S., Malik, P. S., and Elangovan, R. (2022). *RNA profile of immuno-magnetically enriched exosomes isolated from Non-Small Cell Lung Cancer*.

Soltész, B., Lukács, J., Szilágyi, E., Márton, É., Szilágyi Bónizs, M., Penyige, A., et al. (2019). Expression of CD24 in plasma, exosome and ovarian tissue samples of serous ovarian cancer patients. *J. Biotechnol.* 298, 16–20. doi:10.1016/j.jbiotec.2019.03.018

Su, Y. Y., Sun, L., Guo, Z. R., Li, J. C., Bai, T. T., Cai, X. X., et al. (2019). Upregulated expression of serum exosomal miR-375 and miR-1307 enhance the diagnostic power of CA125 for ovarian cancer. *J. Ovarian Res.* 12, 6. doi:10.1186/s13048-018-0477-x

Tang, X., Liu, S., Liu, Y., Lin, X., Zheng, T., Liu, X., et al. (2019). Circulating serum exosomal aHIF is a novel prognostic predictor for epithelial ovarian cancer. *OncoTargets Ther.* 12, 7699–7711. doi:10.2147/OTT.S220533

Taylor, D. D., and Gercel-Taylor, C. (2008). MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecol. Oncol.* 110, 13–21. doi:10.1016/j.ygyno.2008.04.033

Bartosh, T. J., Ullah, M., Zeitouni, S., Beaver, J., and Prockop, D. J. (2016). Cancer cells enter dormancy after cannibalizing mesenchymal stem/stromal cells (MSCs). *Proc. Natl. Acad. Sci.* 113 E6447–E6456. doi:10.1073/pnas.1612290113

van Baal, J., van Noorden, C. J. F., Nieuwland, R., Van de Vijver, K. K., Sturk, A., van Driel, W. J., et al. (2018). Development of peritoneal carcinomatosis in epithelial ovarian cancer: A review. *J. Histochem. Cytochem. official J. Histochem. Soc.* 66, 67–83. doi:10.1369/0022155417742897

Wang, B., Xing, D., Zhu, Y., Dong, S., and Zhao, B. (2019). The state of exosomes research: A global visualized analysis. *BioMed Res. Int.* 2019, 1495130. doi:10.1155/2019/1495130

Wang, Q., Sun, L., Wang, Y., and Wang, S. (2016). *A bibliometric study on review over 1992-2014 literature on life quality of ovarian cancer patients in China*. Modern Clinical Nursing, 47–50.

Wang, W.-T., and Chen, Y.-Q. (2014). Circulating miRNAs in cancer: From detection to therapy. *J. Hematol. Oncol.* 7, 86–89. doi:10.1186/s13045-014-0086-0

Wang, Y., Wang, Q., Wei, X., Shao, J., Zhao, J., Zhang, Z., et al. (2017). Global scientific trends on exosome research during 2007–2016: A bibliometric analysis. *Oncotarget* 8, 48460–48470. doi:10.18632/oncotarget.17223

Wanyama, F. M., Tauber, R., Mokomba, A., Nyongesa, C., and Blanchard, V. (2022). The burden of hepatitis B, hepatitis C, and human immunodeficiency viruses in ovarian cancer patients in nairobi, Kenya. *Infect. Dis. Rep.* 14, 433–445. doi:10.3390/idr14030047

Wu, Q., Wu, X., Ying, X., Zhu, Q., Wang, X., Jiang, L., et al. (2017). Suppression of endothelial cell migration by tumor associated macrophage-derived exosomes is reversed by epithelial ovarian cancer exosomal lncRNA. *Cancer Cell Int.* 17, 62–13. doi:10.1186/s12935-017-0430-x

Xiao, F., Xiao, S., and Xue, M. (2019). miR-139 controls viability of ovarian cancer cells through apoptosis induction and exosome shedding inhibition by targeting ATP7A. *Onco Targets Ther.* 12, 10727–10737. doi:10.2147/OTT.S221236

Xiong, C., Sun, Z., Yu, J., and Lin, Y. (2021). Exosome component 4 promotes epithelial ovarian cancer cell proliferation, migration, and invasion via the wnt pathway. *Front. Oncol.* 11, 797968. doi:10.3389/fonc.2021.797968

Xiong, J., He, X., Xu, Y., Zhang, W., and Fu, F. (2021). MiR-200b is upregulated in plasma-derived exosomes and functions as an oncogene by promoting macrophage M2 polarization in ovarian cancer. *J. Ovarian Res.* 14, 74. doi:10.1186/s13048-021-00826-9

Xu, C., and Li, X. (2022). Research trends in the early diagnosis of ovarian cancer during 2001–2020: A bibliometric analysis. *Eur. J. Gynaecol. Oncol.* 43, 321–334.

Yang, S., Zhao, H., Xiao, W., Shao, L., Zhao, C., and Sun, P. (2022). Extracellular vesicle-packaged miR-181c-5p from epithelial ovarian cancer cells promotes M2 polarization of tumor-associated macrophages via the KAT2B/HOXA10 axis. *J. Gene Med.* 24, e3446. doi:10.1002/jgm.3446

Ying, X., Wu, Q., Wu, X., Zhu, Q., Wang, X., Jiang, L., et al. (2016). Epithelial ovarian cancer-secreted exosomal miR-222-3p induces polarization of tumor-associated macrophages. *Oncotarget* 7, 43076–43087. doi:10.18632/oncotarget.9246

Yousafzai, N. A., Wang, H., Wang, Z., Zhu, Y., Zhu, L., Jin, H., et al. (2018). Exosome mediated multidrug resistance in cancer. *Am. J. cancer Res.* 8, 2210–2226.

Zeh, N., Schneider, H., Mathias, S., Raab, N., Kleemann, M., Schmidt-Hertel, S., et al. (2019). Human CAP cells represent a novel source for functional, miRNA-loaded exosome production. *PLoS One* 14, e0221679. doi:10.1371/journal.pone.0221679

Zhang, H., and Lu, B. (2020). microRNAs as biomarkers of ovarian cancer. *Expert Rev. anticancer Ther.* 20, 373–385. doi:10.1080/14737140.2020.1760095

Zhang, H., Xu, S., and Liu, X. (2019). MicroRNA profiling of plasma exosomes from patients with ovarian cancer using high-throughput sequencing. *Oncol. Lett.* 17, 5601–5607. doi:10.3892/ol.2019.10220

Zhang, S., Zhang, X., Fu, X., Li, W., Xing, S., and Yang, Y. (2018). Identification of common differentially-expressed miRNAs in ovarian cancer cells and their exosomes compared with normal ovarian surface epithelial cell cells. *Oncol. Lett.* 16, 2391–2401. doi:10.3892/ol.2018.8954

Zhang, W., Ou, X., and Wu, X. (2019). Proteomics profiling of plasma exosomes in epithelial ovarian cancer: A potential role in the coagulation cascade, diagnosis and prognosis. *Int. J. Oncol.* 54, 1719–1733. doi:10.3892/ijo.2019.4742

Zhang, W., Peng, P., Kuang, Y., Yang, J., Cao, D., You, Y., et al. (2016). Characterization of exosomes derived from ovarian cancer cells and normal ovarian epithelial cells by nanoparticle tracking analysis. *Tumor Biol.* 37, 4213–4221. doi:10.1007/s13277-015-4105-8

Zhang, W., Yang, J., Cao, D., You, Y., Shen, K., and Peng, P. (2016). Regulation of exosomes released from normal ovarian epithelial cells and ovarian cancer cells. *Tumor Biol.* 37, 15763–15771. doi:10.1007/s13277-016-5394-2

Zhankina, R., Baghban, N., Askarov, M., Saipiyeva, D., Ibragimov, A., Kadirova, B., et al. (2021). Mesenchymal stromal/stem cells and their exosomes for restoration of spermatogenesis in non-obstructive azoospermia: A systemic review. *Stem Cell Res. Ther.* 12, 229–312. doi:10.1186/s13287-021-02295-9

Zhao, L., Corvigno, S., Ma, S., Celestino, J., Fleming, N. D., Hajek, R. A., et al. (2022). Molecular profiles of serum-derived extracellular vesicles in high-grade serous ovarian cancer. *Cancers* 14, 3589. doi:10.3390/cancers14153589

Zhao, Z., Shuang, T., Gao, Y., Lu, F., Zhang, J., He, W., et al. (2022). Targeted delivery of exosomal miR-484 reprograms tumor vasculature for chemotherapy sensitization. *Cancer Lett.* 530, 45–58. doi:10.1016/j.canlet.2022.01.011

Zhou, J., Li, X., Wu, X., Zhang, T., Zhu, Q., Wang, X., et al. (2018). Exosomes released from tumor-associated macrophages transfer miRNAs that induce a Treg/Th17 cell imbalance in epithelial ovarian cancer. *Cancer Immunol. Res.* 6, 1578–1592. doi:10.1158/2326-6066.CIR-17-0479

Zhu, X.-L., Wang, H.-J., Wang, X.-R., Wu, D., Ji, X., Xu, L., et al. (2022). IL-6 secretion of CD4+ T cells stimulated by LC3-positive extracellular vesicles in human epithelial ovarian cancer. *Clin. Transl. Oncol.* 24, 2222–2230. doi:10.1007/s12094-022-02883-y

Zhu, X., Shen, H., Yin, X., Yang, M., Wei, H., Chen, Q., et al. (2019). Macrophages derived exosomes deliver miR-223 to epithelial ovarian cancer cells to elicit a chemoresistant phenotype. *J. Exp. Clin. Cancer Res.* 38, 81–14. doi:10.1186/s13046-019-1095-1

Zhu, Z., Chen, Z., Wang, M., Zhang, M., Chen, Y., Yang, X., et al. (2022). Detection of plasma exosomal miRNA-205 as a biomarker for early diagnosis and an adjuvant indicator of ovarian cancer staging. *J. Ovarian Res.* 15, 27. doi:10.1186/s13048-022-00961-x

Zupic, I., and Čater, T. (2015). Bibliometric methods in management and organization. *Organ. Res. Methods* 18, 429–472. doi:10.1177/1094428114562629

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Patterns of acute poisoning for children during outbreak of Corona virus in Makkah region Saudi Arabia

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Background: Poisoning occurs when a person is exposed to an external substance at a too high dose for them. It is possible for young children to be exposed to chemicals. Lungs, the heart, CNS, the digestive tract, and kidneys can be poisoned. In 2004, over 45,000 children and teenagers died from acute poisoning, representing 13% of all accidental poisoning deaths worldwide. Poisoning patterns vary by exposure type, age group, poison type, and dose.
Aim: This study assessed the pattern of acute poisoning with drugs, chemicals, and natural toxins among children (<12 years old). The study was done in Makkah region and registered in the poison control center in Makkah, the forensic chemistry center in Haddah during 2020–2021.
Methods: A retrospective cohort study was done on 122 children exposed to toxic substances in Makkah. The children were 12 years old and had good health for a maximum of one year. Stratified random sampling was used to divide cases into groups of similar poisons (pharmaceutical products, household products, plant envenomation, and animal envenomation). Then each group got a random samples. The data were analysed with SPSS software.
Results: The mean age of children was 5.2 years, with 59% being boys. The mean temperature, pulse, systolic, diastolic, and respiratory rates were 36.77, 98.29, 109.1, 69.17, and 21.49. The most documented pharmaceutical products (200 mg) were carbamazepine (5 mg), methanol, risperidone (5 mg), propranolol (5 mg), and olanzapine (5 mg). The most common poison forms were tablets (42.6%), syrups (15.6%), capsules (13.9%), and solutions (13.1%). The most common poisoning routes were ingestion (82.8%), dermal (5.7%), injection (4.9%), and inhalation (6.6%). Accidental poisoning was 83%, with a 30-minute lag for 30.3% of children, and most (69.7%) occurred at home. Benzodiazepines were the most commonly used category class drug (18%), with normal pupils and an ECG of 85.2%. Sixty-seven percent had blood tests. Sickness was 9.48, and the positive result was 213.01. The most common presenting symptoms were GIT and neurological (23.8%). 31.1% had mild, moderate, or severe toxicity. Most cases (68%) were complex. 34.4% were intubated, 9.8% had repeated-dose-activated charcoal for enhanced elimination, and 27.8% were on IV fluids. Children with GIT, CVS, respiratory, dermal, and neurological symptoms had a higher percentage of severe toxicity ($p < 0.05$). Slight toxicity was associated with whole bowel irrigation, intubation for oxygen therapy, N-acetylcysteine or

sedation, fluids, and phenytoin ($P < 0.05$). Complicated cases had a higher mean AST/IUL than non-complicated cases (75.5 vs. 20.08, $p < 0.05$). The level of toxicity did not correlate with the mean of all lab tests ($p > 0.05$). The age of the children correlated positively with their systolic BP ($r = 0.22$, $p < 0.01$).

Conclusion: The results show how important it is to teach the public about poisoning and make rules for tracking and dealing with poisonings in Saudi Arabia.

KEYWORDS

patterns, acute, poisoning, children, outbreak, Corona virus, makkah

Introduction

Accidental poisonings take place when a person, typically a child, ingests a poisonous substance without intending to do so (as opposed to purposeful poisoning or overdosing) (1). Children are more likely to experience serious repercussions from poisoning due to the fact that their bodies are smaller, have a faster metabolic rate, and are less capable of neutralizing harmful substances (2). Children who are poisoned might experience psychological and physical repercussions over a long period of time, and the costs to society can be quite high (3).

Poisons ingested can be classified into medications (prescription or non-prescription), household items, and plants Their level of toxicity could be mild, moderate, or severe (4). Poisoning patterns vary depending on the type of exposure, age group, nature, and dose of the poison (5, 6).

Acute poisoning is a common occurrence in emergency rooms worldwide, necessitating extensive medical care and significant financial investment (7). A high number of acute poisoning cases were caused by drug poisoning. Natural poisons, such as toxic plants and animals and acute chemical poisonings in the home, are frequent, especially in children (8). There are various variations in the pattern and etiology of acute poisoning, even within the same geographical region (8).

The pattern and types of poisons vary depending on numerous factors such as demography, education, socioeconomic level, and local beliefs and practices in different parts of the world. As a result, each country needs its epidemiological surveillance to establish the scope and pattern of the disease so that preventative steps can be taken (9).

Knowing the overall trend of poisoning in a certain area can aid in identifying risk factors and enabling early discovery and treatment of such cases, lowering morbidity and mortality (8). Poisoning occurrences were treated differently depending on the patient's condition, the type of poisoning, and the length of exposure (10).

In Saudi Arabia (KSA), acute poisoning in children and adults has been reported in several Saudi cities, including Jeddah, Hafr Al Batin, Abha, and Al Riyadh (11). In Abha, there were 114 acute poisoning incidents in children between January 2000 and October 2003 (12). At King Khaled Hospital in the Al Majmaah region of Saudi Arabia, a study done in 2014 found that most instances were caused by animal envenomation (13). Alghadeer et al., 2018 found that most instances were asymptomatic, and most of the youngsters arrived at the hospital in under three hours (5).

Another perspective study conducted in Riyadh in 2019 found that toxic household goods were the most implicated substance class in children under the age of six (13), and recently in 2020, a study conducted at East Jeddah Hospital in Jeddah city found that unintentional poisoning occurred in 56.5% of recorded instances and 92.8% of incidents occurred at home (14).

This study aimed to assess the pattern of acute poisoning with drugs, chemicals, and natural toxins of children (≤12) in the Makkah region and registered in the poison control center in Makkah, Saudi Arabia. This study can provide additional information about the agents most commonly involved in poisoning in this region and prevention and management guidelines to avoid them.

Subjects and methods

Study design

A retrospective cohort study was done.

Study population, setting and time frame

122 children with toxicity from the Children's Hospital in Makkah region of Saudi Arabia and registered in the poison control center in Makkah, forensic chemistry center in Haddah during 2020–2021 were included. The inclusion criteria were all children taking toxic substances any way route, of both genders, and children who live in Makkah. The exclusion criteria were forensic toxicology children with poisoning, adults, those that do not need medical management or recommendation, and children from regions other than Makkah.

Sampling methodology

Stratified Random Sampling was done by dividing cases into groups according to the type of poisons with similar attributes (Pharmaceutical products-Household products-plant envenomation-animal envenomation). Then, from each group, a random sample was drawn. Children were classified into having mild, moderate, and severe toxicity as follows: (1) *Mild*; transient and spontaneously resolving symptoms, (2) *Moderate*; pronounced or prolonged symptoms, and (3) *Severe* or life-

threatening symptoms. The sample size was calculated by this website (Qualtrics XM of sample size calculator). This equation was done at a confidence level of (95%) and a (5%) margin of error. The ideal sample size was (one hundred twenty-two).

