POSEIDON'S STRATIFICATION OF "LOW PROGNOSIS PATIENTS IN ART": THE WHY, THE WHAT, AND THE HOW

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EDITORIAL
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Editorial: POSEIDON's Stratification of 'Low Prognosis' Patients in ART: The WHY, the WHAT, and the HOW

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Editorial on the Research Topic

POSEIDON's Stratification of 'Low Prognosis' Patients in ART: The WHY, the WHAT, and the HOW

Management of patients with infertility and poor or suboptimal ovarian response to exogenous gonadotropin stimulation has challenged reproductive specialists for a long time. Apart from the limited understanding of its pathophysiology, there is wide heterogeneity in the definition of poor responders and overall disappointing outcomes when these patients undergo assisted reproductive technology (ART).

The **P**atient-**O**riented **S**trategies **E**ncompassing **I**ndividualize**D** **O**ocyte **N**umber (POSEIDON) criteria were introduced in 2016 with the primary goal of underlining differences related to a poor or suboptimal infertility treatment outcome in terms of oocyte quantity and quality, and possibly creating more homogenous groups for clinical management and research (1, 2). The POSEIDON criteria classify patients with infertility undergoing ART into four groups of 'low-prognosis' based on female age, ovarian reserve markers (antral follicle count and/or anti-Mullerian hormone), and the number of oocytes retrieved after a standard ovarian stimulation (**Figure 1**). By contrast, patients with adequate ovarian reserve markers and normal response to ovarian stimulation (>9 oocytes retrieved) can be classified as having a 'normal' prognosis (non-POSEIDON patients).

POSEIDON patients are presumed to be at a higher risk of failing to achieve a live birth after *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) treatment than non-POSEIDON patients for two main reasons, namely, reduced number of oocytes and, consequently, embryos; and poor oocyte/embryo quality, due to advanced female reproductive age (3–6).

Given its novelty and potential clinical and research utility, we developed a Research Topic fully dedicated to the POSEIDON criteria. We aimed to provide clinicians and scientists involved in the study and care of infertile couples a thoughtful and comprehensive review of the significance of the POSEIDON concept and its implications for practice and research. This Frontiers Research Topic on 'POSEIDON Stratification of Low-Prognosis Patients in ART: The WHY, the WHAT, and the HOW' comprises the seminal work of 65 renowned clinicians, embryologists, and scientists from 30 Institutions and fifteen countries on four continents. In 21 articles, authoritative reviews, original

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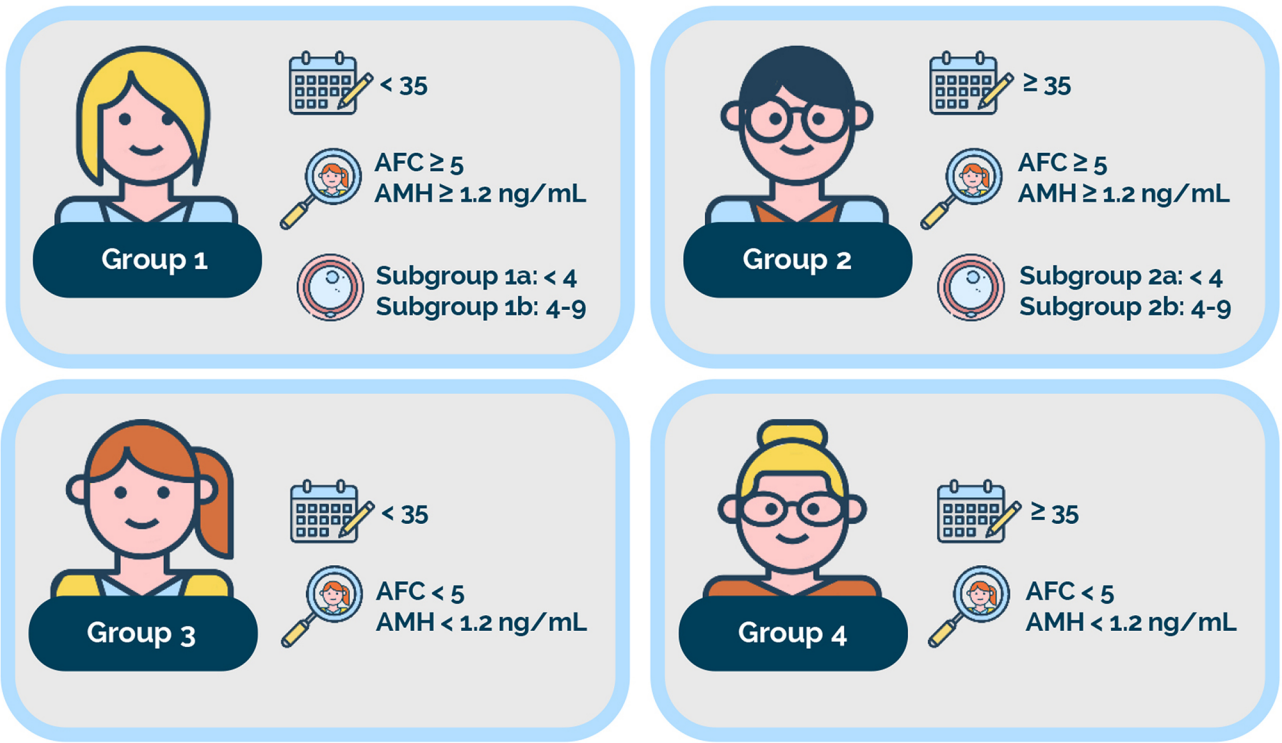

FIGURE 1 | POSEIDON criteria. Four distinct groups of low-prognosis patients can be established based on quantitative and qualitative parameters, namely: 1. The age of the patient and its related embryo aneuploidy rate; 2. Ovarian biomarkers [antral follicle count (AFC) and/or anti-Müllerian hormone (AMH)], and 3. The ovarian response in terms of oocyte quantity (if a previous cycle of conventional ovarian stimulation was carried out). Group 1: Patients <35 years with sufficient pre-stimulation ovarian reserve parameters (AFC ≥5, AMH ≥1.2 ng/ml) and with an unexpected poor (<4 oocytes) or suboptimal (four to nine oocytes) ovarian response. This group is further divided into subgroup 1a, constituted by patients with fewer than four oocytes; and subgroup 1b, constituted by patients with four to nine oocytes retrieved after standard ovarian stimulation, who, at any age, have a lower cumulative delivery rate than age-matched normal responders. Group 2: Patients ≥35 years with sufficient pre-stimulation ovarian reserve parameters (AFC ≥5, AMH ≥1.2 ng/ml) and with an unexpected poor or suboptimal ovarian response. This group is further divided into subgroup 2a, constituted by patients with fewer than four oocytes; and subgroup 2b, constituted by patients with four to nine oocytes retrieved after standard ovarian stimulation, who, at any age, have a lower cumulative delivery rate than age matched normal responders. Group 3: Patients <35 years with poor ovarian reserve pre-stimulation parameters (AFC <5, AMH <1.2 ng/ml). Group 4: Patients ≥35 years with poor ovarian reserve pre-stimulation parameters (AFC <5, AMH <1.2 ng/ml). Art drawing courtesy of Chloé Xilinas, Med.E.A., Rome, Italy. Reprint from: Copyright © 2021 Esteves SC, Conforti A, Sunkara SK, Carbone L, Picarelli S, Vaiarelli A, Cimadomo D, Rienzi L, Ubaldi FM, Zullo F, Andersen CY, Orvieto R, Humaidan P and Alviggi C (2021) Improving Reporting of Clinical Studies Using the POSEIDON Criteria: POSORT Guidelines. Front. Endocrinol. 12:587051. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

articles, and commentaries dissect the POSEIDON criteria from various angles, including epidemiology, pathophysiology, genetics, ovarian biomarkers, ovarian stimulation strategies, and other treatment modalities.

The first section (The 'WHY') explains the reasons why the POSEIDON criteria were developed. Five articles (Esteves, Roque et al., Grisendi et al., Cimadomo et al., Alviggi, Conforti et al., Grynberg and Labrosse) clarify this aspect and provide further insights into parameters used to classify patients fulfilling the POSEIDON criteria. One of them goes further by proposing a new maker –termed Follicle-to-Oocyte (FOI) Index– to identify the patients with ovarian hyporesponse to exogeneous gonadotropin stimulation (Alviggi, Conforti et al.).

The second section (The 'WHAT') comprises articles (Esteves, Alviggi et al., Esteves, Carvalho et al., Esteves, Yarali, Ubaldi et al., Fischer and Baukloh, Esteves and Carvalho) explaining in detail what the POSEIDON criteria are and their potential clinical implications for the diagnosis and management of infertility. Two of them (Esteves, Carvalho et al., Esteves, Yarali, Ubaldi et al.) are original articles, related to the development and validation of a novel predictive model to estimate the number of metaphase II (MII) oocytes required to obtain at least one euploid blastocyst for transfer in couples undergoing IVF/ICSI. Notably, the ability to retrieve the number of oocytes needed to achieve at least one euploid embryo for transfer was proposed by the POSEIDON group as an intermediate marker of a successful outcome in IVF/ICSI cycles. The predictive model mentioned above was the backbone of the so-called 'ART calculator'. It is an online tool that makes two types of predictions, one using pretreatment information to estimate the minimum number of

MII oocytes to achieve ≥1 euploid blastocyst, and another based on the actual number of mature oocytes collected/accumulated to estimate the chances of having a euploid blastocyst using that oocyte cohort for IVF/ICSI. The novel ART calculator may assist in clinical counseling and individualized treatment planning regarding the number of oocytes required for at least one euploid blastocyst in IVF/ICSI procedures, all aspects debated in dedicated commentaries (Fischer and Baukloh, Esteves and Carvalho).