Ethical considerations

No issues regarding animal subjects. This study has had the approval of the research ethics committee at the Department of the deanship of postgraduate studies at Umm al Qura University in the Makkah region and the poison control center in Makkah forensic chemistry center. Written informed consent for the research was not required in accordance with National legislation and institutional requirements. No identifiable human images or data was present in the study.

Data analysis

The data were analyzed using a statistical package for social sciences (SPSS) version 26. (Armonk, NY: IBM Corp.). Qualitative data were expressed as numbers and percentages to test the relationship between variables, and the Chi-squared test ($\chi 2$) was used. Quantitative data were expressed as mean and standard deviation (Mean ± SD), and non-parametric variables were tested using the Mann-Whitney (U) and Kruskal Wallis tests. Correlation analysis was performed using the Spearman's test, and a p-value of less than 0.05 was considered statistically significant.

Results

The mean age of the studied children was 5.2 ± 3.74 years, and the mean BMI was 26.67 ± 24.86 kg/m^{2,} respectively. Of the children, 59% were males, and 58.2% had a Saudi nationality. 38.5% of children had mild toxicity, while 31.1% and 30.3% had moderate and severe toxicity. And most of the cases (68%) were complicated. However, 32% of cases were not complicated. For 30.3% of children, the duration since poisoning was 30 min, 26.2% was one hour, 21.3% was 2 h, 8.2% was 3–12 h, and for 13.9%, the duration was 12 h. For most children (69.7%), the poisoning happened at home.

Table 1 shows that the most common documented pharmaceutical products were Depakine, Olanzapine (5 mg), Cannabinoids, Carbamazepine, Hydrogen peroxide (6%), Methanol, Risperidone lorazepam, Propranolol, and valproate sodium (200 mg). A non-significant relationship was found between the level of toxicity and the taken pharmaceutical products ($p > 0.05$).

Table 2 shows that the most common category class drug used were Benzodiazepine (18%), followed by Analgesic non opioid (15.6%), senna leaves rhubarb root (7.4%), and alcohol or NSAID (4.1%). A non-significant relationship was found between the level of toxicity and major category class drugs used ($p > 0.05$).

TABLE 1 Relationship between pharmaceutical products and the level of toxicity.

Variable	Total	Toxicity level			$\chi 2$	p-value
	No. (%)	Mild	Moderate	Severe		
Pharmaceutical products						
– Depakine	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Olanzapine 5 mg	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)		
– Cannabinoids	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)	13.8	0.464
– Carbamazepine	1 (0.8)	0 (0.0)	0 (0.0)	1 (100)		
– Hydrogen peroxide 6%	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Methanol	1 (0.8)	0 (0.0)	0 (0.0)	1 (100)		
– Risperidone	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)		
– Lorazepam	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)		
– Propranolol	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Valproate-Na (200 mg)	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)		
Dosage form						
– Capsule	17 (13.9)	6 (35.3)	8 (47.1)	3 (17.6)	6.1	0.412
– Chewable tablet	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Gas	2 (1.6)	0 (0.0)	1 (50)	1 (50)		
– Injection	6 (4.9)	2 (33.3)	3 (50)	1 (176.7)		
– Mouth wash	1 (0.8)	0 (0.0)	0 (0.0)	1 (100)		
– Powder	3 (4.5)	0 (0.0)	0 (0.0)	3 (100)		
– Solution	16 (13.1)	5 (31.3)	5 (31.3)	6 (37.5)		
– Suppository	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Suspension	3 (2.5)	2 (66.7)	1 (33.3)	0 (0.0)		
– Syrup	19 (15.6)	5 (26.3)	5 (26.3)	9 (47.4)		
– Tablet	52 (42.6)	25 (48.1)	14 (26.9)	13 (25)		
– Inhalation	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		

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TABLE 2 Relationship between major drug's class and level of toxicity.

Major drug class	Total	Toxicity level			$\chi 2$	*p*-value
	No. (%)	Mild	Moderate	Severe		
– 3,4-Methyl one deoxymethamphetamine	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Aerosol spray	1 (0.8)	0 (0.0)	0 (0.0)	1 (100)		
– Alcohol	5 (4.1)	2 (40)	2 (40)	1 (20)		
– Analgesic	2 (1.6)	2 (100)	0 (0.0)	0 (0.0)	84.74	0.145
– Analgesic non-opioid	19 (15.6)	10 (52.6)	8 (42.1)	1 (5.3)		
– Anticholinergic	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)		
– Anticonvulsants	2 (1.6)	0 (0.0)	1 (50)	1 (50)		
– Antidiabetic drug	2 (1.6)	1 (50)	1 (50)	0 (0.0)		
– Antidepressant	2 (1.6)	0 (0.0)	2 (100)	0 (0.0)		
– Antiepileptic, anticonvulsant	2 (1.6)	0 (0.0)	1 (50)	1 (50)		
– Antihistamine	3 (2.5)	2 (50)	1 (50)	0 (0.0)		
– Antipsychotic and antihypertensive	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Antipyretic	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Atypical antipsychotics	3 (2.5)	0 (0.0)	1 (33.3)	2 (66.7)		
– Benzodiazepine	22 (18)	8 (36.4)	10 (45.4)	4 (18.2)		
– Cannabis	3 (2.5)	0 (0.0)	0 (0.0)	3 (100)		
– CNS stimulant	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)		
– CNS stimulants, Hallucinogens	1 (0.8)	0 (0.0)	0 (0.0)	1 (100)		
– Corrosive agent	3 (2.5)	1 (33.3)	0 (0.0)	2 (66.7)		
– Detergent	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)		
– General anesthetics	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Glycopeptide antibiotics	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)		
– Hallucinogens	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Heavy metal	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)		
– Hydrogen peroxide	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– NSAID	5 (4.1)	1 (20)	3 (60)	1 (20)		
– Opioid	4 (3.3)	2 (50)	2 (50)	0 (0.0)		
– Phenethylamine	7 (5.7)	0 (0.0)	2 (29)	5 (71)		
– Proton pump inhibitors	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Psychoactive drugs	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Second-generation antipsychotics	3 (2.5)	0 (0.0)	2 (66.7)	1 (33.3)		
– Sedative-hypnotics	2 (1.6)	0 (0.0)	1 (50)	1 (50)		
– Selective serotonin reuptake inhibitors	3 (2.5)	0 (0.0)	2 (66.7)	1 (33.3)		
– Senna leaves Rhubarb Root Powder	9 (7.4)	3 (33.3)	3 (33.3)	3 (33.3)		
– Supplement food	2 (1.6)	2 (100)	0 (0.0)	0 (0.0)		
– Volatile substances	4 (3.3)	2 (50)	1 (25)	1 (25)		

Table 3 shows that the most common presenting symptoms were both GIT and neurological symptoms (23.8%), followed by only neurological symptoms (16.4%) and only GIT symptoms (13.1%). The table shows that children who were presented with (CVS, dermal and neurological symptoms) or (CVS and neurological symptoms) or (GIT, CVS, respiratory, dermal and neurological symptoms) or (GIT, CVS, dermal and neurological) or (Respiratory, dermal and neurological) had a significantly higher 100% of having severe toxicity ($p < 0.05$).

Table 4 shows that almost one-quarter of children had activated charcoal (25.4%), 8.2% received N-acetylcysteine, (34.4%) were intubated, and (9.8%) had repeated-dose-activated charcoal for enhancement elimination, 9% had IV fluids as pre-hospital management, and 3.3% were ventilated. Almost one-third of children were on IV fluid (27.8%), 4.1% were on Activated charcoal or N-acetylcysteine or antibiotics, 7.4% were on Flumazenil, and 1.6% were on omeprazole injection. Children who had whole bowel irrigation were intubated for oxygen therapy and had N-acetylcysteine or sedation, fluids, and phenytoin had a significantly higher % of having severe toxicity ($p < 0.05$).

Table 5 demonstrated a non-significant relationship between the level of toxicity among studied children and their age, height, weight BM, gender, nationality, pharmaceutical products, or poison forms ($p > 0.05$).

Table 6 shows that the most common poison forms were tablets (42.6%), syrups (15.6%), capsules (13.9%), solutions (13.1%) poisoning by pharmaceutical products (80.3%), household products accounts (12.3%), plant envenomation (5.7%,) and by animal envenomation (1.6%) as for the route of poisoning, ingestion accounts for (82.8%), dermal route (5.7%), injection (4.9%), inhalation (6.6%). Accidental poisoning was (83%), and intentional poisoning accounts for (39%) of cases. A non-significant relationship was found between the level of toxicity and the taken pharmaceutical products, poison forms, route or mode of poisoning ($p => 0.05$).

TABLE 3 Relationship between presenting symptoms and level of toxicity.

Symptoms	Total	Toxicity level			χ2	*p*-value
	No. (%)	Mild	Moderate	Severe		
– Only GIT symptoms	20 (13.1)	14 (62.5)	6 (37.5)	0 (0.0)	70.23	0.004
– GIT, CVS, respiratory, dermal, and neurological	2 (1.6)	0 (0.0)	0 (0.0)	2 (100)		
– GIT, CVS, respiratory, and neurological	2 (1.6)	0 (0.0)	2 (100)	0 (0.0)		
– GIT, CVS, dermal, and neurological	2 (1.6)	0 (0.0)	0 (0.0)	2 (100)		
– GIT, CVS, and neurological	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)		
– GIT and respiratory	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– GIT, respiratory, and dermal	2 (1.6)	0 (0.0)	2 (100)	0 (0.0)		
– GIT, respiratory, dermal, and neurological	5 (4.1)	1 (20)	4 (80)	0 (0.0)		
– GIT, respiratory and neurological	12 (9.8)	5 (41.7)	3 (25)	4 (33.3)		
– GIT and dermal symptoms	3 (2.5)	2 (66.7)	0 (0.0)	1 (33.3)		
– GIT, dermal, and neurological	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– GIT and neurological	29 (23.8)	16 (55.2)	8 (27.6)	5 (17.2)		
– Only CVS	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)		
– CVS, respiratory, and neurological	3 (2.5)	1 (33.3)	0 (0.0)	2 (66.7)		
– CVS, dermal, and neurological	2 (1.6)	0 (0.0)	0 (0.0)	2 (100)		
– CVS and neurological symptoms	1 (0.8)	0 (0.0)	0 (0.0)	1 (100)		
– Only respiratory	5 (4.1)	1 (20)	1 (20)	3 (60)		
– Respiratory, dermal, and neurological	1 (0.8)	0 (0.0)	0 (0.0)	1 (100)		
– Respiratory and neurological	5 (4.1)	0 (0.0)	2 (40)	3 (60)		
– Dermal and neurological	3 (2.5)	0 (0.0)	1 (33.3)	2 (66.7)		
– Only neurological symptoms	21 (16.4)	5 (25)	6 (30)	10 (45)		

Table 7 shows that complicated cases had a significantly higher mean level of AST (IU/l) than non-complicated cases (75.5 vs. 20.08 ($p < 0.05$). On the other hand, a non-significant relationship was found between the presence of complicated cases and all other laboratory test results of the studied children ($p > 0.05$).

Discussion

This study aimed to assess and analyse the patterns of acute poisoning cases with drugs, chemicals, and natural toxins for children in the Makkah region of Saudi Arabia registered in the Makkah poison control center and the Haddah forensic chemistry center.

In this study, male children accounted for 59% of the affected cases compared to 41% females. And the mean age was 5.2 ± 3.74 years. A previous Saudi study done in 2018 in Riyadh city found a prevalence of 49.7% among females, and the mean age of children was 2.7 ± 2.1 years (8).

Between 2010 and 2016, a review study conducted in the Riyadh region found that more than half of poisoning cases (62%) happened in youngsters under two (5). Another study conducted in the Jeddah region discovered that most cases occurred in male children (1), which was comparable with international studies (15, 16). Previously, the study that conducted in Sri Lanka reported that the majority of children who ingested poisons were under the age of five years (16). The WHO also reported an overall higher rate of poisoning in boys than girls in different world regions (15).

Paracetamol was shown to be the most dangerous pharmaceutical product (94%), followed by other pharmaceuticals such as Depakine, Olanzapine 5 mg, and Risperidone lorazepam. In the present study, the most common category class drug used were Benzodiazepine (18%), followed by analgesic non opioid (15.6%), senna leaves rhubarb root (7.4%), and alcohol or NSAID (4.1%). A study conducted in Jeddah in 2020 found that ingested medicines were the leading cause of acute poisoning (73.9%), a finding that was consistent with a previous study (14) in which medicinal products were the leading cause of poisoning. Previously, the most frequently involved drug class was weak analgesics dominated by paracetamol ($n = 91$, 35%), followed by opioids and benzodiazepines (17).

Many reports, especially from Saudi Arabia, support this finding, highlighting medicine's role in self-poisoning (5, 18). The drug administration providing a reason this to delivering medication in envelopes rather than child-resistant containers, easy access to drugs without prescriptions, and irresponsible home drug storage (19). Furthermore, Saudi Arabia's higher rating for unintended drug poisoning may be related to Saudi families' habit of storing unused prescriptions for future usage (5). The ready availability of medications and chemicals in various forms at home and a lack of parental monitoring to keep these materials in a safe place and out of reach of children were the most common causes of childhood poisoning.

In the present work, it was observed that the most common dosage forms were tablets (41.8%). According to a previous study conducted in Abha (20), tablets' most common poison types (19). The present work found that ingestion was the most common rote of poisoning (93.4%), followed by cutaneous poisoning (13.9%), inhalation (9.8%), and injection (9.8%). This result agrees with previous studies done in KSA (5, 20).

This work observed that accidental poisoning accounted for (68%) of cases compared to 31.1% for intentional poisoning. The

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TABLE 4 Relationship between GIT decontamination, antidote, oxygen therapy, enhancement elimination, pre-hospital management, and current medications and level of toxicity.

Variable	Total	Toxicity level			χ2	*p*-value
	No. (%)	Mild	Moderate	Severe		
GIT decontamination						
– No GIT decontamination	71 (58.2)	19 (26.8)	22 (31)	30 (42.3)		
– Activated charcoal	31 (25.4)	20 (64.5)	8 (25.8)	3 (9.7)	23.17	0.01
– Dilution	3 (2.5)	2 (66.7)	1 (33.3)	0 (0.0)		
– Gastric lavage	15 (12.3)	5 (33.3)	7 (46.7)	3 (20)		
– Ipecac syrup	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Whole bowel irrigation	1 (0.8)	0 (0.0)	0 (0.0)	1 (100)		
Antidote						
– No antidote	97 (79.5)	42 (43.3)	28 (28.9)	27 (27.8)	18.69	0.177
– Atropine	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)		
– Deferoxamine	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Flumazenil	7 (5.7)	1 (14.3)	1 (14.3)	5 (71.4)		
– N-Acetylcysteine	10 (8.2)	2 (20)	5 (50)	3 (30)		
– Naloxone	4 (3.3)	0 (0.0)	2 (50)	2 (50)		
– Physostigmine	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)		
– Thiamin	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
Oxygen therapy						
– No oxygen therapy	80 (65.6)	39 (48.4)	22 (27.5)	19 (23.8)	10.61	0.005
– Intubated	42 (34.4)	8 (19)	16 (38.1)	18 (42.9)		
Enhancement Elimination						
– No enhancement elimination	103 (84.4)	40 (38.8)	31 (30.1)	32 (31.1)		
– Forced acidic diuresis	2 (1.6)	0 (0.0)	2 (100)	0 (0.0)		
– Forced alkaline diuresis	3 (2.5)	1 (33.3)	1 (33.3)	1 (33.3)	9.68	0.468
– Hemodialysis	1 (0.8)	0 (0.0)	0 (0.0)	1 (100)		
– Hemoperfusion	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)		
– Repeated-dose activated charcoal	12 (9.8)	6 (50)	3 (25)	3 (25)		
Pre-hospital Management						
– No pre-hospital management	97 (79.5)	43 (44.3)	24 (24.7)	30 (30.9)		
– Activated charcoal	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Atropine-Diazepam	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)	37.45	0.039
– Diazepam, phenytoin and phenobarbitone	1 (0.8)	0 (0.0)	0 (0.0)	1 (100)		
– Drink juice	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)		
– Drink water	2 (1.6)	0 (0.0)	2 (100)	0 (0.0)		
– Flumazenil	1 (0.8)	0 (0.0)	0 (0.0)	1 (100)		
– IV fluid	10 (8.2)	1 (10)	8 (80)	1 (10)		
– IV fluid and vitamin	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)		
– Keppra 500 mg	1 (0.8)	1 (10)	0 (0.0)	0 (0.0		
– N-acetylcysteine	1 (0.8)	0 (0.0)	0 (0.0)	1 (100)		
– Sedation, fluids, and phenytoin	1 (0.8)	1 (100)	0 (0.0)	1 (100)		
– Ventilated	4 (3.3)	1 (25)	1 (25)	2 (50)		
Current medications						
– No current medications	48 (39.3)	18 (37.5)	14 (29.2)	16 (33.3)		
– Activated charcoal	5 (4.1)	2 (40)	1 (20)	2 (40)	51.84	0.326
– Acyclovir and ceftriaxone	3 (2.5)	1 (33.3)	1 (33.3)	1 (33.3)		
– Antibiotic	5 (4.1)	3 (60)	0 (0.0)	2 (40)		
– Ceftriaxone, acyclovir, and vancomycin	1 (0.8)	0 (0.0)	0 (0.0)	1 (100)		
– Fentanyl and midazolam	1 (0.8)	0 (0.0)	0 (0.0)	1 (100)		
– Flumazenil	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– IV fluid	32 (26.2)	12 (37.5)	15 (46.9)	5 (15.6)		
– IV fluid, thiamin	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– IV fluid, vitamin	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Laxative	2 (1.6)	1 (50)	1 (50)	0 (0.0)		
– Mannitol, dezocine	1 (0.8)	0 (0.0)	0 (0.0)	1 (100)		
– Mechanical ventilation	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Methylprednisolone	1 (0.8)	0 (0.0)	1 (100)	0 (0.0)		
– N-acetylcysteine	5 (4.1)	1 (20)	2 (40)	2 (40)		
– NAD	2 (1.6)	0 (0.0)	2 (100)	0 (0.0)		

(*continued*)

TABLE 4 Continued

Variable	Total	Toxicity level			χ2	*p*-value
	No. (%)	Mild	Moderate	Severe		
– Naloxone	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Observation	2 (1.6)	1 (50)	1 (50)	0 (0.0)		
– Omeprazole injection	2 (1.6)	0 (0.0)	0 (0.0)	2 (100)		
– Pain killer, antibiotic	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Supportive treatment	1 (0.8)	0 (0.0)	1 (0.0)	0 (0.0)		
– Tazocine and IV fluid	1 (0.8)	0 (0.0)	0 (0.0)	1 (100)		
– Vancomycin	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Ventolin, Methylprednisolone, phenytoin, vancomycin, and acyclovir	1 (0.8)	1 (100)	0 (0.0)	0 (0.0)		
– Vitamin	2 (1.6)	2 (100)	0 (0.0)	0 (0.0)		

TABLE 5 Relationship between level of the toxicity and the children's characters, weight, BMI. pharmaceutical products and poison forms ($n = 122$).

Variable	Toxicity level			χ2-one-way Anova	*p*-value
	Mild	Moderate	Severe		
Age	5.63 ± 3.93	4.86 ± 3.4	5.18 3.65	2[a]	0.652
Height	10,308 ± 31.73	96.4 ± 25.99	104.1 ± 25.12	2[a]	0.386
Weight	27.38 ± 14.6	22.03 ± 9.49	23.59 ± 9.56	2[a]	0.398
BMI	29.66 ± 33.29	27.11 ± 23.61	22.43 ± 7.6	2[a]	0.191
Gender					
– Female	20 (40)	18 (36)	12 (24)	1.8	0.405
– Male	27 (37.5)	20 (27.8)	25 (34.7)		
Nationality					
– Non-Saudi	17 (33.3)	15 (29.4)	19 (37.3)	2.08	0.353
– Saudi	30 (42.3)	23 (32.4)	18 (25.4)		
Pharmaceutical products (1)					
– Depakin	1 (100)	0 (0.0)	0 (0.0)		
– Olanzapine 5 mg	0 (0.0)	1 (100)	0 (0.0)	13.8	0.464
– Cannabinoids	1 (100)	0 (0.0)	0 (0.0)		
– Carbamazepine	0 (0.0)	0 (0.0)	1 (100)		
– Hydrogen peroxide 6%	1 (100)	0 (0.0)	0 (0.0)		
– Methanol	0 (0.0)	0 (0.0)	1 (100)		
– Two pharmaceutical products (1)	44 (38.3)	36 (31.3)	35 (30.4)		
– Risperidone	0 (0.0)	1 (100)	0 (0.0)		
Pharmaceutical products (2)					
– Lorazepam	0 (0.00	1 (100)	0 (0.0)		
– Two pharmaceutical products (2)	46 (38.7)	36 (30.3)	37 (31.1)	6.07	0.415
– Propranolol	1 (100)	0 (0.0)	0 (0.0)		
– Valproate sodium 200 mg	0 (0.0)	1 (100)	0 (0.0)		
Poison forms					
– Capsule	0 (0.0)	1 (100)	0 (0.0)		
– Inhalation	1 (100)	0 (0.0)	0 (0.0)	6.1	0.412
– Two poison forms	46 (38.7)	37 (31.1)	36 (30.3)		
– Tablet	0 (0.0)	0 (0.0)	1 (100)		

[a]N.B.: = Kruskal Wallis test.

same result was found in a previous study done in KSA (1, 8) and others (21). The study showed that for (30.3%) of children, the time of exposure was 30 min, for (26.2%) it was one hour, and for (21.3%) it was 2 h. A study done in India found that the time interval between exposure to poison and admission to the hospital was less than 3 h in 73 (35.96%) cases, 3 to 6 h in 92 (45.32%) cases, and more than 6 h in 38 (18.71%) cases (22). Another study in Iran discovered that the average time from incident to hospitalization was 144.3171 min (23). The poisoning occurred at home for the majority of the youngsters (69.7%). The same result was revealed from other studies (1, 21).

In the present study, most symptoms appear as GIT symptoms. In the study done in Majmaah, 25.6% of children had GIT symptoms (8). In Abha, the most common symptoms of poisoning were nausea, vomiting (40.4%), and (16.7%) abdominal pain (18). In an Iranian study, 28.2 percent of youngsters experienced gastrointestinal symptoms (23).

In the current study, 38.5 percent of children experienced mild toxicity, whereas 31.1% and 30.3% had moderate and severe

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TABLE 6 Relationship between level of toxicity and poison forms, route and mode of poisoning, time of exposure, and place of poisoning (No.:122).

Variable	Toxicity level			χ2	*p*-value
	Mild	Moderate	Severe		
Type of poisons	No. (%)	No. (%)	No. (%)		
All types					
Animal envenomation	1 (50)	1 (50)	0 (0.0)	4.74	0.577
Household products	3 (20)	5 (33.3)	7 (46.7)		
Pharmaceutical products	40 (40.8)	29 (29.6)	29 (29.6)		
Plant envenomation	3 (42.9)	3 (42.9)	1 (14.3)		
Pharmaceutical products					
No	7 (29.2)	9 (37.5)	8 (33.3)	1.43	0.487
Yes	40 (40.8)	29 (29.6)	29 (29.6)		
Household products					
No	44 (41.9)	33 (31.4)	30 (26.7)	5.58	0.061
Yes	3 (20)	5 (33.3)	7 (46.7)		
Plant envenomation					
No	44 (38.3)	35 (30.4)	36 (31.3)	2.74	0.254
Yes	3 (42.9)	3 (42.9)	1 (14.3)		
Animal envenomation					
No	46 (38.3)	37 (30.8)	37 (30.8)	0.91	0.632
Yes	1 (50)	1 (50)	0 (0.0)		
Route of poisons					
Ingestion					
No	6 (28.6)	8 (38.1)	7 (33.3)	2.62	0.269
Yes	41 (40.6)	30 (29.7)	30 (29.7)		
Dermal					
No	45 (39.1)	36 (31.3)	34 (29.6)	2.02	0.363
Yes	2 (28.6)	2 (28.6)	3 (42.8)		
Injection					
No	46 (39.6)	35 (30.2)	35 (30.2)	1.52	0.467
Yes	1 (16.7)	3 (50)	2 (33.3)		
Inhalation					
No	44 (38.6)	35 (30.7)	35 (30.7)	2.49	0.288
Yes	3 (37.5)	3 (37.5)	2 (25)		
Mode of poisoning					
Accidental					
No	16 (41)	12 (30.8)	11 (28.2)	0.18	0.914
Yes	31 (37.3)	26 (31.3)	26 (31.3)		
Intentional					
No	31 (37.3)	26 (31.3)	26 (31.3)	0.48	0.787
Yes	16 (41)	12 (31.8)	11 (28.2)		
Time of exposure					
30 min					
No	32 (37.6)	26 (30.6)	27 (31.8)	0.27	0.872
Yes	15 (40.5)	12 (324)	10 (27)		
One hour					
No	31 (34.4)	30 (33.3)	29 (32.2)	241	0.299
Yes	16 (50)	8 (25)	8 (25)		
Two hour					
No	39 (40.6)	28 (29.2)	29 (30.2)	1.08	0.581
Yes	8 (30.8)	10 (38.5)	8 (30.80		
3-12 h					
No	46 (41.1)	35 (31.3)	31 (27.7)	5.46	0.065
Yes	1 (10)	3 (30)	6 (60)		
More than 12 h					
No	42 (40)	31 (29.5)	32 (30.5)	1.06	0.586
Yes	5 (29.4)	7 (41.2)	5 (29.4)		
Place of poisoning					
Home					
No	15 (40.5)	10 (27)	12 (32.4)	0.42	0.809
Yes	32 (37.6)	28 (32.9)	25 (29.4)		
Hospital					
No	44 (37.6)	36 (30.8)	37 (31.6)	2.33	0.311
Yes	3 (60)	2 (40)	0 (0.0)		
Outside home					
No	36 (39.6)	30 (33)	25 (27.5)	1.44	0.486
Yes	11 (35.5)	8 (25.8)	12 (38.7)		

TABLE 7 Relationship between complicated cases and laboratory results (No.:122).

Variable	Complications		U-test	*p*-value
	Complicated cases	Non-complicated case		
	Mean ± SD	Mean ± SD		
pH	7.34 ± 0.14	7.35 ± 0.09	0.29	0.771
HCO3 (mmol)	22.43 ± 5.39	23.16 ± 4.46	0.55	0.581
PaO2 (mmHg)	83.2 ± 17.3	80.08 ± 16.75	0.91	0.359
So2	93.48 ± 6.21	94.14 ± 7.17	0.65	0.512
Paco2 (mmHg)	39.49 ± 13.35	38.31 ± 9.06	0.09	0.923
Na (mEqL)	138.38 ± 6.24	138.51 ± 4.18	0.27	0.784
K (mEqL)	4.31 ± 0.7	4.08 ± 0.06	1.76	0.078
Cl (mEqL)	103.07 ± 10.63	101.21 ± 5.76	0.89	0.369
Mg (mg/dl)	1.7 ± 0.46	1.73 ± 0.41	0.63	0.527
Cholinesterase level (IUL)	3053.38 ± 2942.96	3019.71 ± 2197.65	0.52	0.599
ALT (IUL)	68.66 ± 162.63	30.01 ± 9.13	0.6	0.549
AST (IUL)	75.5 ± 216.05	20.08 ± 13.08	2.8	**0.005**
S.bilirubin (mg/dl)	3.39 ± 14.79	0.95 ± 1.07	0.25	0.8
RBCs (Cell/ml)	4.65 ± 0.52	4.86 ± 0.99	1.12	0.259
Hb (gm/dl)	13.28 ± 2.06	13.67 ± 2.46	0.53	0.594
WBCs (Cell/ml)	12,793.84 ± 26,637.39	19,314.81 ± 45,475.02	1.2	0.229
Urea (mg/dl)	30.08 ± 9.96	31.9 ± 8.84	0.96	0.336
BUN (mg/dl)	18 ± 39.31	11.74 ± 5.8	0.34	0.733
INR	1.11 ± 0.26	1.09 ± 0.21	1.31	0.19

toxicity, respectively. Furthermore, most cases (68%) were complicated. However, 32% of cases were uncomplicated. The high prevalence of mild cases was found in previous studies (1, 16, 24).

Almost one-quarter of children had activated charcoal (25.4%), (12.3%) had gastric lavage, and (2.5%) had dilution. Activated charcoal was found to limit the absorption of various toxins and medications in the stomach and intestine, including antipsychotics, antiepileptics, and salicylates (5, 25). Clinical trials have shown that multi-dose administration of activated charcoal can prevent severe intoxication from carbamazepine, quinine, phenobarbital, and theophylline (26).

In a previous study, only 6% of cases received a specific antidote, which could be attributed to the short time between poisoning and arrival at the hospital (25). Approximately 28% of children were treated by gut decontamination with activated charcoal in Riyadh, and only 1.8% were given specific antidotes (5).

The limitation of this study was having a retrospective study design and singular location. Incomplete and missing data may further affect the study's findings' generalization.

Conclusion

Based on the present study results, acute poisoning among children is a major public health issue in Saudi Arabia, so implementing a national policy for adequate and prompt management will result in a favorable outcome. In addition, children's exposure to harmful chemicals necessitates more attention, particularly among families, to raise their awareness of safety requirements within the home. Future studies are needed to clarify the role of various factors involved in childhood poisoning.

Limitations

Due to its retrospective design and single-center setting, the study has limited general validity. The small sample size may also reduce the study's generalizability; therefore, we intend to conduct additional research involving multiple centres in different geographic regions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the medical research ethics committee at the Faculty of medicine, Umm al Qura University and the poison control center in Makkah forensic chemistry center. Written informed consent from the participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Al Hazmi AM. Patterns of accidental poisoning in children in Jeddah, Saudi Arabia. *Ann Saudi Med.* (1998) 18:457–9. doi: 10.5144/0256-4947.1998.457

2. Afifi RM, Zaytoun SS. Distribution Patterns of Severe Pediatric Trauma: Mandated vs. Non-Mandated Trauma Systems. *Surg. Sci.* (2013) 4(9):385–92. doi: 10.4236/ss.2013.49076

3. Alazab RM, Elmougy MT, Fayad RA, Abdelsalam HF, Mohamed AS. Risk factors of acute poisoning among children: a study at a poisoning unit of a university hospital in Egypt. *South East Asia J Public Health.* (2012) 2:41–7. doi: 10.3329/seajph.v2i2.15943

4. Branche C, Ozanne-Smith J, Oyebite K, Hyder AA. *World report on child injury prevention*. Geneva, Switzerland: World Health Organization, 2008 - Business & Economics - 211 (2008).

5. Alghadeer S, Alrohaimi M, Althiban A, Kalagi NA, Balkhi B, Khan AA. The patterns of children poisoning cases in community teaching hospital in Riyadh, Saudi Arabia. *Saudi Pharm J.* (2018) 26:93–7. doi: 10.1016/j.jsps.2017.10.007

6. Rahimzadeh MR, Rahimzadeh MR, Kazemi S, Moghadamnia AA. Cadmium toxicity and treatment: an update. *Caspian J Intern Med.* (2017) 8:135. doi: 10.22088/cjim.8.3.135

7. Mehrpour O, Akbari A, Jahani F, Amirabadizadeh A, Allahyari E, Mansouri B, et al. Epidemiological and clinical profiles of acute poisoning in patients admitted to the intensive care unit in eastern Iran. *BMC Emerg Med.* (2018) 18:1–9. doi: 10.1186/s12873-018-0181-6

8. Abd-Elhaleem ZAE, Al Muqhem BA. Pattern of acute poisoning in Al Majmaah region, Saudi Arabia. *Am J Clin Exp Med.* (2014) 2:79–85. doi: 10.11648/j.ajcem.20140204.15

9. Yadav S, Yadav SP, Agrawal J, Shah G. Pattern of acute poisoning in children in a tertiary care hospital in eastern Nepal. *Inter J Contempor Pediatr.* (2016) 3:1001–5. doi: 10.18203/2349-3291.ijcp20162380

10. Abbas SK, Tikmani SS, Siddiqui NT. Accidental poisoning in children. *Mercury.* (2012) 3:7.0. PMID: 22755274.

11. Saddique A. Poisoning in Saudi Arabia: ten-year experience in king khaled university hospital. *Ann Saudi Med*. (2001) 21:88–91. doi: 10.5144/0256-4947.2001.88

12. Alruwaili ND, Halimeh B, Al-Omar M, Alhatali B, Sabie II, Alsaqoub M. An epidemiological snapshot of toxicological exposure in children 12 years of age and younger in Riyadh. *Ann Saudi Med*. (2019) 39:229–35. doi: 10.5144/0256-4947.2019.229

13. Naseem A, Khurram D, Khan D, Gari S, Lalani N. Accidental poisoning its magnitude and implications in children. *Int J Pediatr Res*. (2016) 3:400–09. doi: 10.17511/ijpr.2016.i06.06

14. Tobaiqy M, Asiri BA, Sholan AH, Alzahrani YA, Alkatheeri AA, Mahha AM, et al. Frequency and management of drug and chemical poisoning among children attending an emergency department in a single hospital in Saudi Arabia. *medRxiv*. (2020) 8 (4):189. doi: 10.3390/pharmacy8040189

15. Jullien S. Prevention of unintentional injuries in children under five years. *BMC Pediatr*. (2021) 21:1–11. doi: 10.1186/s12887-020-02457-3

16. Dayasiri M, Jayamanne S, Jayasinghe CY. Patterns and outcome of acute poisoning among children in rural Sri Lanka. *BMC Pediatr*. (2018) 18:1–8. doi: 10.1186/s12887-018-1246-0

17. Andersen C.U., Nielsen L.P., Møller J.M., Olesen A.E., Acute drug poisonings leading to hospitalization. *Basic Clin Pharmacol Toxicol* 130 (2022) 328–36. doi: 10.1111/bcpt.13688

18. Khan LA, Khan SA, AI-Hateeti HS, Bhat AR, Bhat KS, Sheikh FS. Clinical profile and outcome of poisoning in najran. *Ann Saudi Med*. (2003) 23:205–7. doi: 10.5144/0256-4947.2003.205

19. Al-Shehri MA. Pattern of childhood poisoning in abha city–southwestern Saudi Arabia. *J Family Community Med*. (2004) 11:59. PMID: 23012050.