Another original article within section two relates to a multicenter and multinational prevalence study of more than 13,000 patients who have undergone ART (Esteves, Yarali, Vuong et al.). This article confirms that POSEIDON patients are very common in the Fertility Clinic, representing about 43.0% of all treated patients. In this study, most POSEIDON patients were poor (<4 oocytes retrieved) or suboptimal (4-9 oocytes retrieved) responders despite having adequate ovarian reserve markers (Groups 1 and 2), thus highlighting opportunities for refining the clinical management of this vulnerable patient population. Additionally, this big data study showed that POSEIDON patients were older, had a higher body mass index, lower ovarian reserve markers, and a higher frequency of female factor as the primary treatment indication than non-POSEIDON patients, i.e, those patients not fulfilling the POSEIDON criteria. Lastly, this study showed that POSEIDON patients required larger doses of gonadotropin for ovarian stimulation, despite achieving a 2.5 times lower number of retrieved oocytes than normal responders with adequate ovarian markers (non-POSEIDON patients).

The last part (The 'HOW') is devoted to the clinical management of POSEIDON patients and how to conduct research using the POSEIDON classification. This section contains ten articles, including reviews (Conforti et al., Haahr et al.), original papers (Drakopoulos et al., Vaiarelli et al.), commentaries (Polyzos and Drakopoulos, Bühler, Sunkara et al., Fischer and Baukloh), future perspectives (Humaidan et al.), and guidelines (Esteves, Conforti, Sunkara et al.). The latter 'Policy and Practice Reviews' article (Esteves, Conforti, Sunkara et al.) is timely because the number of published studies using the POSEIDON criteria has increased steadily; however, inconsistent and incomplete reporting of critical outcomes are commonly seen.

At the time of writing (May 21), the entry 'POSEIDON criteria' on PubMed retrieved more than 50 articles of all sorts. Failure to recognize the critical pillars of the POSEIDON criteria, as mentioned above, might limit the clinical utility of such studies, notably when the essential endpoints are incompletely reported – or not reported at all. With this in mind, the 'Improving Reporting of Clinical Studies Using the POSEIDON Criteria' (POSORT) guidelines aim at help researchers improve the quality of reporting in studies applying the POSEIDON classification system (Esteves, Conforti, Sunkara et al.).

Other studies outside this Research Topic have substantiated the validity of the POSEIDON criteria in identifying relevant subpopulations with low-prognosis in IVF/ICSI treatment. It has recently been reported that either AFC or AMH can be used as the ovarian marker criterion for patient classification within the context of POSEIDON (7). Based on 9484 patients whose baseline ovarian reserves had been assessed by both AFC and AMH, a strong agreement (kappa~0.8) between AMH and AFC was found to classify POSEIDON patients. Approximately 75% of individuals were classified under the same patient group using both biomarkers. Importantly, virtually all patients with discordant biomarker results remained within the broad category of 'low prognosis' as defined by the POSEIDON criteria. Another finding of the study was that the optimal AFC and AMH (by Gen II assay) thresholds to predict the retrieval of <4 oocytes were 5 and 1.27 ng/mL, respectively, and thus like those established by the POSEIDON criteria (see **Figure 1**). Also, for the first time AFC and AMH thresholds were provided to identify suboptimal responders, i.e., patients who end up with an oocyte yield between 4 and 9 after standard ovarian stimulation. An AFC of 12 and an AMH value of 2.95 ng/mL (Gen II assay) were the optimal thresholds below which the risk of retrieving a 'suboptimal' (4-9 oocytes) oocyte number after standard ovarian stimulation is increased. Collectively, this study (7) showed that both biomarkers provide acceptable and equivalent accuracy in predicting oocyte yield further supporting their use and proposed thresholds in daily clinical practice for patient classification according to the POSEIDON criteria.

As previously stated, POSEIDON patients are presumed to be at a higher risk of failing to achieve a live birth after IVF/ICSI than normal responders with an adequate ovarian reserve. The cumulative delivery rate (CDR) per initiated/aspiration cycle after the transfer of all fresh and frozen–thawed/warmed embryos has been suggested to be the critical endpoint that sets these groups apart. This metric is increasingly recognized as an appropriate way to report ART success (8, 9) and has been selected as a critical efficacy outcome marker in the ESHRE 2019 guideline on ovarian stimulation for IVF/ICSI (10). It is considered the most meaningful outcome from the patients' perspective because it adequately reflects the prognosis of achieving a live birth after one initiated/aspirated ART cycle (11). Along these lines, a 2021 multinational study including over 9,000 patients showed that the CDR is, on average, ~50% lower in POSEIDON patients than non-POSEIDON patients, and it varied across POSEIDON groups (12). Interestingly, the CDR was twice as high in suboptimal responders (4-9 oocytes retrieved) as in poor responders (<4 oocytes), an effect that was primarily modulated by female age. Furthermore, logistic regression analysis showed that the POSEIDON stratification, the number of embryos obtained, the number of embryo transfer cycles per patient, the number of oocytes retrieved, female age, duration of infertility, and body mass index were relevant predictors of CDR (12).

This Research Topic has a broad appeal, and we hope it stimulates further research in terms of early diagnosis, prevention, and identification of specific interventions that could benefit POSEIDON patients. We, as guest editors, are grateful to the Chief Editors and the Editorial staff of Frontiers in Endocrinology (Reproduction) for their outstanding support. We recommend this Research Topic to clinicians involved in the management of infertile couples, including reproductive endocrinologists, gynecologists, reproductive urologists, andrologists, embryologists, as well as other healthcare

professionals providing care to infertility patients. Also, students and researchers in the biological and medical sciences, interested in following the exponential growth in knowledge involving female infertility and ART might greatly benefit from this collection of articles. We hope our readers will appreciate this Frontiers Research Topic and that they share our excitement in studying infertility and assisted reproductive technology.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

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PERSPECTIVE
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Double Stimulation in the Same Ovarian Cycle (DuoStim) to Maximize the Number of Oocytes Retrieved From Poor Prognosis Patients: A Multicenter Experience and SWOT Analysis

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A panel of experts known as the POSEIDON group has recently redefined the spectrum of poor responder patients and introduced the concept of suboptimal response. Since an ideal management for these patients is still missing, they highlighted the importance of tailoring the ovarian stimulation based on the chance of each woman to obtain an euploid blastocyst. Interestingly, a novel pattern of follicle recruitment has been defined: multiple waves may arise during a single ovarian cycle. This evidence opened important clinical implications for the treatment of poor responders. For instance, double stimulation in the follicular (FPS) and luteal phase (LPS) of the same ovarian cycle (DuoStim) is an intriguing option to perform two oocyte retrievals in the shortest possible time. Here, we reported our 2-year experience of DuoStim application in four private IVF centers. To date, 310 poor prognosis patients completed a DuoStim protocol and underwent IVF with blastocyst-stage preimplantation-genetic-testing. LPS resulted into a higher mean number of oocytes collected than FPS; however, their competence (i.e., fertilization, blastocyst, euploidy rates, and clinical outcomes after euploid single-embryo-transfer) was comparable. Importantly, the rate of patients obtaining at least one euploid blastocyst increased from 42.3% (n = 131/310) after FPS to 65.5% (n = 203/310) with the contribution of LPS. A summary of the putative advantages and disadvantages of DuoStim was reported here through a Strengths–Weaknesses–Opportunities–Threats analysis. The strengths of this approach make it very promising. However, more studies are needed in the future to limit its weaknesses, shed light on its putative threats, and realize its opportunities.

Keywords: duostim, double stimulation, dual-stimulation, low prognosis patients, poor responder, IVF, euploid blastocyst, Poseidon

INTRODUCTION

In IVF, poor response to controlled ovarian stimulation (COS) represents an important issue, which may affect 9–24% of the infertile women (1). Such a wide range is indeed indicative of a heterogeneous population of patients. Hence, several definitions have been proposed to classify "poor responders," namely up to 41 according to the systematic review by Polyzos and Devroey (2), and numerous protocols have been adopted to treat these women. The Bologna criteria (3) represented the first successful attempt to outline some guidelines in the definition of poor ovarian response. At least two of the following characteristics must be present to define "a poor responder patient": advanced maternal age (>40 years) and/or scarce response to a previous conventional stimulation (≤3 oocytes) and/or reduced ovarian reserve (antral follicle count, AFC < 5–7 follicles, and/or AMH < 1.1 ng/ml).

Yet, some criticism arose, since oocyte competence may be severely affected from numerous factors, among which maternal age is the most important (4, 5), to point out that the classification should be more patient-oriented and match the putative number of retrievable oocytes with their putative chance to develop as an euploid blastocyst. Hard evidence support that both the number of retrieved oocytes and woman age are indeed the most important parameters to predict the chance to conceive after IVF for all patients, including poor prognosis ones (6–9). An efficient prediction of the ovarian response is, therefore, pivotal to define a tailored-COS for each patient, especially poor responders, which should be based upon AFC and AMH, namely, the most widely used biomarkers at present (10).