20. Kandeel F, El-Farouny R. Study of acute poisoning cases in children admitted to menoufia poison control center (MPCC) during the year (2016). *Ain Shams J Forensic Med Clin Toxicol*. (2017) 29:89–99. doi: 10.21608/ajfm.2017.18213

21. Fang J, Wang M, Gong S, Cui N, Xu L. Increased 28-day mortality due to fluid overload prior to continuous renal replacement in sepsis associated acute kidney injury. *Ther Apher Dial*. (2022) 26:288–96. doi: 10.1111/1744-9987.13727

22. bdulaziz Al-Sekait M. Accidental poisoning of children in Riyadh, Saudi Arabia. *J R Soc Health*. (1989) 109:204–5. doi: 10.1177/146642408910900609

23. Shirkosh S, Esmaeilidooki M, Nakhjavani N, Hadipour A, Osia S, Hajiahmadi M. Epidemiological and clinical pattern of acute poisoning in children: a hospital based study in northern Iran. *Caspian J Pediatr*. (2019) 5:334–41. doi: 10.22088/CJP.BUMS.5.1.334

24. Elshoura AIA, Sherif MM, Noor El-Deen TM, Ali MA, Abbod MA, Ghanem MA. Assessment of acute poisoning among children in damietta governorate. *Al-Azhar Med J*. (2016) 45:631–44. doi: 10.12816/0033129

25. Zhu Y, Q WU. The current situation of acute poisoning in children. *Chin Pediatr Emerg Med*. (2018) 12:81–3. doi: 10.3760/cmajissn1673-4912.2018.02.001

26. American Academy of Clinical Toxicology, European Association of Poisons Centres and Clinical Toxicologists. Position statement and practice guidelines on the use of multi-dose activated charcoal in the treatment of acute poisoning. *J Toxicol*. (1999) 37:731–51. doi: 10.1081/CLT-100102451

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Drug exposure during pregnancy: Current understanding and approaches to measure maternal-fetal drug exposure

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Prescription drug use is prevalent during pregnancy, yet there is limited knowledge about maternal-fetal safety and efficacy of this drug use because pregnant individuals have historically been excluded from clinical trials. Underrepresentation has resulted in a lack of data available to estimate or predict fetal drug exposure. Approaches to study fetal drug pharmacology are limited and must be evaluated for feasibility and accuracy. Anatomic and physiological changes throughout pregnancy fluctuate based on gestational age and can affect drug pharmacokinetics (PK) for both mother and fetus. Drug concentrations have been studied throughout different stages of gestation and at or following delivery in tissue and fluid biospecimens. Sampling amniotic fluid, umbilical cord blood, placental tissue, meconium, umbilical cord tissue, and neonatal hair present surrogate options to quantify and characterize fetal drug exposure. These sampling methods can be applied to all therapeutics including small molecule drugs, large molecule drugs, conjugated nanoparticles, and chemical exposures. Alternative approaches to determine PK have been explored, including physiologically based PK modeling, *in vitro* methods, and traditional animal models. These alternative approaches along with convenience sampling of tissue or fluid biospecimens can address challenges in studying maternal-fetal pharmacology. In this narrative review, we 1) present an overview of the current understanding of maternal-fetal drug exposure; 2) discuss biospecimen-guided sampling design and methods for measuring fetal drug concentrations throughout gestation; and 3) propose methods for advancing pharmacology research in the maternal-fetal population.

KEYWORDS

maternal-fetal pharmacology, pregnancy, fetal drug exposure, prenatal testing, pharmacokinetics

Introduction

Prescription medication use during pregnancy is widespread. At least 70% of individuals take at least one prescription medication during pregnancy (Lupattelli et al., 2014; Haas et al., 2018; Centers for Disease Control and Prevention, 2022). These medications may be prescribed to treat an individual's chronic conditions (e.g., depression, epilepsy, hypertension, thyroid disorders), acute illnesses (e.g., infections), and pregnancy-related illnesses (e.g., pre-eclampsia or gestational diabetes) (Wesley et al., 2021). Many of these drugs will cross the placenta and expose the fetus. The extent and impact of fetal exposure is unknown for most drugs.

In order to optimize drug dosing in pregnant individuals and prevent harm to the fetus, it is critical to understand physiologic changes during pregnancy that determine fetal drug exposure. However, determining fetal drug exposure is challenging. *In utero* sampling procedures to directly measure fetal drug concentrations are invasive and place both mother and baby at increased risk for adverse events. Preclinical *in vitro* and animal models are not always translatable to humans. Opportunistic samples obtained during prescribed clinical care leverages standard of care procedures (e.g., collecting amniotic fluid at the time of routine amniocentesis) and collect non-invasive surrogate samples related to fetal exposure (e.g., fetal hair or meconium) as an alternative approach to assessing fetal drug transfer.

When formulating this manuscript, we essentially wanted to answer the question, "How do we obtain drug levels from pregnant individuals for clinical studies or trials to measure fetal drug exposure?" To help answer this, we provide narrative for the current understanding of maternal-fetal drug transfer, evaluate the pros and cons of different opportunistic sampling approaches, and investigate potential alternative methods to better characterize fetal pharmacology.

Current understanding of maternal-fetal drug transfer

Maternal anatomic and physiological changes during pregnancy

Human gestation length is about 280 days and is divided into three trimesters. The first trimester is usually dated from the start of the mother's last menstrual period, which is 2 weeks before the estimated date of conception, and continues through week 12. This is often designated as the embryonic period. The second trimester comprises the most prolonged period and is defined as weeks 13–28. The third trimester begins at week 29 and continues until delivery, typically at week 40 for a full term delivery (Andersen et al., 2018). Each trimester is marked by maternal changes in anatomy and physiology, such as renal function. For example, the glomerular filtration rate and renal plasma flow increase up to 50% and 80%, respectively, during pregnancy (Cheung and Lafayette, 2013). As another example, increases in estradiol and progesterone are initiated at the beginning of pregnancy and are regulated by the placenta starting at week 10 (Weissgerber and Wolfe, 2006; Kumar and Magon, 2012). Pregnancy related changes in these hormones can, both directly and indirectly, affect the pharmacokinetics (PK) of drugs through competition for binding to plasma proteins, changes in the activity of metabolic enzymes (Table 1), and other anatomical and physiological changes such as changes in gastrointestinal motility. (Dickinson et al., 1989; Gerdin et al., 1990; Prevost et al., 1992; Tomson et al., 1994; Hakkola et al., 1996; Collier et al., 2002; McGready et al., 2003; Nishimura et al., 2003; De Haan et al., 2004; Dempsey et al., 2004; Franco et al., 2008; Hebert et al., 2008; Ke et al., 2014; Fa et al., 2018; Goh et al., 2021). These types of changes can affect drug absorption, distribution, metabolism, and excretion (ADME) as highlighted in Table 2 (Ke et al., 2014; Feghali et al., 2015; Kazma et al., 2020).

Fetal drug exposure

The placenta performs vital functions for the developing fetus and has several structural components. The basic structural unit of this disk-shaped organ is the chorionic villi that project into the intervillous space (Griffiths and Campbell, 2015). Chorionic villi are surrounded by the chorion which consists of the outer syncytiotrophoblast and inner cytotrophoblast layers (Griffiths and Campbell, 2015). Placental structural components and activity are vital for normal embryonic development to ensure sufficient oxygen, nutrient, and waste exchange between mother and fetus (Grigsby, 2016). Maternal-fetal drug exposure and PK are largely moderated by the placenta. Drugs in maternal blood can reach fetal blood by passing through the placental intervillous space, syncytiotrophoblast layer, and fetal connective tissue to reach the endothelium of fetal capillaries and enter the fetal circulation (Figure 1) (Griffiths and Campbell, 2015). Drugs in fetal circulation can also re-enter maternal blood in small amounts (Syme et al., 2004; Griffiths and Campbell, 2015).

Placental transfer of drugs can occur *via* passive diffusion, facilitated diffusion, or active transport (Griffiths and Campbell, 2015; Pemathilaka et al., 2019). Minute transfer may occur *via* pinocytosis and phagocytosis, but these mechanisms are too slow to play a significant effect on fetal drug concentrations (Syme et al., 2004). Passive diffusion of drugs occurs for neutral, lipophilic, and unbound drugs with a molecular weight less than 500 Daltons (Pavek et al., 2009a; Feghali et al., 2015). Facilitated diffusion occurs when drugs are structurally related to endogenous compounds such as glucocorticoids (Griffiths and Campbell, 2015; Pemathilaka et al., 2019). Drug transporters, such as multidrug resistance proteins (MRPs), P-glycoprotein (P-gp), and breast cancer resistance protein (BCRP), require energy, usually in the form of adenosine triphosphate, to actively transfer drugs (Myllynen et al., 2009; Iqbal et al., 2012; Griffiths and Campbell, 2015; Pemathilaka et al., 2019). Drug transporters present in the placenta allow drug transfer from mother to fetus and *vice versa* (Griffiths and Campbell, 2015).

Placental transfer of drugs can be further complicated as the placenta contains a broad range of enzymatic activity (Prouillac and

TABLE 1 Summary of drug metabolizing enzyme activity by gestational age.

Metabolizing enzyme	Change in activity during gestation by trimester			Expressed in placenta	References
	First[a]	Second[a]	Third[a]		
CYP1A1	-	-	-	yes	Collier et al. (2002), Ke et al. (2014), Fa et al. (2018), Goh et al. (2021)
CYP1A2	+	-	-	no	Hakkola et al. (1996), Nishimura et al. (2003), Goh et al. (2021)
CYP2C9	+	+	+	yes	Dickinson et al. (1989), Tomson et al. (1994)
CYP2C19		-	-	yes	McGready et al. (2003), Ke et al. (2014)
CYP2A6		+	+	no	Dempsey et al. (2004)
CYP2B6		+	+	no	Hakkola et al. (1996), Ke et al. (2014)
CYP3A4	+	+	+	no	Prevost et al. (1992), Hebert et al. (2008)
CYP2D6	+	+	+	yes	Hakkola et al. (1996)
UGT1A4	+	+	+	yes	Collier et al. (2002), De Haan et al. (2004), Franco et al. (2008)
UGT2B7			+	yes	Gerdin et al. (1990), Collier et al. (2002)

[a]Blank spaces indicate no information found for metabolizing enzyme expression in indicated gestational trimester; - indicates a decrease; + indicates an increase.

TABLE 2 Selected maternal organ system changes that affect pharmacokinetics during pregnancy.

Specific organ system	Change during pregnancy	PK effect	References
Renal plasma flow	Increase (up to 80%)	Increase CL	Feghali et al. (2015), Kazma et al. (2020)
Glomerular filtration rate	Increase (up to 50%)	Increase CL	Kazma et al. (2020)
Gastrointestinal tract motility	Decrease (not reported)	Delay K_a	Feghali et al. (2015), Kazma et al. (2020)
Cardiac output	Increase (20%–45%)	Increase K_a and V_d	Feghali et al. (2015)
Creatinine clearance	Increase (26%–28%)	Increase CL	Ke et al. (2014)
Uterine blood flow	Increase (923%–2,721%)	Increase K_a	Ke et al. (2014)
Total fat mass	Increase (6%–23%)	Increase V_d	Ke et al. (2014)

CL, clearance, K_a, absorption; *PK*, pharmacokinetic, V_d, volume of distribution.

Lecoeur, 2010). Several cytochrome P450 (CYP) drug metabolizing enzymes have been isolated from the placenta and include CYP1, CYP2, and CYP3 (Myllynen et al., 2009; Prouillac and Lecoeur, 2010). These enzymes, along with active drug transporters, alter fetal exposure to varying amounts of parent drug, metabolites, and byproducts (Dallmann et al., 2019a).

The importance of placental effects is exemplified by a study that investigated illicit drug exposure in monozygotic and dizygotic twins (Boskovic et al., 2001). Similar concentrations of cocaine and cannabinoids were found for monozygotic twins who share the same placenta. More significant differences in drug concentrations were observed in dizygotic twins with separate placentas. Notably, one dizygotic twin tested positive for drugs while the other twin did not. This study demonstrates the variation in drug transfer across the placenta that can alter fetal concentrations.

Once a drug reaches the fetus, fetal ADME can impact fetal drug exposure. Fetal ADME differs substantially from maternal ADME and even infant ADME (Feghali et al., 2015; Allegaert and Van Calsteren, 2016). For example, expression levels of fetal CYP enzymes mature over the course of pregnancy and, in general, are much lower than infant and maternal expression levels (Lacroix et al., 1997; Hines, 2008). In addition, drugs and metabolites can become trapped in fetal tissues *via* two processes: 1) reabsorption from amniotic fluid and 2) ionization. First, drugs that are renally excreted by the fetus can recirculate through the amniotic fluid and be reabsorbed through fetal swallowing (Pritchard, 1966; Blackburn and Loper, 1992; Pavek et al., 2009b; Abduljalil et al., 2019). The fetal swallow reflex begins as soon as week 10 of gestation (De Vries et al., 1985). Second, he pH of fetal blood is slightly more acidic than maternal blood leading to ionization of weak bases. When ionized, these weak bases usually do not pass from the fetus back to the mother *via* the placenta (Pavek et al., 2009b). These fetal-specific aspects confound generalizations and complicate measurement of fetal drug exposure.

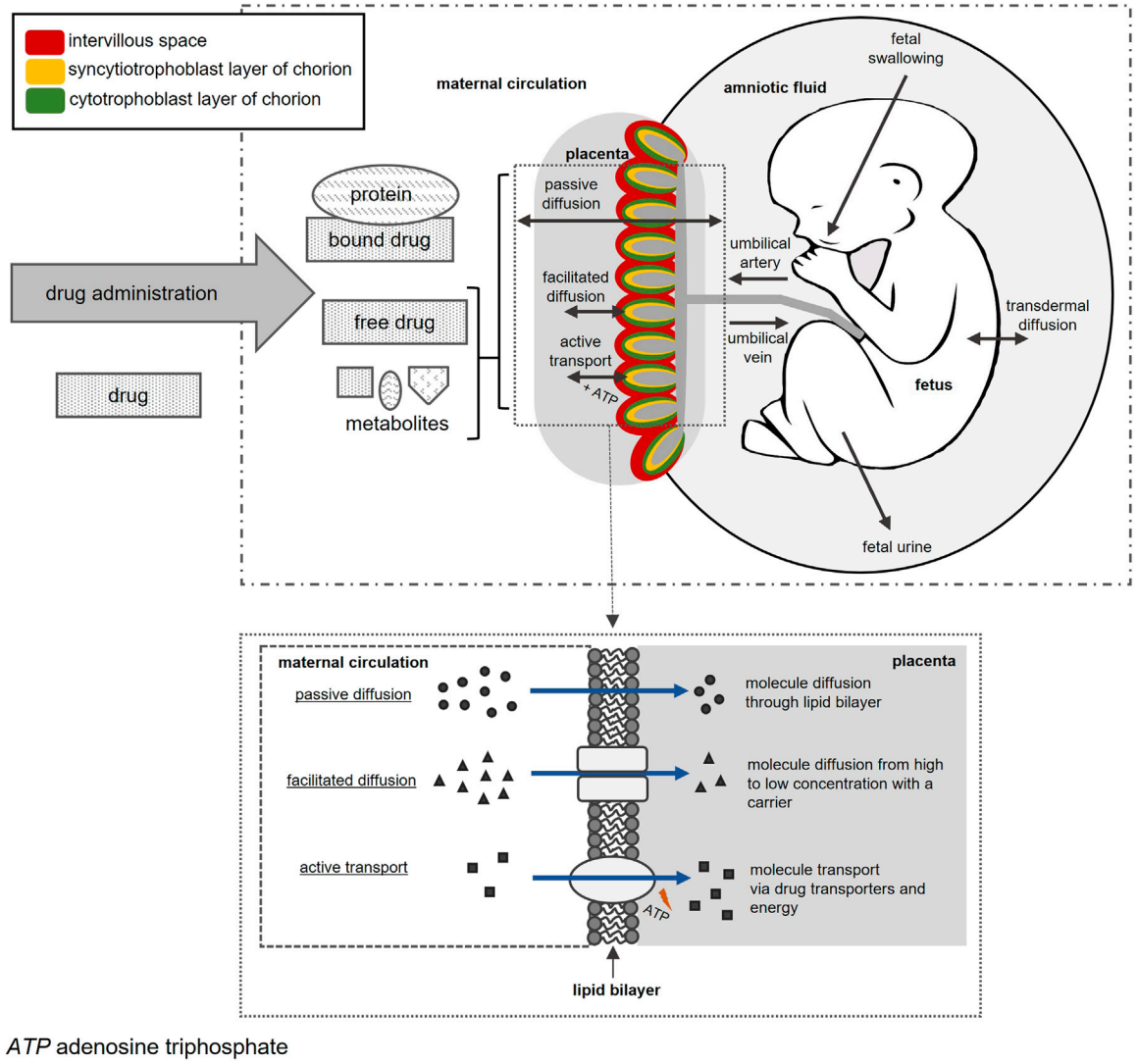

FIGURE 1
In utero fetal drug transfer following maternal drug administration.

Fetal drug detection from biological fluid and tissue specimens

Methods for measuring fetal drug concentrations are invasive in nature and pose risks to both mother and fetus. To minimize risks, the collection of opportunistic surrogate samples during standard of care procedures increases feasibility for measuring fetal drug exposure. Several of these surrogate options are illustrated in Figure 2.

Amniotic fluid

Background and sampling

Amniotic fluid provides protection and temperature regulation during fetal development, and its composition changes as pregnancy progresses (Beall et al., 2007). During early embryogenesis before fetal kidneys start to function, amniotic fluid is predominately thought to derive from maternal plasma (Beall et al., 2007; Orczyk-Pawilowicz et al., 2016). Its composition shifts following the first trimester with increased creatinine, urea, and uric acid concentrations, most likely a consequence of fetal swallowing and renal excretion (Brace and Wolf, 1989; Bloomfield et al., 2017). *In utero* sampling of amniotic fluid, known as amniocentesis, is performed for specific diagnostic testing. Amniocentesis is typically conducted after weeks 15–16 of gestation when the amnion and chorion have fused (Jindal and Chaudhary, 2020). Other options for obtaining amniotic fluid would be in cases of miscarriage, planned termination of pregnancy, or at delivery.

Maternal-fetal drug transfer

Drug concentrations have been evaluated in amniotic fluid from early and mid-gestation as well as at delivery (Table 3) (Bernard et al., 1977a; Bernard et al., 1977b; Szeto et al., 1978; Mandelbrot

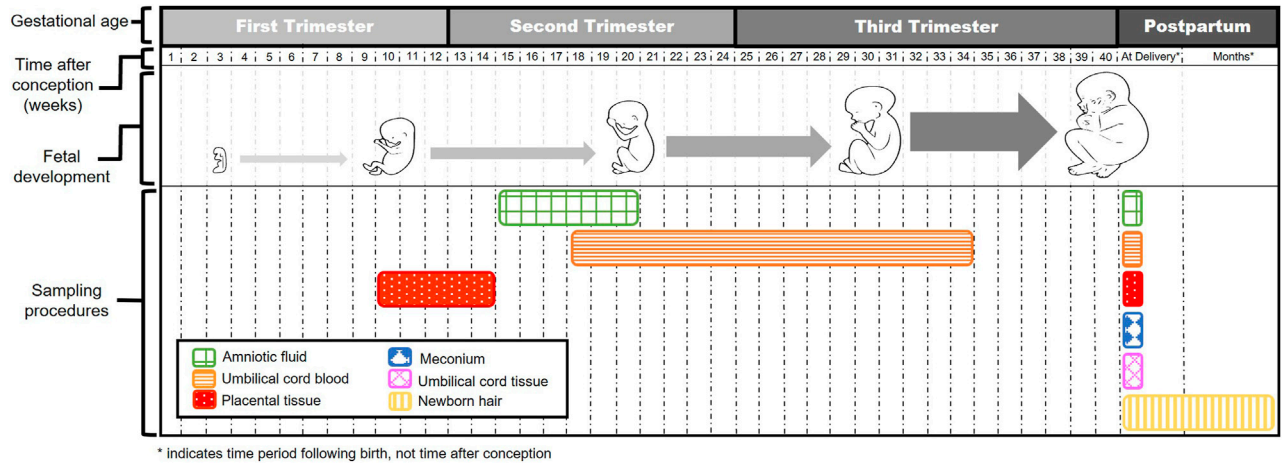

FIGURE 2
Illustration of surrogate sampling times throughout the stages of gestation.

TABLE 3 Description of studies that reported drug concentrations in surrogate specimens by gestational age and at delivery.

Surrogate specimen	Gestational age by trimester				References
	Frist[a]	Second[a]	Third[a]	At delivery[a]	
Amniotic fluid	x	x		x	Bernard et al. (1977a), Bernard et al. (1977b), Szeto et al. (1978), Pons et al. (1991), Siu et al. (2000), Mandelbrot et al. (2001), Chappuy et al. (2004a), Chappuy et al. (2004b), Fokina et al. (2016), Paulzen et al. (2017a), Paulzen et al. (2018), Paulzen et al. (2020)
Umbilical cord blood	x	x		x	Kauffman et al. (1975), Bernard et al. (1977a), Bernard et al. (1977b), Pons et al. (1991), Mandelbrot et al. (2001), Hendrick et al. (2003), Paulzen et al. (2017a), Paulzen et al. (2017b), Veit et al. (2017), Paulzen et al. (2018), Paulzen et al. (2020)
Placental tissue	x	x		x	Bernard et al. (1977a), Bernard et al. (1977b), de Barros Duarte et al. (2009), Duarte et al. (2011)
Meconium				x	Ostrea et al. (1989), Maynard et al. (1991), Ostrea et al. (2001), Bar-Oz et al. (2003), Eyler et al. (2005), Montgomery et al. (2006), Gray and Huestis (2007), Montgomery et al. (2008), Concheiro et al. (2010), Marin et al. (2014), Colby (2017)
Umbilical cord tissue				x	Montgomery et al. (2006), Montgomery et al. (2008), Concheiro et al. (2010), Concheiro et al. (2013), Marin et al. (2014), Colby (2017)
Newborn hair				x	Eliopoulos et al. (1996), Klein and Koren (1999), Boskovic et al. (2001), Ostrea et al. (2001), Bar-Oz et al. (2003), Gray and Huestis (2007)

[a]Blank spaces indicate no studies found for surrogate specimens; x indicates reported surrogate specimen analysis was reported for the trimester.

et al., 2001; Chappuy et al., 2004a; Chappuy et al., 2004b; Fokina et al., 2016; Paulzen et al., 2017a; Paulzen et al., 2018; Paulzen et al., 2020). While amniocentesis is not typically carried out prior to week 15 of gestation, drug concentrations in amniotic fluid have been reported during the first trimester from older practices. Dependent on gestational age and drug evaluation, conflicting results are reported between drug concentrations in amniotic fluid versus fetal tissue, fetal plasma, and maternal blood. For example, diclofenac and amikacin concentrations measured in amniotic fluid were lower than concentrations measured in fetal tissue samples. In contrast, ritodrine and quetiapine concentrations measured in amniotic fluid and umbilical cord blood were similar at delivery (Bernard et al., 1977a; van Lierde and Thomas, 1982; Siu et al., 2000; Paulzen et al., 2018). These discrepancies highlight crucial factors when considering amniotic fluid as a biospecimen, including drug permeability to fetal skin, amniotic fluid composition, and effects of fetal and maternal hepatic metabolism throughout pregnancy (Ward and Varner, 2019).

Limitations

Amniocentesis is an invasive test and carries certain risks to the mother and fetus. Risks of mid-trimester amniocentesis include rupturing the amniotic sac, miscarriage, needle injury to the fetus, Rh sensitization, and infection (Pruthi, 2020). Amniocentesis before week 15 of gestation is associated with a higher rate of miscarriages than mid-term amniocentesis and is rarely performed unless the benefits outweigh the risks (Wilson, 1995; Steinfort et al., 2021). With advancing gestation, additional risks include preterm birth,

chorioamnionitis, and stillbirth (Daum et al., 2019). Therefore, amniocentesis would only be viable option in cases where an amniocentesis was performed for clinical indications. In these cases, the fetus often has anomalies or suspected genetic abnormalities, which may influence drug metabolism. Because amniocentesis is typically carried out mid-gestation, sampling opportunities may be limited during early and late pregnancy.

Umbilical cord blood

Background and sampling

The umbilical vein provides blood from mother to fetus with flow established within the umbilical cord by the end of week 5 of gestation (Spurway et al., 2012). Umbilical cord blood has a unique composition as it contains blood cells with varying stem cell markers, and differs from both newborn and maternal peripheral blood (Pranke et al., 2001). Composition is also influenced by fetal sex, gestational age, and mode of delivery (Glasser et al., 2015). Fetal gender appears to influence red blood cell values and white blood cells are reported to increase with gestational age and vaginal births (Glasser et al., 2015). Cord blood can be collected *in utero* (cordocentesis-usually of the fetal vein), typically between week 18–34 of gestation, and at the time of delivery (Jindal and Chaudhary, 2020). Other options for obtaining cord blood would be in cases of miscarriage, planned termination of pregnancy, or at delivery.

Maternal-fetal drug transfer

Umbilical cord blood measurements are predominantly reported mid-to late-gestation or at delivery (Kauffman et al., 1975; Pons et al., 1991; Mandelbrot et al., 2001; Hendrick et al., 2003; Paulzen et al., 2017a; Paulzen et al., 2017b; Paulzen et al., 2018; Paulzen et al., 2020). Most studies assumed cord blood was informative of fetal exposure. This assumption is supported by one study that measured similar gentamicin concentrations in fetal and cord serum following elective second trimester abortion (Kauffman et al., 1975). However, most of the reported studies only compared cord blood measurements with maternal serum. Because of differences in PK between the mother and the fetus, single pairs of samples from the mother and the umbilical cord blood can show ratios that vary widely depending on the interval after drug administration (Ward, 1995). Data evaluating the relationship between drug concentrations in cord blood and other fetal samples may provide further insight into fetal exposure.

Limitations

Cordocentesis is an invasive test with risks to the pregnancy. Procedure-related risks include bleeding from the puncture site (most common), fetal distress, pregnancy loss, and rarely vertical transmission of maternal infection (Society for Maternal-Fetal et al., 2013). Therefore, cordocentesis would only be a viable option when performed for clinical indications. In these cases, the fetus may have anemia, which may influence the activity of enzymes involved in drug metabolism. Like amniocentesis, cordocentesis is typically carried out mid-gestation, but can extend to late pregnancy. Technical aspects of cordocentesis limit its use during early pregnancy.

Placental tissue

Background and sampling

Placental chorionic villi serve as the functional and structural unit of the human placenta and are involved in the exchange of gas and nutrients between mother and fetus (Gude et al., 2004). During fetal development, chorionic villi grow and form branches as pregnancy progresses with high variability in vascularization, the degree of branching, and budding (Gude et al., 2004; Hannibal et al., 2018). Chorionic villus sampling (CVS) is conventionally conducted between weeks 10 and 14 during the first trimester (Jindal and Chaudhary, 2020). Other options for obtaining a chorionic villus biospecimen would be in cases of miscarriage, planned termination of pregnancy, or at delivery.

Maternal-fetal drug transfer

Studies to evaluate drug concentrations from the placental tissue by CVS have not been explored extensively. Some studies have evaluated concentrations of bupivacaine enantiomers, lidocaine, and fentanyl from the placental intervillous space following term deliveries (de Barros Duarte et al., 2009; Duarte et al., 2011). While these studies reported relatively high drug and drug metabolite concentrations, the translation of this work to chorionic villi samples rather than placental intervillous space is uncertain. In addition, CVS is typically conducted in early gestation, and the cited studies were carried out in late gestation following term deliveries. Measuring drug concentration in CVS biospecimens should be explored for estimating fetal drug exposure in the first trimester of pregnancy using convenience samples obtained as part of clinically indicated sampling.

Limitations

CVS is an invasive test with risks to pregnancy. Risks of CVS include infection, membrane rupture, and fetal loss (Jindal and Chaudhary, 2020). Therefore, collection of chorionic villi biospecimens is only an option in cases where a CVS is performed for clinical indications. This restricts *in utero* CVS biospecimen collection to early pregnancy. Overall, our understanding of drug concentrations measured from chorionic villi are quite limited.

Meconium

Background and sampling

Meconium is the initial substance present in the intestines of a developing fetus and constitutes the first stools of a newborn (Skelly et al., 2020). Meconium accumulates during the second trimester (weeks 13–16) when fetal swallowing begins (Skelly et al., 2020). Drug concentrations detected in meconium represent cumulative exposure from the second trimester through birth. Collection of meconium can typically be conducted within the first 24 to 48 h following birth dependent on the timing of the first newborn stool (Skelly et al., 2020).

Maternal-fetal drug transfer

Meconium is frequently used for detecting fetal drug exposure concentrations in newborns for suspected maternal

illicit drug use. It has been studied extensively (Ostrea et al., 1989; Maynard et al., 1991; Ostrea et al., 2001; Bar-Oz et al., 2003; Eyler et al., 2005; Montgomery et al., 2006; Gray and Huestis, 2007; Montgomery et al., 2008; Concheiro et al., 2010; Marin et al., 2014; Colby, 2017). Although used extensively to detect illicit perinatal drug use, the convenience of this sampling supports the use of this biospecimen to determine *in utero* fetal drug transfer of non-illicit drugs. Meconium has been recognized as a sensitive biospecimen to detect *in-utero* drug exposure (Ostrea et al., 2001; Bar-Oz et al., 2003; Eyler et al., 2005; Gray and Huestis, 2007).

Limitations

Sampling of meconium can be limited if meconium is passed early *in utero* before birth (Farst et al., 2011). Meconium is also frequently contaminated with urine from diaper collection, complicating drug concentration interpretation (Gray and Huestis, 2007). While meconium sampling offers a wide window of drug detection, it is impossible to distinguish a single concentration time-point of drug exposure (Gareri et al., 2006). Drug concentrations measured in meconium represent the accumulation of drug exposure *in utero* over many weeks to months. Drug use just prior to delivery may not have had time to distribute and thus may relay inaccurate results (Farst et al., 2011). Furthermore, it is not clear when during pregnancy drugs first appear in meconium, or how the meconium concentration compares to the extent of maternal drug use.

Umbilical cord tissue

Background and sampling

The umbilical cord provides a pathway for blood transport from the placenta to the fetus (Spurway et al., 2012). Development of the umbilical cord begins between weeks 4 and 8 of pregnancy with the amnion enveloping tissue from the body stalk (Schöni-Affolter et al., 2007; Spurway et al., 2012). As an option for monitoring *in utero* fetal drug exposure, cord tissue can be collected following birth. Collection of cord tissue can be conducted relatively quickly as it does not require an invasive procedure, utilizes an otherwise discarded specimen, and may reflect a relatively long window of drug detection (Price et al., 2020).