A new classification by a panel of experts, known as the POSEIDON (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number) group (11), has been introduced to better categorize the spectrum of poor responder patients. Currently, the treatment for this heterogeneous group is not evidence-based, yet, and the prognosis is highly dependent upon patients' specific characteristics, rather than upon the COS protocol chosen (9). The POSEIDON group highlighted instead the importance of tailoring the stimulation based on the chance of each woman to obtain an euploid blastocyst, proposed as novel main goal of COS. Indeed, blastocyst transfer (12), especially of euploid embryos (13), showed to date the most promising results per transfer achievable in IVF. The POSEIDON group then introduced the concept of "sub-optimal response." In this group of patients collecting 4–9 oocytes, 4 sub-clusters were outlined according to both the ovarian reserve and the maternal age. Specifically, groups 3 and 4 are represented from women younger than 35 or older than 35, respectively, with a compromised ovarian reserve (AFC < 5 and AMH < 1.2 ng/ml), an issue, which cannot be resolved pharmacologically, as already reported in several studies (14–21).

The aim of this paper is to provide an update about the IVF management of poor responders, as well as to describe and encourage the use of novel strategies, especially for the patients of POSEIDON groups 3 and 4, to increase the cumulative live birth per IVF treatment.

THEORIES OF FOLLICLE RECRUITMENT

Follicular development is an extremely dynamic process. According to the classic theory (*single recruitment episode theory*), a single cohort of antral follicles grows during the follicular phase of the ovarian cycle after luteal regression. However, this theory has been overtaken by the evidence of multiple waves arising during an ovarian cycle in many mammals. Such evidence, at first reported in large animal models (22–27), was confirmed also in humans leading to the definition of two further theories of follicle recruitment (28): *the continuous recruitment theory*, according to which the follicles start growing and regress continuously during the ovarian cycle; and *the waves theory*, according to which 2–3 cohorts of antral follicles are recruited per ovarian cycle. However, the mechanisms underlying follicular recruitment have not been fully elucidated yet. Several intraovarian regulators, FSH and progesterone levels, inflammatory markers (e.g., serum C reactive protein) were all proposed as modulators of the dynamics behind the origin of follicular waves (28–30). From a clinical perspective, the growing knowledge of human ovarian follicular waves, opened new options for COS to improve the efficiency and possibly the efficacy of IVF.

DuoStim: CONSIDERATIONS, INDICATIONS, AND FRAMEWORK

Currently, there is insufficient evidence to recommend an ideal management of poor responders as defined through the Bologna Criteria. Indeed, regardless the COS protocol adopted, consistently low live birth rates were achieved in this population of patients (31–33). The choice of COS for patients with poor ovarian reserve markers and/or of advanced maternal age can be challenging. Yet, the number as well as the quality of the oocytes retrieved are important factors to increase the cumulative live birth rate. Moreover, these women have a limited time left to attempt to conceive with their own eggs: their "follicular heritage" suffers from a dramatic physiological decline of oocyte quantity and quality. The gonadotrophins can only support the growth of cohorts of follicles already present in the ovaries, but they cannot induce the *de novo* production of follicles. Therefore, increasing the dose of gonadotrophins administered or even adopting more powerful drugs will never compensate a reduced ovarian reserve.

In this scenario, a novel COS strategy has been proposed: double stimulation in the same ovarian cycle (DuoStim). Such protocol particularly suits poor prognosis and oncological patients, who require maximizing the exploitation of their ovarian reserve in a limited time (34–36). DuoStim, by combining conventional follicular phase stimulation (FPS) with luteal phase stimulation (LPS), can be considered a valuable option in patients with reduced ovarian reserve and/or advanced maternal age to maximize the number of oocytes retrieved in a single ovarian cycle, and for patients who did not collect oocytes or did not produce competent embryos after conventional FPS (37).

The very first experience with double stimulation has been reported by Kuang and colleagues (36) who showed that COS conducted in both the FPS and LPS of the same ovarian cycle results in the collection of oocytes with similar developmental

competence (36). The drugs used for COS in the Shanghai protocol, as it was called in the paper, were clomiphene citrate 25 mg/day, letrozole 2.5 mg/day, and mild dose of human menopausal gonadotrophin 150–225 IU/day. Moreover, the final oocytes maturation was induced with triptorelin followed by ibuprofen 0.6 g the day of trigger and the day after, in both FPS and LPS. In 2016, we published our proof-of-concept study where a DuoStim protocol was adopted together with a pre-implantation genetic testing (PGT-A) program in poor prognosis patients (34). The most important outcome outlined by this study was that the application of DuoStim in this thorny patient population increased the chance of obtaining at least one euploid blastocyst in a single ovarian cycle from 40 to 70%. Contrary to the Shanghai protocol, the DuoStim protocol consists in a co-treatment with maximal dose of FSH plus LH and GnRh antagonist to prevent ovulation in both FPS and LPS. The rationale of administrating FSH 300 IU/day plus LH 75 IU/day in an antagonist protocol, instead of adopting a mild stimulation, is to limit the risk for cycle cancelation and possibly decrease time-to-pregnancy by maximizing the number of oocytes collected per stimulation. To this regard, mild stimulation has been associated with a reduced number of oocytes retrievable per COS cycle (38). Therefore, even if no randomized controlled trial (RCT) has been performed to compare mild versus conventional COS in a DuoStim protocol, it is reasonable to hypothesize that while the cost of the former COS approach might involve lower expense than the latter (39), effectiveness is questionable. This is especially true if we account cumulative live birth rate per started cycle as the measure of success in IVF (40, 41).

The patient drop-out is then another very important issue in the treatment of poor prognosis patients. It has been reported largely variable (20–60%) among couples undergoing IVF worldwide (42–44). Still, a generally valid information cannot be produced due to heterogeneity in terms of cost, reimbursement policies, accessibility to IVF, indication for PGT-A, etc., among the different countries (45, 46). Importantly, the most significant drop-out rate involves the second attempt after a first failed IVF cycle. Furthermore, when a second attempt is performed, ~10 months often pass from the former retrieval, while the time is crucial especially for poor prognosis patients (47). These cases might be rescued *via* the application of a DuoStim approach, which would at least allow to conduct two retrievals in a single ovarian cycle. A future RCT comparing double FPS versus DuoStim and entailing also the drop-out rate among the outcomes under investigation might provide an answer to this issue.

Indications to Duostim

Since October 2015, DuoStim has been proposed at our four centers, after extensive counseling, to all patients matching at least two of the following criteria: AMH < 1.5 ng/mg, AFC ≤ 6 follicles, $\leq$5 metaphase II (MII) oocytes retrieved in a previous cycle, advanced maternal age ($\geq$35 years). Importantly, a single parameter is insufficient to outline an indication to DuoStim, since AFC evaluation *per se* might be limited from large inter-operator variability and AMH measurement *per se* might be affected from sample handling, storage, and low inter-laboratory reproducibility (48–50).

Another possible application of this strategy is urgent fertility preservation, in case few mature oocytes are collected after conventional COS and the time left before starting cancer therapy allows it (51).

Framework of a Duostim Protocol

To all patients undergoing Duostim, luteal estradiol priming (4 mg/day of estradiol valerate) was started in day 21 of the previous menstrual cycle to promote the synchronization and coordination of follicular growth (52, 53). After the transvaginal ultrasound and basal assessment of the ovaries, on day 2 to day 3 of the menstrual cycle, luteal estradiol priming was stopped, and FPS was started with fixed dose of rec-FSH 300 IU/day plus LH 75 IU/day for 4 days. Follicular growth was monitored on day 5 and then every 2–3 days. GnRh antagonist was administered daily after the identification of a leading follicle with a diameter $\geq$ 13–14 mm in FPS and LPS until the day of ovulation trigger. The final maturation of oocytes was triggered by a subcutaneous bolus of buserelin (dose 0.5 ml) to reduce the time of luteolysis. Egg retrieval was performed 35 h after the trigger. ICSI, blastocyst culture, trophectoderm biopsy, and vitrification, were performed as described in detail elsewhere (8, 54–56). Five days after the first retrieval, namely, the time needed to complete luteolysis (57), LPS was started with the same protocol and daily dose regardless of the number of antral follicles visible through ultrasound scan in the anovulatory wave. A freeze-all approach was adopted and the biopsy fragments from both stimulations were shipped together and analyzed in the same run at an external genetic lab (Igenomix, Italy). In presence of euploid blastocyst(s), frozen single embryo transfers were performed in a modified-natural or artificial cycle (58).

MULTICENTER EXPERIENCE AT G.EN.E.R.A. CENTERS FOR REPRODUCTIVE MEDICINE (ROME, NAPLES, UMBERTIDE, AND MAROSTICA, ITALY) TO DATE

DuoStim was suggested to 353 consecutive couples approaching G.EN.E.R.A. centers for reproductive medicine (Rome, Naples, Marostica, and Umbertide, Italy) between October 2015 and December 2017. All the related data were prospectively recorded in a relational database [Fertilab Manager (FLM), Italy]. Among them, 17 did not respond to FPS and were excluded from this analysis (4.8%). Then, 336 patients underwent LPS and 26 (7.7%) did not respond. The 43 patients who did not respond to either FPS or LPS were stopped after 8–9 days of gonadotrophins administration. Overall, 310 patients completed the DuoStim approach with two oocyte retrievals of at least one cumulus-oocyte-complex in a single menstrual cycle and were included in this analysis (Figure S1A in Supplementary Material). The maternal age of the patients included in the analysis was 40.0 ± 3.0 years (33.0–44.0), the AFC was 5.3 ± 2.5 (3–13), the AMH was 1.0 ± 1.0 (0.1–2), and they already underwent 1.0 ± 1.3 (0–6) previous IVF cycles collecting 4.0 ± 2.6 (0–14) MII oocytes. LPS was on average 1 day longer

than FPS. No increase of the post-oocyte retrieval complications has been reported so far compared to FPS-only cycles.

299 FPS- (96.5%) and 298 LPS-derived (96.1%) oocyte retrievals resulted in at least one MII oocyte collected (Figure S1B in Supplementary Material). **Figure 1A** displays the number of MII oocytes, fertilized oocytes, and blastocysts obtained on average after each LPS and FPS, respectively. Interestingly, a higher number of oocytes was collected after LPS, which involved also a higher number of fertilized oocytes and blastocysts (Wilcoxon Signed Rank test: $p < 0.01$). No difference was reported to date in terms of mean number of euploid blastocysts obtained from this cohort. The mean fertilization, blastocyst, and euploid blastocyst rates calculated per number of MII oocytes collected from each cycle (FPS- and LPS-derived ones, respectively) are reported in **Figure 1B** and were similar in the two groups. The overall fertilization, blastocyst, and euploid blastocyst rates of the 1,229 and 1,442 MII oocytes obtained after FPS and LPS, respectively, were also similar (**Figure 1C**).

229 (73.9%) and 230 (74.2%) patients obtained at least one blastocyst after FPS and LPS, respectively. This resulted in 280 patients who obtained at least one blastocyst in a single menstrual cycle due to DuoStim (90.3%). 131 (42.3%) and 129 (41.6%) patients obtained at least one euploid blastocyst after FPS and LPS, respectively. This resulted in 203 (65.5%) patients who obtained at least one euploid blastocyst in a single menstrual cycle due to DuoStim (Figure S1B in Supplementary Material).

81 and 83 FPS-derived and LPS-derived single euploid blastocyst transfers have been performed, respectively. In presence of euploid blastocysts from both FPS and LPS, the embryo to transfer was randomly chosen. The positive pregnancy rates were 48.1% ($n = 39/81$) and 59.0% ($n = 49/83$; Fisher's exact test: $p = $ NS). The biochemical pregnancy loss rates were 7.7% ($n = 3/39$) and 8.2%

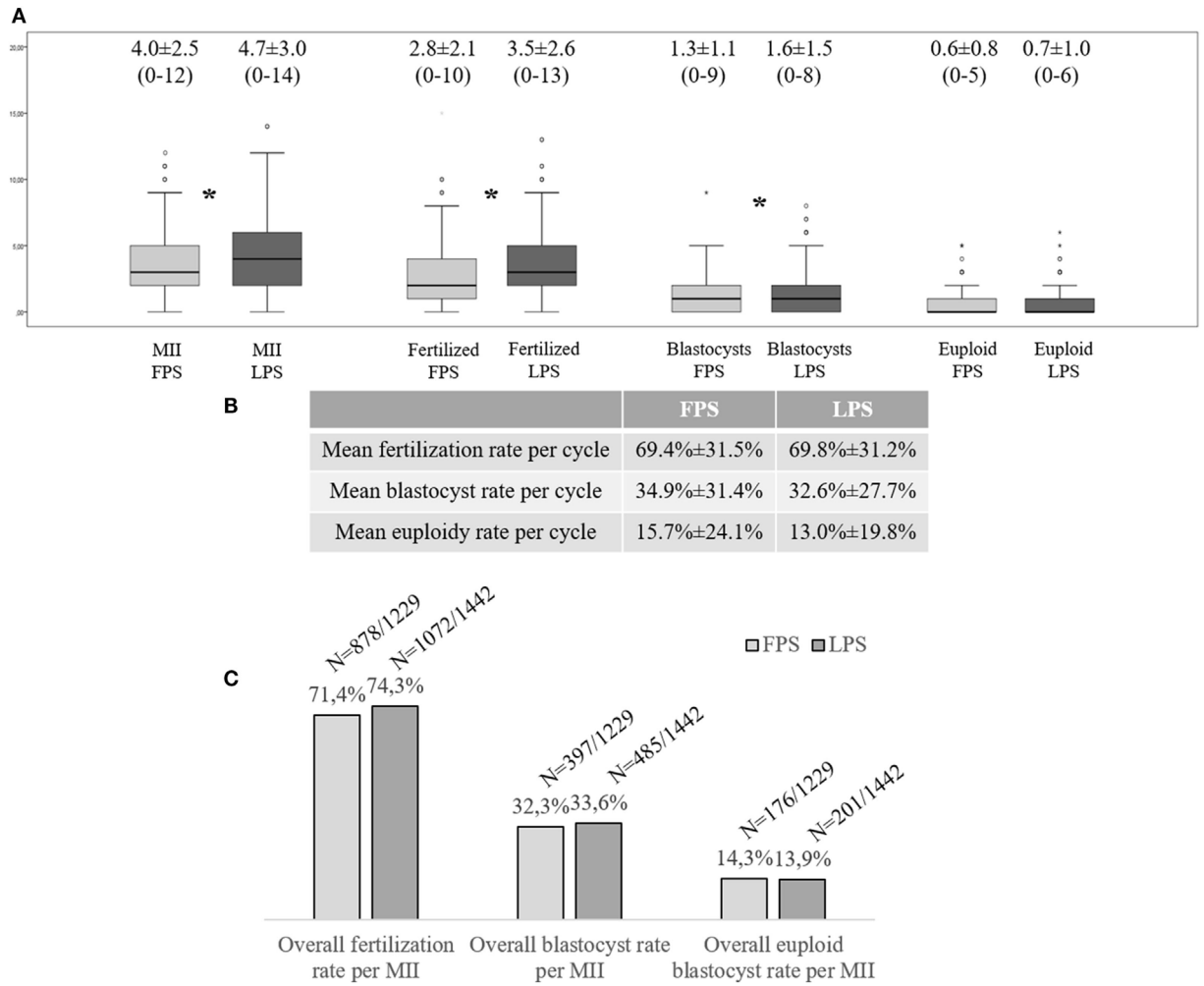

FIGURE 1 | Multicenter clinical experience at the G.EN.E.R.A. centers for reproductive medicine (Rome, Naples, Marostica, and Umbertide) with the application of a DuoStim approach. **(A)** Mean number of metaphase (MII) oocytes, fertilized embryos, blastocysts, and euploid blastocysts obtained per cycle after follicular phase stimulation (FPS) and luteal phase one (LPS); **(B)** Mean embryological results calculated per MII oocyte retrieved and inseminated in FPS- and LPS-derived cycles; **(C)** Overall embryological results of the MII oocytes collected after FPS and LPS, respectively. The stars identify statistically significant differences. The non-Gaussian distribution of the data was assessed through the Shapiro–Wilk test. Wilcoxon signed-rank test and Fisher's exact test were used to test for significant differences between FPS- and LPS-derived data.

($n = 4/49$; p = NS). The miscarriage rates were 11.1% ($n = 4/36$) and 8.9% ($n = 4/45$; p = NS). Therefore, the ongoing pregnancy rates were 39.5% ($n = 32/81$) and 49.4% ($n = 41/83$; p = NS) (Table S1 in Supplementary Material).

DISCUSSION

This perspective paper dealing with the definition and implementation of DuoStim highlights the value of this strategy in treating poor prognosis patients. Importantly, the competence of the oocytes collected after both stimulations conducted in the FP and LP is similar in terms of fertilization, blastulation, and euploidy rates, as well as clinical outcomes after single euploid blastocyst transfer. However, the LPS seems to induce a better exploitation of the ovarian reserve with almost one more oocyte on average collected with respect to the FPS. Interestingly, these data further support the exploitation of anovulatory waves of follicle recruitment to obtain competent oocytes (34, 59–64). This practice is in countertendency with respect to the ovarian physiological behavior, but apparently it may be very successful. However, more stimulation cycles were canceled in the LP due to no response to the stimulation with respect to the FP.

The idea of DuoStim has been initially proposed to manage patients with poor ovarian reserve. However, the POSEIDON group highlighted the importance of obtaining at least one euploid embryo after COS as novel primary outcome in IVF. Therefore, based on this new concept, DuoStim in the future could be proposed not only *a priori* according to the inclusion criteria previously defined in this paper but also *post hoc* according to the number of blastocysts obtained after FPS. Clearly, the decision to perform also LPS (i.e., DuoStim) should depend on the expected euploidy rate of those FPS-derived blastocysts. To this end, the combination between the maternal age at oocyte retrieval and the number of embryos obtained after FPS represent the most predictive scheme to make a more appropriate choice (6, 7). Instead, in case of unexpectedly positive outcomes after FPS only (i.e., higher blastocyst rate than expected), we can consider avoiding LPS. Future studies should be properly designed to validate this putative strategy.