Maternal-fetal drug transfer

Several studies have compared samples from the umbilical cord tissue versus meconium to assess fetal concentrations following prescribed medication intake and illicit drug use (Montgomery et al., 2006; Montgomery et al., 2008; Concheiro et al., 2010; Concheiro et al., 2013; Marin et al., 2014; Colby, 2017). Among these studies, investigators have suggested similar sensitivity and specificity between meconium and cord tissue, yet cord tissue may offer some advantages. For example, meconium collection varies based on newborn passage while cord tissue can be sent for testing immediately following delivery (Montgomery et al., 2006). Cord tissue has been utilized in standard clinical practice for estimating fetal drug exposure, which supports its use as a suitable biospecimen.

Limitations

Umbilical cord tissue sampling can only be performed following birth or termination of pregnancy. This results in a significant limitation in sampling, with no ability to use cord tissue when conducting fetal drug exposure analysis before birth. An important consideration for use of cord tissue is the possibility for drug metabolites to passively diffuse from cord plasma to cord tissue *in utero* and confound measured drug concentrations (Concheiro et al., 2010). Several studies reported possible "false negatives" from cord tissue because drug metabolites were found rather than the parent compound. Therefore, variations in maternal and fetal kinetic patterns suggest cord tissue drug concentrations may not accurately reflect the extent of maternal to fetal drug transfer (Ward, 1995).

Neonatal hair

Background and sampling

Fetal hair aids in *utero* skin protection and temperature regulation. Hairs project from all skin surface areas and the hair shaft becomes fully formed by the beginning of the third trimester (Holbrook and Odland, 1978). The foremost advantage of fetal hair as a biospecimen is its collection at any point during the first 3 months of life. After 3 months, neonatal hair is replaced with infant hair (Gray and Huestis, 2007).

Maternal-fetal drug transfer

Neonatal hair testing has identified fetal drug exposure from specific drugs of abuse (Eliopoulos et al., 1996; Klein and Koren, 1999; Boskovic et al., 2001; Ostrea et al., 2001; Bar-Oz et al., 2003; Gray and Huestis, 2007). A high correlation was reported for drug concentrations in paired maternal and neonatal hair specimens (Klein and Koren, 1999). These concentrations would be reflective of drug exposure relatively late in pregnancy as fetal hair grows during the third trimester.

Limitations

Similar to meconium and cord tissue, neonatal hair can only be collected following birth. Sampling may be limited in newborns born with limited hair or baldness (Gray and Huestis, 2007). In some cases, mothers are unwilling to consent to fetal hair collection for cosmetic or cultural reasons (Gray and Huestis, 2007). Drug concentrations measured in neonatal hair represent the accumulation of drug exposure *in utero* relatively late in pregnancy. It is not possible to distinguish a single concentration time-point of drug exposure. Furthermore, differing amounts of melanin in neonatal hair may confound measured drug concentrations. Higher amounts of melanin present in dark colored hair can bind more drug than lighter colored hair (Slawson et al., 1998).

Alternative approaches to estimate maternal-fetal drug transfer

While *in utero* PK studies are ideal, decreases in prenatal testing limit access to biospecimens collected before birth. The difficulties associated with biological fluid and tissue sampling during pregnancy have motivated the development of alternative methods to study fetal drug exposure.

Physiologically based pharmacokinetic (PBPK) modeling

Background

PBPK models are mathematical tools that integrate drug-specific information (e.g., metabolism, protein binding) and system-specific information (e.g., organ size, blood flow) to predict the effect of physiological conditions (e.g., pregnancy) on drug exposure (Edginton et al., 2006; Zhao et al., 2011; Dallmann et al., 2019a; Dallmann et al., 2019b; Silva et al., 2022). To model drug exposure in pregnant individuals, pregnancy-related virtual organs can be linked to the PBPK model. Model parameters (e.g., increased GFR) can then be modified to reflect pregnancy physiology (Dallmann et al., 2018). One advantage of PBPK models includes the ability to use published or opportunistic PK study data to predict fetal drug exposure. This combined approach allows for the simulation of clinical trials, improved trial design, and reduced number of pregnant individuals needed for PK dosing studies.

Maternal-fetal drug transfer

Pregnancy PBPK models have demonstrated excellent capabilities in the last few decades as predictive tools for maternal and fetal populations. These models build on existing information and data to describe maternal-fetal drug transfer throughout pregnancy. There is an increasing focus on methodologies for including placental transfer physiology to describe fetal exposure (De Sousa Mendes et al., 2017; Zhang et al., 2017; Zhang et al., 2018; George et al., 2020; Liu et al., 2020; Mian et al., 2020; Gingrich et al., 2021; Abduljalil et al., 2022a; Bukkems et al., 2022; Peng et al., 2022). Methodologies capitalize on available *in vitro*, *in vivo*, and *ex vivo* studies in animals and humans to inform models for fetal exposure. These combined advancements have allowed for the consolidation of physiological changes into reference databases for pregnancy models (Dallmann et al., 2017; Dallmann et al., 2019a; Abduljalil et al., 2022b). PBPK models and databases provide a quantitative framework for placental transfer and examining fetal exposure throughout pregnancy. This framework has the flexibility to incorporate changes in drug-specific and physiology-specific components to advance our understanding of maternal PK and fetal drug exposure.

Limitations

PBPK model validation still requires biologic sampling. While smaller sample sizes are required for PBPK modeling, pronounced physiological changes necessitate dynamic assumptions for model building. Additional data are needed throughout gestation to improve model accuracy, build inter-individual and intra-individual variability, and validate the PBPK models (Center for Drug Evaluation and Research, 2019).

Placenta-on-a-chip

Background

The placenta is responsible for regulating drug transfer to the fetus during pregnancy. To explore this, a "placenta-on-a-chip" system that mimics the structure and function of the human placenta has been assessed. This microdevice concept typically includes the static culture of trophoblast monolayers in Transwell inserts to mimic the placental passage of compounds (Poulsen et al., 2009). Some advanced models include human trophoblast cells and villous endothelial cells cultured in apposition on a semipermeable membrane under flow conditions (Blundell et al., 2018). This *in vitro* device offers the opportunity to carry out non-invasive experiments that do not interfere with the care of the mother or fetus.

Maternal-fetal drug transfer

An advanced placenta-on-a-chip model has been developed to study transporter-mediated drug efflux. The placental barrier's multilayered architecture and hemodynamic environment were mimicked with a single device *in vitro* (Blundell et al., 2018). Examination of the model assessing glyburide transfer was consistent with some *in vivo* studies (Elliott et al., 1991; Langer et al., 2000). This model for drug transfer is appealing as it gains the capacity to precisely control and manipulate critical parameters of placental drug transport. Placenta-on-a-chip models have explored the transfer of other compounds, including caffeine and nanoparticles (Nadanaciva et al., 2011; Pemathilaka et al., 2019).

Limitations

These studies offer reasonable contributions to assessing the maternal-fetal transfer of different compounds using *in vitro* strategies; however, additional research is needed to confirm these models. Future development requires the incorporation of changes in drug transporters and metabolizing enzymes throughout gestation.

Discussion

A better understanding of maternal-fetal pharmacology is critical for both the mother and fetus. Changes in anatomy and physiology during pregnancy can result in supra- or subtherapeutic dosing. In current practice, dosage adjustments for medications during pregnancy are rare due to limitations in literature and dosing guidance. Dosing adjustments may be necessary for drugs that put the fetus at increased risk. In particular, additional data are needed for drugs or medications that concentrate in the fetal compartment. Further investigation of fetal drug PK in pregnancy is a priority area with implications for both mother and fetus.

Improved methods and protocols are needed to collect concentration data throughout gestation. Convenience sampling is a method that would allow sample collection during already indicated *in utero* procedures. By utilizing multiple procedures, concentration time-point measurements can be collected during each trimester. For example, CVS is typically conducted in the first trimester, while amniocentesis is carried out during the second trimester and cordocentesis is available in the early third trimester. Further collection of these biospecimens in addition to cord tissue, meconium, and neonatal hair at or after delivery can provide additional PK data. Incorporation of PK data with alternative approaches can inform fetal drug exposure.

Given the inherent limitations of invasive sampling, alternative approaches are necessary to supplement our understanding of fetal drug disposition. Examples of alternative approaches include traditional animal models as well as *in silico* and *in vitro* methods. Historically, animal models have been utilized to study

the passage of drugs from mother to fetus, yet these results are not always transferrable to humans (Bracken, 2009). Animal placental anatomy, gestation lengths, and translatability to the clinical setting should be considered when using this approach (Grigsby, 2016). PBPK modeling used to describe medications administered during pregnancy is becoming more popular, but data to validate this approach is essential. In attempt to provide pregnancy exposure data to the public, the FDA organizes registries that collect information on exposure to medical products during pregnancy (Food and Drug Administration, 2023). However, limited concentration data for validation of fetal exposure is available through these post-marketing registries. It is therefore necessary to supplement this data with well-designed, opportunistic trials as well as share study results from academic and government institutions. Placenta-on-a-chip and other experimental *in vitro* approaches (Myllynen and Vahakangas, 2013) have the potential to provide important information; however, these techniques currently lack integration of changes that occur throughout pregnancy. Excellent examples of combining multiple approaches to estimate human fetal drug exposure have recently been published (Balhara et al., 2022; Roelofsen et al., 2022). Alternative approaches can provide insight into fetal drug exposure during human pregnancy and inform dosing in clinical trials that include pregnant individuals.

The importance of including pregnant individuals in drug therapy studies cannot be overstated. The U.S. Food and Drug Administration (FDA) has recently focused attention on the importance of including pregnant individuals in clinical trials (Vasisht et al., 2021), and drawn attention to their extensive Final Rule on drug labeling for use during pregnancy (Food and Drug Administration, 2014). In addition, the FDA recommends that clinical research including pregnant individuals meet all ten conditions specified in the U.S. Department of Health and Human Services regulations (Food and Drug Administration, 2018). These regulations acknowledge the variations in local regulations involving pregnant minors in pregnancy-related research and outline how to consider risks to both the mother and fetus. Regulations for considering the fetal effects of new drugs are extremely limited, as pediatric regulations (21 CFR subpart D) do not apply to the fetus (Green et al., 2021). Only U.S. Health and Human Service regulations (45 CFR Part 46) apply to the fetus (Green et al., 2021). Rules and regulations from the FDA, European Medicines Agency, and other agencies outline ethical considerations associated with conducting clinical trials involving pregnant individuals (European Medicines Agency, 2005; International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), 2016; Food and Drug Administration, 2022).

Ethical considerations in fetal medicine are complex, involving the interests of the mother, the father, and the fetus. Medications administered to the mother during pregnancy cross the placenta to reach the fetus in varying amounts. Fetal exposure may be below or above the NOAEL (no observable adverse effect level), which is the threshold for an adverse fetal effect. Prospective studies to determine NOAEL without therapy intended to benefit the mother, fetus, or both is unethical. The extent of maternal drug disposition and the amount of maternal-fetal drug transfer varies for specific pathways throughout pregnancy. By utilizing available data, convenience biospecimen sampling, and alternative approaches, we can optimize clinical care and minimize risk to the mother and fetus during pregnancy.

Conclusion

Ethical considerations are unavoidable when considering pregnant individuals in clinical trials and research studies. Notably, anatomical and physiological changes throughout pregnancy can impact risk associated with medication or illicit drug use. Here, we present different sampling options from various biospecimens *in utero* and following birth to aid in quantifying maternal-fetal drug transfer. Biospecimen samples may opportunistically be collected during a procedure for a prenatal standard of care medical decision. Non-invasive approaches, including animal models, PBPK modeling, and *in vitro* methods, provide a gateway for scientists to explore fetal drug transfer without putting the mother or fetus at risk. These and other innovative methods are necessary to advance the field of maternal-fetal pharmacology.

Nonetheless, future exploration is necessary when investigating medications in pregnant populations.

Author contributions

RH, KW, and KJ contributed to manuscript conception, design, and preparation. RH, TM, RW, AM, EE, CS, and KJ contributed to the literature search, interpretation of data, and manuscript writing. RH, TM, RW, and KJ worked on the discussion and conclusion sections. RH, TM, RW, EE, CS, KW, and KJ made substantial contributions and conducted revision of the manuscript for valuable intellectual content. All authors have read and approved the final version of the manuscript.

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Conflict of interest

We declare that EE and KJ are Associate Editors for the Obstetric and Pediatric Pharmacology section of Frontiers in Pharmacology. We declare that CS is a Specialty Chief Editor in Frontiers Obstetric and Pediatric Pharmacology for the Frontiers in Pharmacology Journal.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abduljalil, K., Gardner, I., and Jamei, M. (2022a). Application of a physiologically based pharmacokinetic approach to predict theophylline pharmacokinetics using virtual non-pregnant, pregnant, fetal, breast-feeding, and neonatal populations. *Front. Pediatr.* 10, 840710.

Abduljalil, K., Jamei, M., and Johnson, T. N. (2019). Fetal physiologically based pharmacokinetic models: Systems information on the growth and composition of fetal organs. *Clin. Pharmacokinet.* 58, 235–262.

Abduljalil, K., Ning, J., Pansari, A., Pan, X., and Jamei, M. (2022b). Prediction of maternal and fetoplacental concentrations of cefazolin, cefuroxime, and amoxicillin during pregnancy using bottom-up physiologically based pharmacokinetic models. *Drug Metab. Dispos.* 50, 386–400. doi:10.1124/dmd.121.000711

Allegaert, K., and Van Calsteren, K. (2016). Maternal, fetal, and neonatal pharmacokinetics. *Oxf. Textb. Obstetric Anaesth.* 68.

Andersen, M. D., Alstrup, A. K. O., Duvald, C. S., Mikkelsen, E. F. R., Vendelbo, M. H., Ovesen, P. G., et al. (2018). Animal models of fetal medicine and obstetrics. In *Experimental animal models of human diseases-an effective therapeutic strategy*, 10.

Balhara, A., Kumar, A. R., and Unadkat, J. D. (2022). Predicting human fetal drug exposure through maternal-fetal PBPK modeling and *in vitro* or *ex vivo* studies. *J. Clin. Pharmacol.* 62, S94–S114. doi:10.1002/jcph.2117

Bar-Oz, B., Klein, J., Karaskov, T., and Koren, G. (2003). Comparison of meconium and neonatal hair analysis for detection of gestational exposure to drugs of abuse. *Arch. Dis. Child. Fetal Neonatal Ed.* 88, F98–F100. doi:10.1136/fn.88.2.f98

Beall, M. H., Van Den Wijngaard, J. P., Van Gemert, M. J., and Ross, M. G. (2007). Amniotic fluid water dynamics. *Placenta* 28, 816–823. doi:10.1016/j.placenta.2006.11.009

Bernard, B., Abate, M., Thielen, P. F., Attar, H., Ballard, C. A., and Wehrle, P. F. (1977a). Maternal-fetal pharmacological activity of amikacin. *J. Infect. Dis.* 135, 925–932. doi:10.1093/infdis/135.6.925

Bernard, B., Garcia-Cazares, S. J., Ballard, C. A., Thrupp, L. D., Mathies, A. W., and Wehrle, P. F. (1977b). Tobramycin: Maternal-fetal pharmacology. *Antimicrob. Agents Chemother.* 11, 688–694. doi:10.1128/AAC.11.4.688

Blackburn, S. T., and Loper, D. L. (1992). *Maternal, fetal, and neonatal physiology: A clinical perspective*. Philadelphia: Saunders.

Bloomfield, F. H., Alexander, T., Muelbert, M., and Beker, F. (2017). Smell and taste in the preterm infant. *Early Hum. Dev.* 114, 31–34. doi:10.1016/j.earlhumdev.2017.09.012

Blundell, C., Yi, Y. S., Ma, L., Tess, E. R., Farrell, M. J., Georgescu, A., et al. (2018). Placental drug transport-on-a-chip: A microengineered *in vitro* model of transporter-mediated drug efflux in the human placental barrier. *Adv. Healthc. Mater* 7, 1700786. doi:10.1002/adhm.201700786

Boskovic, R., Klein, J., Woodland, C., Karaskov, T., and Koren, G. (2001). The role of the placenta in variability of fetal exposure to cocaine and cannabinoids: A twin study. *Can. J. Physiol. Pharmacol.* 79, 942–945. doi:10.1139/y01-080

Brace, R. A., and Wolf, E. J. (1989). Normal amniotic fluid volume changes throughout pregnancy. *Am. J. Obstet. Gynecol.* 161, 382–388. doi:10.1016/0002-9378(89)90527-9

Bracken, M. B. (2009). Why animal studies are often poor predictors of human reactions to exposure. *J. R. Soc. Med.* 102, 120–122. doi:10.1258/jrsm.2008.08k033

Bukkems, V. E., Van Hove, H., Roelofsen, D., Freriksen, J. J. M., Van Ewijk-Beneken Kolmer, E. W. J., Burger, D. M., et al. (2022). Prediction of maternal and fetal doravirine exposure by integrating physiologically based pharmacokinetic modeling and human placenta perfusion experiments. *Clin. Pharmacokinet.* 61, 1129–1141. doi:10.1007/s40262-022-01127-0

Center for Drug Evaluation and Research (2019). *Population pharmacokinetics (draft guidance)*. [Online]. United States: Food and Drug Administration. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/population-pharmacokinetics (Accessed April 26, 2021).