The data reported in this paper represent a further evidence to support the use of DuoStim to increase the number of poor prognosis patients obtaining an euploid blastocyst in a single menstrual cycle. No embryological, gynecological, or clinical issue has been reported to date. Yet, more biological, obstetrical, and neonatal evidence of safety is required, as well as an analysis of its cost-effectiveness.

SWOT Analysis of DuoStim

To summarize the putative advantages and disadvantages of DuoStim, we conducted a SWOT analysis (**Figure 2**), namely an efficient analytical framework useful to summarize the *Strengths*, *Weaknesses*, *Opportunities*, and *Threats* of a technology. The strengths are: a higher number of oocyte (and embryos) might be obtained per ovarian cycle; more patients obtaining

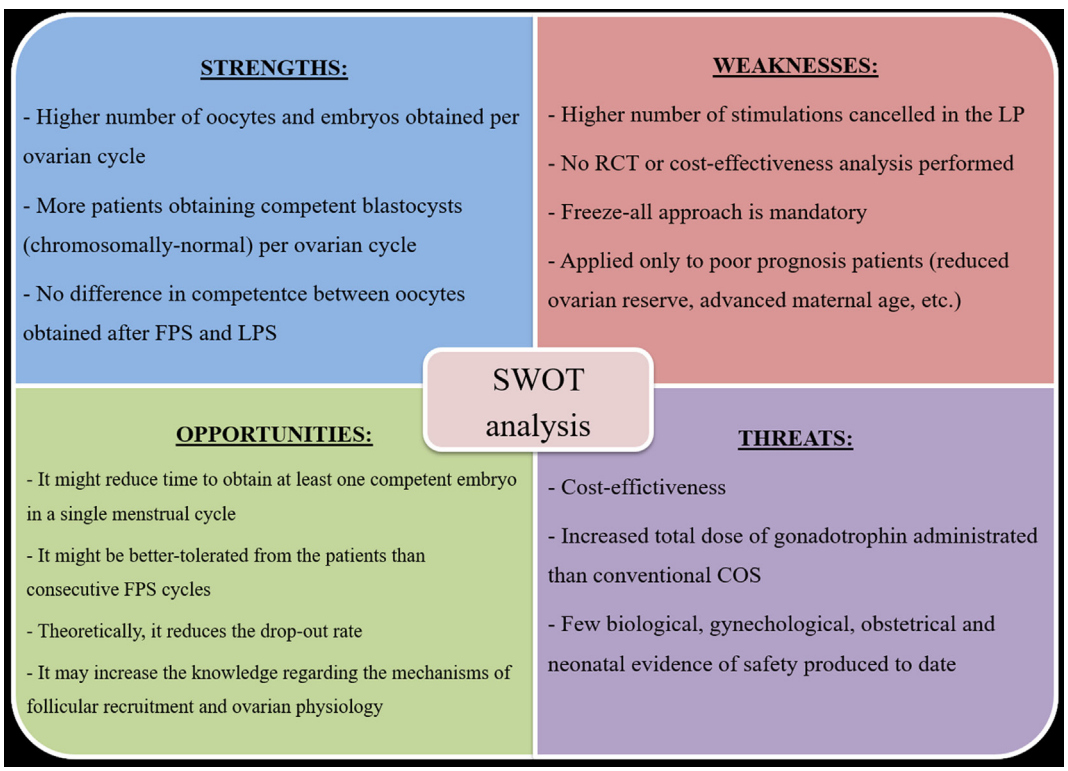

FIGURE 2 | DuoStim SWOT analysis. Abbreviations: FPS, follicular phase stimulation; LPS, luteal phase stimulation; RCT, randomized controlled trial; COS, controlled ovarian stimulation.

a (chromosomally normal) blastocyst per ovarian cycle; no difference has been reported to date in terms of competence between oocytes obtained after FPS and LPS. The weaknesses are: a higher number of stimulations seems to be canceled in the LP than in the FP; no RCT or cost-effectiveness analysis has been performed to date investigating the use of DuoStim; a freeze-all approach is mandatory; it has been applied only to poor prognosis patients. The opportunities are: a decrease in the time and increase in the chance to obtain at least one competent embryo in a single menstrual cycle; the DuoStim protocol might be better-tolerated from the patients than consecutive FPS cycles; the drop-out rate might be reduced; the knowledge regarding the mechanisms of follicular recruitment and ovarian physiology might be increased. The threats are: an analysis of the cost-effectiveness is yet eagerly needed; the total dose of gonadotrophins to be administrated is substantial; few biological, gynecological, obstetrical, and neonatal evidence of safety have been produced to date. The strengths of this approach make it very promising. However, more studies are needed in the future to limit its weaknesses, shed light on its putative threats, and realize its opportunities.

CONCLUSION

The evidence that multiple waves of follicle recruitment may arise during a single ovarian cycle in women opened important clinical implications for the treatment of poor prognosis patients. LPS in general has become a promising protocol for patients who need to collect the highest number of oocytes in the shortest possible time (e.g., oncological patients). DuoStim approach conjugates FPS to LPS with very successful results reported to date. Still, any stimulation protocol, which exploits anovulatory waves of follicle recruitment should undergo a thorough biological and clinical investigation before it can be generally implemented. To this regard, DuoStim still needs a more extensive and wider validation to testify its safety. Interesting future perspectives to investigate its clinical efficacy/efficiency would entail (i) a RCT comparing double-FPS versus DuoStim; (ii) the application of DuoStim in cancer patients for fertility preservation; (iii) as well as in prospective analyses focused on patients clustered according to either the Bologna criteria or the Poseidon stratification. Until such evidence would be produced, DuoStim should be clinically applied only to a population of patients of poor prognosis and/or to whom time represents a critical issue.

ETHICS STATEMENT

The institutional review board of the involved clinics approved this study. The project was reviewed by two different members of each committee, none directly involved in the study to exclude any potential conflict of interest. All members approved the study. The study was performed in accordance with the local regulation and all patients gave written informed consent to it in accordance with the Declaration of Helsinki. This study was considered in line with the clinics' protocols and standard procedures.

AUTHOR CONTRIBUTIONS

AV and DC analyzed the data and drafted the manuscript. All authors contributed to the interpretation and discussion of the data.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fendo.2018.00317/full#supplementary-material.

FIGURE S1 | **(A)** Flowchart and **(B)** cycle outcomes of 2-year multicenter application of DuoStim at G.EN.E.R.A. centers for reproductive medicine (Rome, Naples, Marostica, and Umbertide). FPS, follicular phase stimulation; LPS, luteal phase stimulation; MII, metaphase II oocyte.

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REVIEW
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Impact of Maternal Age on Oocyte and Embryo Competence

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The overall success of human reproduction, either spontaneously or after IVF, is highly dependent upon maternal age. The main reasons for age-related infertility include reduced ovarian reserve and decreased oocyte/embryo competence due to aging insults, especially concerning an increased incidence of aneuploidies and possibly decreased mitochondrial activity. Age-related chromosomal abnormalities mainly arise because of meiotic impairments during oogenesis, following flawed chromosome segregation patterns such as non-disjunction, premature separation of sister chromatids, or the recent reverse segregation. In this review, we briefly discuss the main mechanisms putatively impaired by aging in the oocytes and the deriving embryos. We also report the main strategies proposed to improve the management of advanced maternal age women in IVF: fertility preservation through oocyte cryopreservation to prevent aging; optimization of the ovarian stimulation and enhancement of embryo selection to limit its effects; and oocyte donation to circumvent its consequences.

Keywords: ovarian reserve, oocyte competence, aging, aneuploidies, IVF

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INTRODUCTION

Human reproduction success is highly dependent upon the age at which women attempt to conceive, which is progressively increasing worldwide (1, 2). Fertility decreases as the woman ages, while the incidence of miscarriage and the prevalence of vital chromosomal abnormalities follow an opposite trend (2–4) (**Figure 1**). In IVF, maternal age is among the strongest predictors of success (5). Specifically, advanced maternal age (AMA; defined as ≥35 years) shows just a negligible impact upon fertilization rate (6, 7) and a mild impact upon embryo development to the blastocyst stage (8, 9), but results in a dramatic impact upon blastocyst aneuploidy rate (10, 11) (**Figure 1**). However, the molecular and biochemical mechanisms involved in age-related infertility and their impact on oocyte and embryo quality remain to be clearly elucidated. Up to date, several dysfunctions have been associated with impaired fertility in aged women. Together with a progressive reduction of the ovarian reserve, woman aging involves also a compromised competence of the oocytes/embryos because of defective physiological pathways, such as energy production and balance, metabolism, epigenetic regulation, cell cycle checkpoints, and increased meiotic missegregation (11, 12). In this review, we provide a summary of the main putative causes for the age-related decrease in oocyte/embryo competence, along with the mechanisms underlying aging and the main clinical strategies proposed to prevent/limit the impact of AMA upon IVF success.

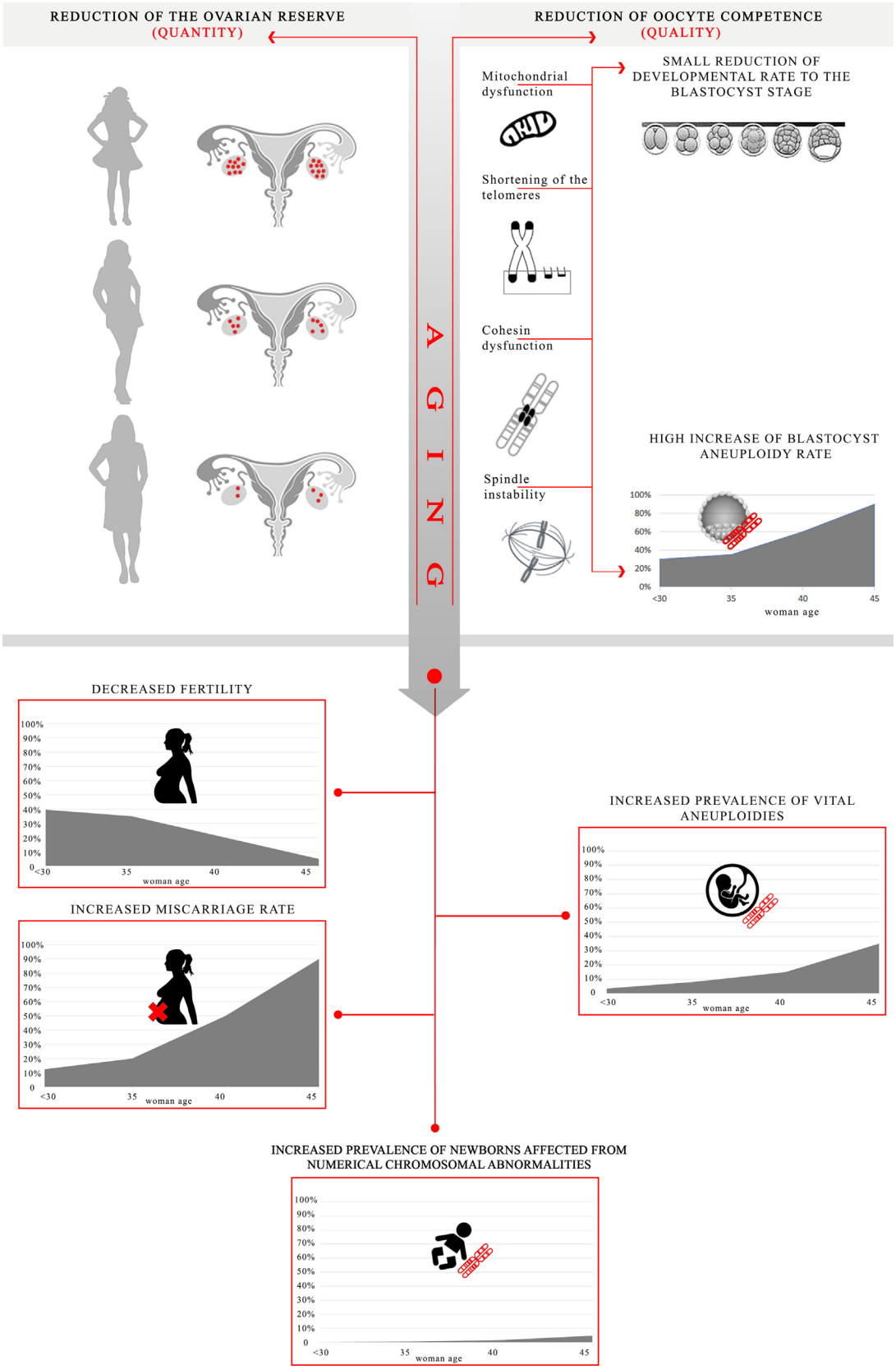

FIGURE 1 | Continued

FIGURE 1 | Effect of advanced maternal age on oocyte/embryo competence and putative mechanisms impaired by aging. Aging in women causes both a reduction of the ovarian reserve and of the oocyte competence. All the processes impaired may result into a lower energy production/balance involving a small reduction of embryo developmental rate to the blastocyst stage, as well as a higher frequency of chromosome missegregation during maternal meiosis leading to a high increase in blastocyst aneuploidy rate (especially in women older than 35) [*data adapted from Franasiak et al*. (10) and *Capalbo et al*. (11)]. Ultimately, these mechanisms converge into a decreased fertility, an increased prevalence of vital chromosomal abnormalities, an increased miscarriage rate, as well as an increased prevalence of numerical chromosomal abnormalities in the newborns [*data adapted from Hassold and Hunt* (13) and *Heffner* (4)]. *The aneuploidy rate is estimated per biopsied blastocyst; the fertility is estimated as number of babies born per 1,000 married women; the overall prevalence of vital aneuploidies is estimated per clinically recognized pregnancy; the miscarriage rate is estimated per clinical pregnancy; at last, the overall prevalence of numerical chromosomal abnormalities is estimated per number of newborns.*

MATERNAL AGING AND ANEUPLOIDIES

The oocyte must sustain embryo development until embryonic genome activation (EGA) (14). To effectively reach EGA, synchronous nuclear and cytoplasmic maturation are required. Any failure in these processes may cause an incorrect transition from a maternal to an embryonic control upon embryo development. However, after birth and until follicle recruitment and ovulation, the oocytes enter a protracted arrest in the prophase of meiosis I, during which they are subject to the detrimental effects of aging, especially impairing the genetic stability (15), and ultimately affecting the chance of success in human reproduction. Indeed, the oocytes hold most of the reproductive potential in humans, as demonstrated by the restored fertility in women who undergo egg donation (16).

Maternal age is the main cause of embryonic aneuploidies (4, 13, 17). More than 90% of these imbalances are indeed of maternal origin caused by chromosomal missegregation during oogenesis (15). Mainly meiosis I errors may occur (>70% of cases), which although can be "corrected" in meiosis II, thereby resolving the initial error (11).

If full-chromosome constitutive aneuploidies are mainly generated by a defective maternal meiosis, structural chromosomal abnormalities (e.g., balanced translocations) seem instead independent from maternal age and may equally affect both the partners together with segmental aneuploidies, copy number variations, microdeletions/microduplications, and post-zygotic mitotic errors. Indeed, they probably arise from *de novo* events during either oogenesis and spermatogenesis or mitosis (18, 19).

The maintenance of the bivalent structure is a critical issue in maternal meiosis. In humans, homologous chromosomes recombine in primary oocytes during fetal development to form a bivalent configuration at meiotic prophase I. This configuration must be maintained for years, along which the oocytes remain arrested at the G2/M transition (dictyate stage) until menarche. At this stage, meiosis resumption and chromosome segregation take place. However, during this extended period of quiescence, the bivalent structure may weaken, leading to the formation of univalents or to sister chromatids splitting at meiosis I. The incidence of both these events indeed correlates with increased maternal age and reduced recombination rate (20–25), but the related causative mechanisms are still unclear. Two hypotheses have been proposed: (i) the univalents originate from bivalents deterioration throughout the dictyate arrest or (ii) the oocytes that underwent deficient recombination are ovulated last from the ovary.

Surprisingly, Ottolini and colleagues recently reported, *via* the karyomapping technique (a method that through the specific parental haplotypes allows the definition of an SNPs-based map of each chromatid) applied to artificially activated human oocytes and their polar bodies, that the most common non-canonical segregation pattern is reverse segregation (26). According to this novel segregation scheme, which cannot be identified by conventional copy number analysis, the non-sister chromatids, instead of the homologous, segregate together in meiosis I. This pattern, even if unconventional, does not result in an unbalanced chromosomal constitution *per se*, unless it is followed by a further error during meiosis II. Therefore, the most common segregation error of maternal meiosis reported in the majority of activated/fertilized oocytes is still the premature separation of sister chromatids (PSSC) in meiosis I (27–30). At last, meiosis I or meiosis II non-disjunction events should be accounted as causes of maternal meiotic impairments, even though probably less frequent than what previously reported (31, 32).

Hereafter, we summarize the molecular and cellular processes that may be affected because of aging in the oocytes (33, 34): mitochondrial dysfunction, shortening of telomeres, cohesins dysfunction, and meiotic spindle abnormalities due to spindle-assembly checkpoint (SAC) impairment. Reduced development to the blastocyst stage and/or chromosomal abnormalities are their putative consequences (**Figure 1**).

PUTATIVE MECHANISMS IMPAIRED BY AGING AND LEADING TO A REDUCED OOCYTE/EMBRYO COMPETENCE

Mitochondrial Dysfunction

Mitochondria are the most numerous organelles in the oocyte and represent its powerhouse. They are characterized by their own genome (mtDNA) and constitute the main maternal contribution to embryogenesis (35). Indeed, the sperm does not provide mitochondria to the offspring. They are considered pivotal especially in the delicate first phases of preimplantation development, when a balanced energy consumption is crucial for an efficient oocyte cytoplasmic and nuclear maturation, throughout processes such as germinal vesicle breakdown, or microtubule assembly and disassembly during meiotic spindle formation (36, 37). Moreover, mitochondria cover an essential role in various signaling pathways, such as Ca^{2+} signaling and regulation of the intracellular red-ox potential, particularly important for fertilization and early development (36, 38).

The adverse effect of aging upon the mitochondria within the oocyte has been widely reported: mitochondrial swelling, vacuolization, and cristae alteration have been described as common structural features of oocytes from AMA patients (39, 40). For instance, the mitochondrial membrane potential, which mirrors mitochondrial activity, is progressively altered (41). Similarly, a reduced ATP production and decreased metabolic activity in aged oocytes has been highlighted, which in turn may contribute to impairments in meiotic spindle assembly, cell cycle regulation, chromosome segregation, embryo development, and finally implantation (40, 42).