Centers for Disease Control and Prevention (2022). *Research on medicines and pregnancy*. [Online]. Available at: https://www.cdc.gov/pregnancy/meds/treatingfortwo/research.html#:~:text=Medicine%20Use%20during%20Pregnancy&text=Almost%201%20in%204%20pregnant,at%20least%20one%20prescription%20medicine.Can (Accessed November 7, 2022).

Chappuy, H., Treluyer, J. M., Jullien, V., Dimet, J., Rey, E., Fouche, M., et al. (2004a). Maternal-fetal transfer and amniotic fluid accumulation of nucleoside analogue reverse transcriptase inhibitors in human immunodeficiency virus-infected pregnant women. *Antimicrob. Agents Chemother.* 48, 4332–4336. doi:10.1128/AAC.48.11.4332-4336.2004

Chappuy, H., Treluyer, J. M., Rey, E., Dimet, J., Fouche, M., Firtion, G., et al. (2004b). Maternal-fetal transfer and amniotic fluid accumulation of protease inhibitors in pregnant women who are infected with human immunodeficiency virus. *Am. J. Obstet. Gynecol.* 191, 558–562. doi:10.1016/j.ajog.2004.01.034

Cheung, K. L., and Lafayette, R. A. (2013). Renal physiology of pregnancy. *Adv. Chronic Kidney Dis.* 20, 209–214. doi:10.1053/j.ackd.2013.01.012

Colby, J. M. (2017). Comparison of umbilical cord tissue and meconium for the confirmation of *in utero* drug exposure. *Clin. Biochem.* 50, 784–790. doi:10.1016/j.clinbiochem.2017.03.006

Collier, A. C., Tingle, M. D., Paxton, J. W., Mitchell, M. D., and Keelan, J. A. (2002). Metabolizing enzyme localization and activities in the first trimester human placenta: The effect of maternal and gestational age, smoking and alcohol consumption. *Hum. Reprod.* 17, 2564–2572. doi:10.1093/humrep/17.10.2564

Concheiro, M., Gonzalez-Colmenero, E., Lendoiro, E., Concheiro-Guisan, A., De Castro, A., Cruz-Landeira, A., et al. (2013). Alternative matrices for cocaine, heroin, and methadone *in utero* drug exposure detection. *Ther. Drug Monit.* 35, 502–509. doi:10.1097/FTD.0b013e31828a6148

Concheiro, M., Jones, H. E., Johnson, R. E., Choo, R., Shakleya, D. M., and Huestis, M. A. (2010). Umbilical cord monitoring of *in utero* drug exposure to buprenorphine and correlation with maternal dose and neonatal outcomes. *J. Anal. Toxicol.* 34, 498–505. doi:10.1093/jat/34.8.498

Dallmann, A., Ince, I., Coboeken, K., Eissing, T., and Hempel, G. (2018). A physiologically based pharmacokinetic model for pregnant women to predict the pharmacokinetics of drugs metabolized via several enzymatic pathways. *Clin. Pharmacokinet.* 57, 749–768. doi:10.1007/s40262-017-0594-5

Dallmann, A., Ince, I., Meyer, M., Willmann, S., Eissing, T., and Hempel, G. (2017). Gestation-specific changes in the anatomy and physiology of healthy pregnant women: An extended repository of model parameters for physiologically based pharmacokinetic modeling in pregnancy. *Clin. Pharmacokinet.* 56, 1303–1330. doi:10.1007/s40262-017-0539-z

Dallmann, A., Liu, X. I., Burckart, G. J., and Van Den Anker, J. (2019a). Drug transporters expressed in the human placenta and models for studying maternal-fetal drug transfer. *J. Clin. Pharmacol.* 59 (1), S70–S81. doi:10.1002/jcph.1491

Dallmann, A., Mian, P., Van Den Anker, J., and Allegaert, K. (2019b). Clinical pharmacokinetic studies in pregnant women and the relevance of pharmacometric tools. *Curr. Pharm. Des.* 25, 483–495. doi:10.2174/1381612825666190320135137

Daum, H., Ben David, A., Nadjari, M., Zenvirt, S., Helman, S., Yanai, N., et al. (2019). Role of late amniocentesis in the era of modern genomic technologies. *Ultrasound Obstet. Gynecol.* 53, 676–685. doi:10.1002/uog.20113

De Barros Duarte, L., Moises, E. C., Carvalho Cavalli, R., Lanchote, V. L., Duarte, G., and Da Cunha, S. P. (2009). Distribution of fentanyl in the placental intervillous space and in the different maternal and fetal compartments in term pregnant women. *Eur. J. Clin. Pharmacol.* 65, 803–808. doi:10.1007/s00228-009-0645-4

De Haan, G.-J., Edelbroek, P., Segers, J., Engelsman, M., Lindhout, D., Devile-Notschaele, M., et al. (2004). Gestation-induced changes in lamotrigine pharmacokinetics: A monotherapy study. *Neurology* 63, 571–573. doi:10.1212/01.wnl.0000133213.10244.fd

De Sousa Mendes, M., Lui, G., Zheng, Y., Pressiat, C., Hirt, D., Valade, E., et al. (2017). A physiologically-based pharmacokinetic model to predict human fetal exposure for a drug metabolized by several CYP450 pathways. *Clin. Pharmacokinet.* 56, 537–550. doi:10.1007/s40262-016-0457-5

De Vries, J. I., Visser, G., and Prechtl, H. F. (1985). The emergence of fetal behaviour. II. Quantitative aspects. *Early Hum. Dev.* 12, 99–120. doi:10.1016/0378-3782(85)90174-4

Dempsey, D., Tutka, P., Jacob, P., Iii, Allen, F., Schoedel, K., Tyndale, R. F., et al. (2004). Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. *Clin. Pharmacol. Ther.* 76, 64–72. doi:10.1016/j.clpt.2004.02.011

Dickinson, R., Hooper, W., Wood, B., Lander, C., and Eadie, M. (1989). The effect of pregnancy in humans on the pharmacokinetics of stable isotope labelled phenytoin. *Br. J. Clin. Pharmacol.* 28, 17–27. doi:10.1111/j.1365-2125.1989.tb03501.x

Duarte, L. D., Moises, E. C. D., Cavalli, R. C., Lanchote, V. L., Duarte, G., and Da Cunha, S. P. (2011). Distribution of bupivacaine enantiomers and lidocaine and its metabolite in the placental intervillous space and in the different maternal and fetal compartments in term pregnant women. *J. Clin. Pharmacol.* 51, 212–217. doi:10.1177/0091270010365551

Edginton, A. N., Schmitt, W., and Willmann, S. (2006). Development and evaluation of a generic physiologically based pharmacokinetic model for children. *Clin. Pharmacokinet.* 45, 1013–1034. doi:10.2165/00003088-200645100-00005

Eliopoulos, C., Klein, J., Chitayat, D., Greenwald, M., and Koren, G. (1996). Nicotine and cotinine in maternal and neonatal hair as markers of gestational smoking. *Clin. Investigative Medicine-Medecine Clinique Exp.* 19, 231–242.

Elliott, B. D., Langer, O., Schenker, S., and Johnson, R. F. (1991). Insignificant transfer of glyburide occurs across the human placenta. *Am. J. Obstet. Gynecol.* 165, 807–812. doi:10.1016/0002-9378(91)90421-m

European Medicines Agency (2005). *Guideline on the exposure to medicinal products during pregnancy: Need for post-authorisation data*. [Online]. Available at: https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/guideline-exposure-medicinal-products-during-pregnancy-need-post-authorisation-data_en.pdf (Accessed December 21, 2022).

Eyler, F. D., Behnke, M., Wobie, K., Garvan, C. W., and Tebbett, I. (2005). Relative ability of biologic specimens and interviews to detect prenatal cocaine use. *Neurotoxicol Teratol.* 27, 677–687. doi:10.1016/j.ntt.2005.04.001

Fa, S., Larsen, T. V., Bilde, K., Daugaard, T. F., Ernst, E. H., Lykke-Hartmann, K., et al. (2018). Changes in first trimester fetal CYP1A1 and AHRR DNA methylation and mRNA expression in response to exposure to maternal cigarette smoking. *Environ. Toxicol. Pharmacol.* 57, 19–27. doi:10.1016/j.etap.2017.11.007

Farst, K. J., Valentine, J. L., and Hall, R. W. (2011). Drug testing for newborn exposure to illicit substances in pregnancy: Pitfalls and pearls. *Int. J. Pediatr.* 2011, 951616. doi:10.1155/2011/951616

Feghali, M., Venkataramanan, R., and Caritis, S. (2015). "Pharmacokinetics of drugs in pregnancy," in *Seminars in perinatology* (Elsevier), 512–519.

Fokina, V. M., West, H., Oncken, C., Clark, S. M., Ahmed, M. S., Hankins, G. D., et al. (2016). Bupropion therapy during pregnancy: The drug and its major metabolites in umbilical cord plasma and amniotic fluid. *Am. J. Obstet. Gynecol.* 215, 497.e1–497.e7. doi:10.1016/j.ajog.2016.05.016

Food, and Drug Administration, H. (2023). *List of Pregnancy Exposure Registries* [Online]. Available: https://www.fda.gov/science-research/womens-health-research/list-pregnancy-exposure-registries [Accessed January 20 2023].

Food, and Drug Administration, H. (2014). Content and format of labeling for human prescription drug and biological products; requirements for pregnancy and lactation labeling. Final rule. *Fed. Regist.* 79, 72063–72103.

Food, and Drug Administration, H. (2018). Pregnant women: Scientific and ethical considerations for inclusion in clinical trials. [Online]. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/pregnant-women-scientific-and-ethical-considerations-inclusion-clinical-trials (Accessed July 1, 2022).

Food, and Drug Administration (2022). *International counterparts*. [Online]. Available at: https://www.fda.gov/about-fda/cvm-offices/international-counterparts (Accessed December 21, 2022).

Franco, V., Mazzucchelli, I., Gatti, G., Specchio, L. M., La Neve, A., Papantonio, A., et al. (2008). Changes in lamotrigine pharmacokinetics during pregnancy and the puerperium. *Ther. drug Monit.* 30, 544–547. doi:10.1097/FTD.0b013e318178e2a9

Gareri, J., Klein, J., and Koren, G. (2006). Drugs of abuse testing in meconium. *Clin. Chim. Acta* 366, 101–111. doi:10.1016/j.cca.2005.10.028

George, B., Lumen, A., Nguyen, C., Wesley, B., Wang, J., Beitz, J., et al. (2020). Application of physiologically based pharmacokinetic modeling for sertraline dosing recommendations in pregnancy. *NPJ Syst. Biol. Appl.* 6, 36. doi:10.1038/s41540-020-00157-3

Gerdin, E., Salmonson, T., Lindberg, B., and Rane, A. (1990). *Maternal kinetics of morphine during labour*.

Gingrich, J., Filipovic, D., Conolly, R., Bhattacharya, S., and Veiga-Lopez, A. (2021). Pregnancy-specific physiologically-based toxicokinetic models for bisphenol A and bisphenol S. *Environ. Int.* 147, 106301. doi:10.1016/j.envint.2020.106301

Glasser, L., Sutton, N., Schmeling, M., and Machan, J. T. (2015). A comprehensive study of umbilical cord blood cell developmental changes and reference ranges by gestation, gender and mode of delivery. *J. Perinatol.* 35, 469–475. doi:10.1038/jp.2014.241

Goh, J. J. N., Behn, J., Chong, C.-S., Zhong, G., Maurer-Stroh, S., Fan, H., et al. (2021). Structure-based virtual screening of CYP1A1 inhibitors: Towards rapid tier-one assessment of potential developmental toxicants. *Archives Toxicol.* 95, 3031–3048. doi:10.1007/s00204-021-03111-2

Gray, T., and Huestis, M. (2007). Bioanalytical procedures for monitoring *in utero* drug exposure. *Anal. Bioanal. Chem.* 388, 1455–1465. doi:10.1007/s00216-007-1228-9

Green, D. J., Park, K., Bhatt-Mehta, V., Snyder, D., and Burckart, G. J. (2021). Regulatory considerations for the mother, fetus and neonate in fetal pharmacology modeling. *Front. Pediatr.* 9, 698611. doi:10.3389/fped.2021.698611

Griffiths, S. K., and Campbell, J. P. (2015). Placental structure, function and drug transfer. *Continuing Educ. Anaesth. Crit. Care and Pain* 15, 84–89. doi:10.1093/bjaceaccp/mku013

Grigsby, P. L. (2016). "Animal models to study placental development and function throughout normal and dysfunctional human pregnancy," in *Seminars in reproductive medicine* (Thieme Medical Publishers), 011–016.

Gude, N. M., Roberts, C. T., Kalionis, B., and King, R. G. (2004). Growth and function of the normal human placenta. *Thromb. Res.* 114, 397–407. doi:10.1016/j.thromres.2004.06.038

Haas, D. M., Marsh, D. J., Dang, D. T., Parker, C. B., Wing, D. A., Simhan, H. N., et al. (2018). Prescription and other medication use in pregnancy. *Obstet. Gynecol.* 131, 789–798. doi:10.1097/AOG.0000000000002579

Hakkola, J., Raunio, H., Purkunen, R., Pelkonen, O., Saarikoski, S., Cresteil, T., et al. (1996). Detection of cytochrome P450 gene expression in human placenta in first trimester of pregnancy. *Biochem. Pharmacol.* 52, 379–383. doi:10.1016/0006-2952(96)00216-x

Hannibal, R. L., Cardoso-Moreira, M., Chetty, S. P., Lau, J., Qi, Z., Gonzalez-Maldonado, E., et al. (2018). Investigating human placentation and pregnancy using first trimester chorionic villi. *Placenta* 65, 65–75. doi:10.1016/j.placenta.2018.03.005

Hebert, M. F., Easterling, T., Kirby, B., Carr, D., Buchanan, M., Rutherford, T., et al. (2008). Effects of pregnancy on CYP3A and P-glycoprotein activities as measured by disposition of midazolam and digoxin: A university of Washington specialized center of research study. *Clin. Pharmacol. Ther.* 84, 248–253. doi:10.1038/clpt.2008.1

Hendrick, V., Stowe, Z. N., Altshuler, L. L., Hwang, S., Lee, E., and Haynes, D. (2003). Placental passage of antidepressant medications. *Am. J. Psychiatry* 160, 993–996. doi:10.1176/appi.ajp.160.5.993

Hines, R. N. (2008). The ontogeny of drug metabolism enzymes and implications for adverse drug events. *Pharmacol. Ther.* 118, 250–267. doi:10.1016/j.pharmthera.2008.02.005

Holbrook, K. A., and Odland, G. F. (1978). Structure of the human fetal hair canal and initial hair eruption. *J. Investig. Dermatol* 71, 385–390. doi:10.1111/1523-1747.ep12556818

International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (2016). *Integrated addedum to ICH E6(R1): Guideline for good clinical practice*. [Online]. Available at: https://database.ich.org/sites/default/files/E6_R2_Addendum.pdf (Accessed December 21, 2022).

Iqbal, M., Audette, M. C., Petropoulos, S., Gibb, W., and Matthews, S. G. (2012). Placental drug transporters and their role in fetal protection. *Placenta* 33, 137–142. doi:10.1016/j.placenta.2012.01.008

Jindal, A., and Chaudhary, C. (2020). Amniocentesis, [Internet]. *StatPearls*.

Kauffman, R. E., Morris, J. A., and Azarnoff, D. L. (1975). Placental transfer and fetal urinary excretion of gentamicin during constant rate maternal infusion. *Pediatr. Res.* 9, 104–107. doi:10.1203/00006450-197502000-00009

Kazma, J. M., Van Den Anker, J., Allegaert, K., Dallmann, A., and Ahmadzia, H. K. (2020). Anatomical and physiological alterations of pregnancy. *J. Pharmacokinet. pharmacodynamics* 47, 271–285. doi:10.1007/s10928-020-09677-1

Ke, A. B., Rostami-Hodjegan, A., Zhao, P., and Unadkat, J. D. (2014). Pharmacometrics in pregnancy: An unmet need. *Annu. Rev. Pharmacol. Toxicol.* 54, 53–69. doi:10.1146/annurev-pharmtox-011613-140009

Klein, J., and Koren, G. (1999). Hair analysis-a biological marker for passive smoking in pregnancy and childhood. *Hum. Exp. Toxicol.* 18, 279–282. doi:10.1191/096032799678840048

Kumar, P., and Magon, N. (2012). Hormones in pregnancy. *Niger. Med. J.* 53, 179–183. doi:10.4103/0300-1652.107549

Lacroix, D., Sonnier, M., Moncion, A., Cheron, G., and Cresteil, T. (1997). Expression of CYP3A in the human liver-evidence that the shift between CYP3A7 and CYP3A4 occurs immediately after birth. *Eur. J. Biochem.* 247, 625–634. doi:10.1111/j.1432-1033.1997.00625.x

Langer, O., Conway, D. L., Berkus, M. D., Xenakis, E. M., and Gonzales, O. (2000). A comparison of glyburide and insulin in women with gestational diabetes mellitus. *N. Engl. J. Med.* 343, 1134–1138. doi:10.1056/NEJM200010193431601

Liu, X. I., Momper, J. D., Rakhmanina, N., Van Den Anker, J. N., Green, D. J., Burckart, G. J., et al. (2020). Physiologically based pharmacokinetic models to predict maternal pharmacokinetics and fetal exposure to emtricitabine and acyclovir. *J. Clin. Pharmacol.* 60, 240–255. doi:10.1002/jcph.1515

Lupattelli, A., Spigset, O., Twigg, M. J., Zagorodnikova, K., Mårdby, A.-C., Moretti, M. E., et al. (2014). Medication use in pregnancy: A cross-sectional, multinational web-based study. *BMJ open* 4, e004365. doi:10.1136/bmjopen-2013-004365

Mandelbrot, L., Peytavin, G., Firtion, G., and Farinotti, R. (2001). Maternal-fetal transfer and amniotic fluid accumulation of lamivudine in human immunodeficiency virus-infected pregnant women. *Am. J. Obstet. Gynecol.* 184, 153–158. doi:10.1067/mob.2001.108344

Marin, S. J., Metcalf, A., Krasowski, M. D., Linert, B. S., Clark, C. J., Strathmann, F. G., et al. (2014). Detection of neonatal drug exposure using umbilical cord tissue and liquid chromatography time-of-flight mass spectrometry. *Ther. Drug Monit.* 36, 119–124. doi:10.1097/FTD.0b013e3182a0d18c

Maynard, E. C., Amoruso, L. P., and Oh, W. (1991). Meconium for drug testing. *Am. J. Dis. Child.* 145, 650–652. doi:10.1001/archpedi.1991.02160060068022

Mcgready, R., Stepniewska, K., Seaton, E., Cho, T., Cho, D., Ginsberg, A., et al. (2003). Pregnancy and use of oral contraceptives reduces the biotransformation of proguanil to cycloguanil. *Eur. J. Clin. Pharmacol.* 59, 553–557. doi:10.1007/s00228-003-0651-x

Mian, P., Allegaert, K., Conings, S., Annaert, P., Tibboel, D., Pfister, M., et al. (2020). Integration of placental transfer in a fetal-maternal physiologically based

pharmacokinetic model to characterize acetaminophen exposure and metabolic clearance in the fetus. *Clin. Pharmacokinet.* 59, 911–925. doi:10.1007/s40262-020-00861-7

Montgomery, D., Plate, C., Alder, S. C., Jones, M., Jones, J., and Christensen, R. D. (2006). Testing for fetal exposure to illicit drugs using umbilical cord tissue vs meconium. *J. Perinatol.* 26, 11–14. doi:10.1038/sj.jp.7211416

Montgomery, D. P., Plate, C. A., Jones, M., Jones, J., Rios, R., Lambert, D. K., et al. (2008). Using umbilical cord tissue to detect fetal exposure to illicit drugs: A multicentered study in Utah and New Jersey. *J. Perinatol.* 28, 750–753. doi:10.1038/jp.2008.97

Myllynen, P., Immonen, E., Kummu, M., and Vahakangas, K. (2009). Developmental expression of drug metabolizing enzymes and transporter proteins in human placenta and fetal tissues. *Expert Opin. Drug Metab. Toxicol.* 5, 1483–1499. doi:10.1517/17425250903304049

Myllynen, P., and Vahakangas, K. (2013). Placental transfer and metabolism: An overview of the experimental models utilizing human placental tissue. *Toxicol Vitro* 27, 507–512. doi:10.1016/j.tiv.2012.08.027

Nadanaciva, S., Lu, S., Gebhard, D. F., Jessen, B. A., Pennie, W. D., and Will, Y. (2011). A high content screening assay for identifying lysosomotropic compounds. *Toxicol Vitro* 25, 715–723. doi:10.1016/j.tiv.2010.12.010

Nishimura, M., Yaguti, H., Yoshitsugu, H., Naito, S., and Satoh, T. (2003). Tissue distribution of mrna expression of human cytochrome P450 isoforms assessedby high-sensitivity real-time reverse transcription PCR. *Yakugaku zasshi* 123, 369–375. doi:10.1248/yakushi.123.369

Orczyk-Pawilowicz, M., Jawien, E., Deja, S., Hirnle, L., Zabek, A., and Mlynarz, P. (2016). Metabolomics of human amniotic fluid and maternal plasma during normal pregnancy. *PLoS One* 11, e0152740. doi:10.1371/journal.pone.0152740

Ostrea, E. M., Jr., Brady, M. J., Parks, P. M., Asensio, D. C., and Naluz, A. (1989). Drug screening of meconium in infants of drug-dependent mothers: An alternative to urine testing. *J. Pediatr.* 115, 474–477. doi:10.1016/s0022-3476(89)80860-1

Ostrea, E. M., Jr., Knapp, D. K., Tannenbaum, L., Ostrea, A. R., Romero, A., Salari, V., et al. (2001). Estimates of illicit drug use during pregnancy by maternal interview, hair analysis, and meconium analysis. *J. Pediatr.* 138, 344–348. doi:10.1067/mpd.2001.111429

Paulzen, M., Goecke, T. W., Kuzin, M., Augustin, M., Grunder, G., and Schoretsanitis, G. (2018). Pregnancy exposure to quetiapine - therapeutic drug monitoring in maternal blood, amniotic fluid and cord blood and obstetrical outcomes. *Schizophrenia Res.* 195, 252–257. doi:10.1016/j.schres.2017.09.043

Paulzen, M., Goecke, T. W., Stickeler, E., Grunder, G., and Schoretsanitis, G. (2017a). Sertraline in pregnancy - therapeutic drug monitoring in maternal blood, amniotic fluid and cord blood. *J. Affect. Disord.* 212, 1–6. doi:10.1016/j.jad.2017.01.019

Paulzen, M., Goecke, T. W., Stingl, J. C., Janssen, G., Stickeler, E., Grunder, G., et al. (2017b). Pregnancy exposure to citalopram - therapeutic drug monitoring in maternal blood, amniotic fluid and cord blood. *Prog. Neuro-Psychopharmacology Biol. Psychiatry* 79, 213–219. doi:10.1016/j.pnpbp.2017.06.030

Paulzen, M., Schoretsanitis, G., Grunder, G., Franz, C., Stingl, J. C., and Augustin, M. (2020). Pregnancy exposure to venlafaxine-Therapeutic drug monitoring in maternal blood, amniotic fluid and umbilical cord blood and obstetrical outcomes. *J. Affect Disord.* 266, 578–584. doi:10.1016/j.jad.2020.02.010

Pavek, P., Ceckova, M., and Staud, F. (2009a). Variation of drug kinetics in pregnancy. *Curr. drug Metab.* 10, 520–529. doi:10.2174/138920009788897993

Pavek, P., Ceckova, M., and Staud, F. (2009b). Variation of drug kinetics in pregnancy. *Curr. Drug Metab.* 10, 520–529. doi:10.2174/138920009788897993

Pemathilaka, R. L., Caplin, J. D., Aykar, S. S., Montazami, R., and Hashemi, N. N. (2019). Placenta-on-a-Chip: *In vitro* study of caffeine transport across placental barrier using liquid chromatography mass spectrometry. *Glob. Chall.* 3, 1800112. doi:10.1002/gch2.201800112

Peng, J., Ladumor, M. K., and Unadkat, J. D. (2022). Estimation of fetal-to-maternal unbound steady-state plasma concentration ratio of P-glycoprotein and/or breast cancer resistance protein substrate drugs using a maternal-fetal physiologically based pharmacokinetic model. *Drug Metab. Dispos.* 50, 613–623. doi:10.1124/dmd.121.000733

Pons, J. C., Taburet, A. M., Singlas, E., Delfraissy, J. F., and Papiernik, E. (1991). Placental passage of azathiothymidine (AZT) during the second trimester of pregnancy: Study by direct fetal blood sampling under ultrasound. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 40, 229–231. doi:10.1016/0028-2243(91)90122-2

Poulsen, M. S., Rytting, E., Mose, T., and Knudsen, L. E. (2009). Modeling placental transport: Correlation of *in vitro* BeWo cell permeability and *ex vivo* human placental perfusion. *Toxicol Vitro* 23, 1380–1386. doi:10.1016/j.tiv.2009.07.028

Pranke, P., Failace, R. R., Allebrandt, W. F., Steibel, G., Schmidt, F., and Nardi, N. B. (2001). Hematologic and immunophenotypic characterization of human umbilical cord blood. *Acta Haematol.* 105, 71–76. doi:10.1159/000046537

Prevost, R. R., Aki, S. A., Whybrew, W. D., and Sibai, B. M. (1992). Oral nifedipine pharmacokinetics in pregnancy-induced hypertension. *Pharmacother. J. Hum. Pharmacol. Drug Ther.* 12, 174–177.

Price, H. R., Chehroudi, C., Knight, S. J., Smith, A. D., Lai, D., Kim, H., et al. (2020). Umbilical cord as an analytical matrix - a technical note. *Placenta* 90, 42–44. doi:10.1016/j.placenta.2019.12.001

Pritchard, J. A. (1966). Fetal swallowing and amniotic fluid volume. *Obstet. Gynecol.* 28, 606–610.

Prouillac, C., and Lecoeur, S. (2010). The role of the placenta in fetal exposure to xenobiotics: Importance of membrane transporters and human models for transfer studies. *Drug Metab. Dispos.* 38, 1623–1635. doi:10.1124/dmd.110.033571

Pruthi, S. (2020). *Amniocentesis*. [Online]. Available at: https://www.mayoclinic.org/tests-procedures/amniocentesis/about/pac-20392914 (Accessed March 10, 2022).

Roelofsen, D., Van Hove, H., Bukkems, V., Russel, F., Eliesen, G., and Greupink, R. (2022). Predicting fetal exposure of crizotinib during pregnancy: Combining human *ex vivo* placenta perfusion data with physiologically-based pharmacokinetic modeling. *Toxicol. Vitro* 85, 105471. doi:10.1016/j.tiv.2022.105471

Schöni-Affolter, F., Dubuis-Grieder, C., and Strauch, E. (2007). *The umbilical cord*.

Silva, L. L., Silvola, R. M., Haas, D. M., and Quinney, S. K. (2022). Physiologically based pharmacokinetic modelling in pregnancy: Model reproducibility and external validation. *Br. J. Clin. Pharmacol.* 88, 1441–1451. doi:10.1111/bcp.15018

Siu, S. S., Yeung, J. H., and Lau, T. K. (2000). A study on placental transfer of diclofenac in first trimester of human pregnancy. *Hum. Reprod.* 15, 2423–2425. doi:10.1093/humrep/15.11.2423

Skelly, C. L., Zulfiqar, H., and Sankararaman, S. (2020). "Meconium,". [Internet] in *StatPearls* (Treasure Island, FL: StatPearls Publishing).

Slawson, M. H., Wilkins, D. G., and Rollins, D. E. (1998). The incorporation of drugs into hair: Relationship of hair color and melanin concentration to phencyclidine incorporation. *J. Anal. Toxicol.* 22, 406–413. doi:10.1093/jat/22.6.406

Society for Maternal-Fetal, M., Berry, S. M., Stone, J., Norton, M. E., Johnson, D., and Berghella, V. (2013). Fetal blood sampling. *Am. J. Obstet. Gynecol.* 209, 170–180. doi:10.1016/j.ajog.2013.07.014

Spurway, J., Logan, P., and Pak, S. (2012). The development, structure and blood flow within the umbilical cord with particular reference to the venous system. *Australas. J. Ultrasound Med.* 15, 97–102. doi:10.1002/j.2205-0140.2012.tb00013.x

Steinfort, K., Van Houtven, E., Jacquemyn, Y., Blaumeiser, B., and Loquet, P. (2021). Difference in procedure-related risk of miscarriage between early and mid-trimester amniocentesis: A retrospective cohort study. *Diagnostics* 11, 1098. doi:10.3390/diagnostics11061098

Syme, M. R., Paxton, J. W., and Keelan, J. A. (2004). Drug transfer and metabolism by the human placenta. *Clin. Pharmacokinet.* 43, 487–514. doi:10.2165/00003088-200443080-00001

Szeto, H. H., Zervoudakis, I. A., Cederqvist, L. L., and Inturrisi, C. E. (1978). Amniotic fluid transfer of meperidine from maternal plasma in early pregnancy. *Obstet. Gynecol.* 52, 59–62.

Tomson, T., Lindbom, U., Ekqvist, B., and Sundqvist, A. (1994). Disposition of carbamazepine and phenytoin in pregnancy. *Epilepsia* 35, 131–135. doi:10.1111/j.1528-1157.1994.tb02922.x

Van Lierde, M., and Thomas, K. (1982). Ritodrine concentrations in maternal and fetal serum and amniotic fluid. *J. Perinat. Med.* 10, 119–124. doi:10.1515/jpme.1982.10.2.119

Vasisht, K. P., Nugent, B. M., and Woodcock, J. (2021). Progress and opportunities for women in clinical trials: A look at recent data and initiatives from the US FDA. *Med* 2, 456–459. doi:10.1016/j.medj.2021.04.010

Veit, F., Erdmann, F., Birngruber, C., and Dettmeyer, R. (2017). Detection of drugs in paired maternal and umbilical cord blood samples. *Romanian J. Leg. Med.* 25, 185–192. doi:10.4323/rjlm.2017.185

Ward, R. M. (1995). Pharmacological treatment of the fetus. Clinical pharmacokinetic considerations. *Clin. Pharmacokinet.* 28, 343–350. doi:10.2165/00003088-199528050-00001

Ward, R. M., and Varner, M. W. (2019). Principles of pharmacokinetics in the pregnant woman and fetus. *Clin. Perinatol.* 46, 383–398. doi:10.1016/j.clp.2019.02.014

Weissgerber, T. L., and Wolfe, L. A. (2006). Physiological adaptation in early human pregnancy: Adaptation to balance maternal-fetal demands. *Appl. Physiol. Nutr. Metab.* 31, 1–11. doi:10.1139/h05-003

Wesley, B. D., Sewell, C. A., Chang, C. Y., Hatfield, K. P., and Nguyen, C. P. (2021). Prescription medications for use in pregnancy-perspective from the US Food and drug administration. *Am. J. Obstet. Gynecol.* 225, 21–32. doi:10.1016/j.ajog.2021.02.032

Wilson, R. D. (1995). Early amniocentesis: A clinical review. *Prenat. Diagn* 15, 1259–1273. doi:10.1002/pd.1970151307

Zhang, H., Kalluri, H. V., Bastian, J. R., Chen, H., Alshabi, A., Caritis, S. N., et al. (2018). Gestational changes in buprenorphine exposure: A physiologically-based pharmacokinetic analysis. *Br. J. Clin. Pharmacol.* 84, 2075–2087. doi:10.1111/bcp.13642

Zhang, Z., Imperial, M. Z., Patilea-Vrana, G. I., Wedagedera, J., Gaohua, L., and Unadkat, J. D. (2017). Development of a novel maternal-fetal physiologically based pharmacokinetic model I: Insights into factors that determine fetal drug exposure through simulations and sensitivity analyses. *Drug Metab. Dispos.* 45, 920–938. doi:10.1124/dmd.117.075192

Zhao, P., Zhang, L., Grillo, J. A., Liu, Q., Bullock, J. M., Moon, Y. J., et al. (2011). Applications of physiologically based pharmacokinetic (PBPK) modeling and simulation during regulatory review. *Clin. Pharmacol. Ther.* 89, 259–267. doi:10.1038/clpt.2010.298

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