Mitochondrial-DNA lacks protective histones and efficient DNA repair mechanisms. Therefore, mtDNA mutation rate is about 25-times higher than nuclear-DNA one (43). Clearly, the longer the quiescent period, the higher the risk for mtDNA errors. Furthermore, also the overall concentration of mtDNA seems to be decreased in the oocytes from older patients (44, 45), thereby concurring to a lower oocyte/embryo competence (46–48). Of note, in humans, mitochondrial biogenesis is physiologically activated only at the blastocyst stage (40, 49) to limit the oxidative phosphorylation-induced stress in the first phases of embryo development. In older patients, the reduced amount and/or faulty activity of the pre-existing mitochondria within the oocyte may induce a compensatory premature initiation of mitochondrial biogenesis (50), which in turn may contribute to early embryo developmental failure (48).

Recently, mtDNA content in trophectoderm biopsies at the blastocyst stage has been proposed as a putative biomarker of implantation potential. However, the clinical studies conducted to date reported controversial results (48, 51–54). Indeed, lately, Humaidan and colleagues warned that it is still difficult to discriminate between "fact and fiction" in the current scenario and mtDNA cannot be considered a new biomarker of embryonic implantation potential (55): extensive validation, as well as more pre-clinical and possibly non-selection data, are yet required. Until then, the quantification of mtDNA from trophectoderm biopsies should be considered still an experimental procedure.

The mitochondria are also present in the granulosa cells (GCs) surrounding the oocyte already in the early phases of oogenesis. GCs are directly involved in establishing oocyte competence during oogenesis thanks to the well-known bi-directional dialog between these two sections of the follicle (56, 57). As for the oocytes, also GCs from AMA women showed higher levels of mtDNA deletions (58) and damaged mitochondria (59). The amount of mtDNA in GCs has been also reported to correlate with embryo quality (60) and poor ovarian reserve. The current hypothesis is that as the mtDNA in the oocyte supports the early embryonic development, similarly the mtDNA on its related GCs supports oocyte maturation, both possibly modulating embryo competence. Such hypothesis is supported by the high correlation between the mtDNA levels in the two compartments of the follicle (61).

In summary, aging can compromise both mtDNA integrity and/or mitochondria morphology or alter the microenvironment within the follicle and perturbate the mutual crosstalk between the oocyte and its GCs (39, 40, 62).

Shortening of the Telomeres

The telomeres are short tandem repeats of specialized-DNA sequences that protect chromosome ends (63). Their function is essential for meiosis since, during the early prophase, the telomeres tether the chromosomes to the nuclear membrane to facilitate homologous pairing and initiate synapsis to form chiasmata, the physical sites of recombination responsible for normal segregation, thereby preventing non-disjunction (64, 65). Age-related telomeres shortening occurs either in dividing or non-dividing cells and has been associated with several age-related diseases (e.g., diabetes, cardiovascular diseases, and cancer) (66, 67). However, telomere dynamics extensively differ according to the cell type and gender. For instance, in the male germline, the length of the telomeres is preserved with aging, probably due to a constant activity of the telomerase (the reverse transcriptase involved in telomeres extension), which is expressed at high levels in the spermatogonia (68). Interestingly, an even increased mean length of the telomeres, as well as a higher length heterogeneity, has been recently reported in aged men with respect to younger patients (69). Conversely, the telomeres in the oocytes begin shortening during fetal oogenesis, and this process is continued in the adult ovary, probably due to the chronic effects of oxidative and genotoxic stress, the late exit of the female gametes from their cell cycle arrest, as well as to a reduced activity of the telomerase (68, 70, 71). Furthermore, it has been demonstrated that the telomeres are shorter in oocytes from women who experienced IVF failure or recurrent miscarriage (72), as well as in oocytes resulting in fragmented (73) or aneuploid embryos (74). To this regard, Keefe and colleagues postulated the evolutionistic "telomere-mediated oocyte aging" theory: preventing AMA women from conceiving would, in turn, prevent them from dying because of childbirth, thereby affecting the reproductive fitness of their offspring (70, 75).

Cohesin Dysfunctions

Loss of cohesion between sister chromatids close to the centromeres is another age-related dysfunction which may cause chromosomal missegregation. Cohesins are a complex of proteins that holds sister chromatids together after DNA replication and is responsible for maintaining the bivalent structure throughout the extended period of quiescence. Only at anaphase, the cohesins are removed to trigger the separation of sister chromatids. Gathering evidence is outlining an age-related disruption of cohesin function leading to missegregation within the oocyte, especially in the presence of low recombination rate (76). For instance, cytogenetic studies of human oocytes and embryos showed that PSSC is often associated with the age-related reduction of cohesins (e.g., Rec8, SA3, and SMC1b) (77, 78). Furthermore, also the activity of the regulatory proteins preventing a precocious removal of the cohesins seems to decline in an age-related fashion (79), regardless their nuclear location, which theoretically should protect them from the insults of mechanical stress and/or reactive-oxygen-species. Finally, a structural and functional interaction exists between cohesins and telomeres in mice (80). Therefore, in AMA patients, the age-related issues

that affect the telomeres may trigger similar dysfunctions in the cohesins' activity (76).

Spindle Instability

The meiotic spindle is responsible for the separation of both homologous chromosomes and sister chromatids, therefore essential to ensure an accurate segregation (81). Aberrations in its assembly seem to contribute to the higher prevalence of aneuploidies in older women (82). These aberrations may also be ascribed to a decreased metabolic activity of mitochondria, resulting into a reduced amount of ATP because of AMA. The spindle of young oocytes is compact, orthogonally oriented with respect to the oolemma and each pole is associated with a ring of centrosome proteins. Conversely, nearly 80% of the oocytes in AMA patients may exhibit abnormal spindles with an elongated and/or smaller profile and few microtubular foci at the cortex (81, 82). To this regard, also the SAC, a ubiquitous safety protein complex that ensures a correct spindle formation (83), shows a reduced stringency with AMA (84–86). Different protein components of SAC (e.g., Mad2 and Bub1) showed indeed lower concentrations in oocytes from older women (84, 87).

Other Putative Mechanisms Impaired by Aging

Gene expression studies in oocytes from several species indicate that the activity of gene products involved in cell cycle regulation, spindle formation, and organelle integrity may be altered in oocytes from older individuals. For instance, in both murine and human oocytes ~5% of all the transcripts detected at the MII stage were found to be affected by aging (88, 89). Possibly, the divergent signatures derive from the altered patterns of epigenetic modifications (e.g., methylation and acetylation), which have been indeed reported in both species (90–94). This field of reproductive genetics requires extensive investigations in the next years to better unveil these mechanisms.

CLINICAL CONSIDERATIONS

A clear correlation exists between increasing maternal age and decreasing success in conceiving both spontaneously and after IVF (4, 5). Both reduced ovarian reserve and oocyte quality contribute to this scenario. Currently, no therapy exists to counteract infertility in AMA patients and we can only try to limit this biological and social issue.

First, fertility preservation *via* oocyte cryopreservation (95, 96) provides a valuable option to all women (not only oncological patients) aiming to prevent the natural decline of oocyte competence. Yet, the age at which fertility preservation is performed is an important effector of the ultimate outcome (<35 years is preferable), and obviously the pregnancy cannot be guaranteed by oocyte banking (97).

Second, the maximization of ovarian reserve exploitation through tailored controlled-ovarian-stimulation (COS) is crucial to increase the number of oocytes collected, thereby also increasing the chance of success after IVF (98, 99). A higher number of oocytes collected per ovarian cycle might indeed compensate for the decrease in both oocyte quantity (i.e., ovarian reserve) and quality (i.e., competence). Therefore, novel COS strategies, such as oocyte/embryo accumulation in consecutive cycles (100) or double ovarian stimulation in the same ovarian cycle [i.e., the Shanghai (101) or the DuoStim protocol (102)], have been recently proposed to shorten the time invested by poor prognosis patients in their pursuit of a live birth. Promising data have been reported to this regard, especially in terms of cost-effectiveness and safety.

Third, the enhancement of embryo selection *via* preimplantation-genetic-testing represents another important option in AMA patients. In fact, the goal of ART is to achieve the birth of a healthy child minimizing the risks for the patient, and this is particularly true in AMA when the incidence of aneuploidies dramatically increases (10). This approach, by avoiding the transfer of aneuploid blastocysts and their related risks (i.e., implantation failures, miscarriages, and affected child), might result in an increased efficiency of each IVF treatment (103, 104). Importantly, once an euploid blastocyst is identified, its implantation potential is independent of maternal age (45–50%), thereby allowing the adoption of a single-embryo-transfer policy also in AMA patients, concurrently lowering the risk for multiple gestations and their related obstetrical/perinatal risks (105, 106). Soon, the implementation of -omic sciences and the pursuit of non-invasiveness and higher cost-effectiveness in this field may converge and bring about intriguing avant-gardes to further improve embryo selection.

Finally, oocyte donation represents an effective approach to circumvent the age-related fertility decline. Recently, the optimization of cryopreservation techniques and the constitution of oocyte-banking facilities and programs allowed us to avoid synchronization between donors and recipients. Indeed, similar success rates derive from either fresh or frozen oocytes (107). Yet, in some countries oocyte donation is still forbidden and ethical/psychological concerns limit its large-scale adoption.

CONCLUSION

Currently in IVF, a panel of experts focused on the management of poor prognosis patients, known as the POSEIDON group (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number), has redefined the aim of ovarian stimulation (108). Specifically, they claimed that COS should be tailored "to retrieve the number of oocytes needed for the specific patient to obtain at least one euploid embryo for transfer." Such statement is based on two important assumptions: (i) aneuploidy rate in human blastocysts increases from a 30% baseline in women younger than 35 to >90% in women older than 44 (10, 11) and (ii) the number of eggs collected and embryos obtained during IVF does not alter this rate (109). In other terms, the definition of the number of oocytes required (quantity) from each patient should entail the estimate of their competence (quality) aiming at obtaining at least one euploid blastocyst. Then, when performed, a euploid blastocyst transfer results into a healthy live birth in ~50% of cases, regardless woman age (103).

To conclude, evidence-based data should always guide the counseling and the patients should be scrupulously informed about their estimated chance to conceive, especially if older than 35.

Indeed, 35 years should be the lowest age-threshold to define AMA and 45 years should be considered the highest age-threshold to undergo IVF with own eggs, at least according to the latest published report (9).

AUTHOR CONTRIBUTIONS

DC and GF drafted the manuscript. All authors contributed in the literature search and discussion of the published evidence.

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ORIGINAL RESEARCH
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The Effect of Dose Adjustments in a Subsequent Cycle of Women With Suboptimal Response Following Conventional Ovarian Stimulation

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Several infertile patients, who may even represent around 40% of the infertile cohort, may respond "suboptimally" (4–9 oocytes retrieved) following IVF, despite being predicted as normal responders. The aim of our longitudinal study was to evaluate the ovarian response of suboptimal responders in terms of the number of oocytes retrieved, following their second IVF cycle, evaluating exclusively patients who had the same stimulation protocol and used the same or higher initial dose of the same type of gonadotropin compared to their previous failed IVF attempt. Overall, our analysis included 160 patients treated with a fixed antagonist protocol in their second cycle with the same [53 (33.1%)] or higher [107 (66.9%)] starting dose of rFSH. The number of oocytes retrieved was significantly higher in the second IVF cycle [6 (5–8) vs. 9 (6–12), $p < 0.001$]. According to our results, a dose increment of rFSH remained the only significant predictor of the number of oocytes retrieved in the subsequent IVF cycle (coefficient 0.02, p-value = 0.007) after conducting GEE multivariate regression, while adjusting for relevant confounders. A regression coefficient of 0.02 for the starting dose implies that an increase of 50 IU of the initial rFSH dose would lead to 1 more oocyte.

Keywords: oocytes, ovarian response, suboptimal responders, number of oocytes, dose adjustments

INTRODUCTION

The number of oocytes retrieved following ovarian stimulation is considered to be a strong surrogate marker for the reproductive outcome. Since the early days of *in-vitro* fertilization (IVF), ovarian stimulation has been applied to compensate for inefficiencies in the IVF procedure by aiming to increase the oocyte yield. While there is scientific evidence to justify the categorization of women as poor responders (≤3 oocytes) or excessive responders (>15 oocytes) based on a uniform prognosis, categorization of patients as normal responders is often based on the exclusion of the aforementioned categories (1).

The homogeneity of this "normal" group has been recently debated, given that patients with 4–9 retrieved oocytes may have substantial different clinical prognosis in comparison to women with a 10–15 oocyte yield (2). This implies that several patients, who may even represent around 40% of the infertile cohort (3), may respond "suboptimally" following ovarian stimulation, despite being predicted as normal responders based on their ovarian reserve markers (4).

Although several explanations may be given for the nature of suboptimal response, the main dilemma is which treatment modality should be implemented in order to increase the number of oocytes in a subsequent IVF cycle (5). In this context, the adjustment of the gonadotropins' dose in a following cycle represents one of the most common treatment measures used in clinical practice. However, in order to be able to evaluate this approach, the naturally existing individual variability in ovarian response between consecutive cycles should be taken into consideration and for such an assessment, repetitive cycles should be evaluated, which would ideally be performed under the same conditions.

Therefore, the aim of our study was to evaluate the ovarian response of suboptimal responders in term of number of oocytes retrieved, following their second IVF cycle, evaluating exclusively patients who had the same stimulation protocol and used the same or higher initial dose of the same type of gonadotropin compared to their previous failed attempt. Allowing each patient to serve as her own control could assess inter-patient variability and would provide potential implications for the management of this difficult group of patients.

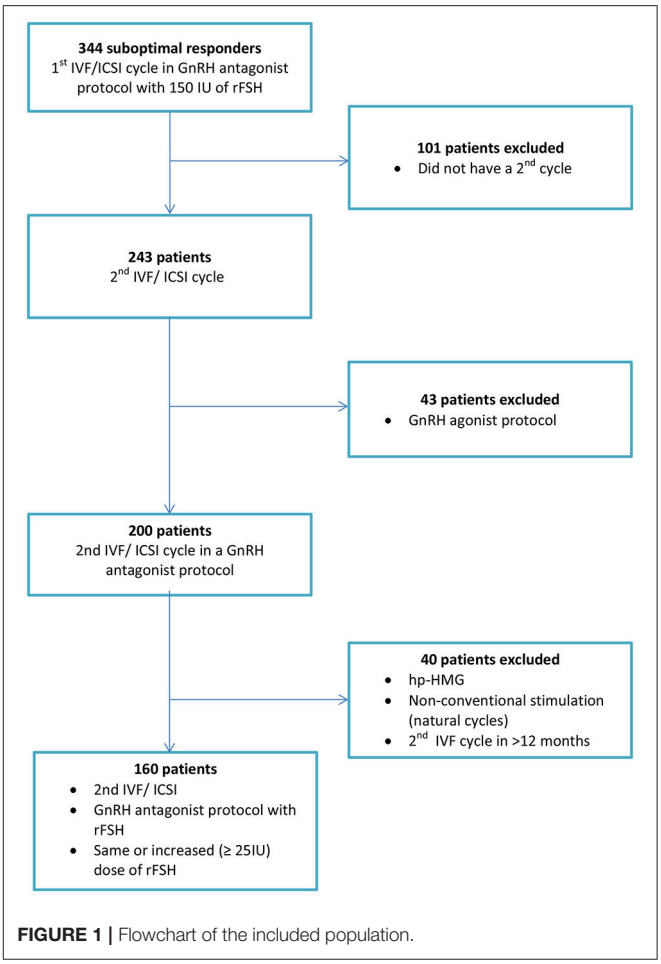

FIGURE 1 | Flowchart of the included population.

MATERIALS AND METHODS

This retrospective study included all consecutive women attending the Centre for Reproductive Medicine (CRG) of the University Hospital of Brussels in Belgium from January 2009 to December 2014. The study was approved by the institutional review board of our hospital (B.U.N. 143201733041).

Patients' Eligibility Criteria

Eligible patients were considered to be all consecutive infertile women less than 40 years undergoing their 2nd ovarian stimulation cycle in a fixed gonadotropin-releasing hormone (GnRH) antagonist protocol with daily recombinant FSH (rFSH) and who had demonstrated suboptimal response (4–9 oocytes retrieved) following their 1st IVF cycle with 150 IU of rFSH in an antagonist setting in a time interval less than 12 months.

All the included patients were supposed to be normal responders based on their ovarian reserve markers [anti-müllerian hormone (AMH) and antral follicle count (AFC)] and may have used the same initial dose or ≥25 IU increase in rFSH in their 2nd IVF cycle, based on clinicians' discretion.

Patients were excluded from the study if they did not proceed to a 2nd IVF attempt, if they had undergone ovarian stimulation with a GnRH agonist protocol, if they had been stimulated with urinary gonadotropins or if the time interval between the two oocyte retrievals was longer than 12 months.

In addition, we excluded women who were planned to undergo ovarian stimulation for pre-implantation genetic diagnosis or screening, oocyte donation, and social or medical freezing of oocytes (**Figure 1**).

Treatment Protocol

Patients received daily injections of rFSH starting on day 2 or 3 of their menstrual cycle, followed by a daily dose of 0.25 mg of GnRH antagonist in fixed protocol starting 6 days later, as described elsewhere (2). Women did not receive any type of priming before starting IVF. Cycle monitoring was performed through serum estradiol (E2), progesterone and luteinizing hormone (LH) assessments, and serial transvaginal ultrasound examinations. Dose adjustments were not allowed during ovarian stimulation.

Ovulation triggering was performed with the administration of human (10.000IU) or recombinant (250 μcg) chorionic gonadotropin (hCG) as soon as three follicles of 17 mm diameter were observed. Oocyte retrieval took place 36 h later.

Main Outcome Measures

The primary objective was to determine the variation between the two treatment cycles following gonadotropin dose adjustment of the initial stimulation dose, in term of number of oocytes

retrieved and to investigate whether the changes in the ovarian response per cycle could be explained by several predictors, in suboptimal responders. Secondary endpoint was the total number of good quality Day 3 embryos between cycles. EQ was classified similar to what is described in a previous study performed by De Munck et al. (6), with a minor update in the classification (good quality embryos included up to <50% fragmentation).

Statistical Methods

Continuous data are presented as the mean value ± standard deviation (SD) and median with interquartile range (IQR). Categorical data are described by number of cases, including numerator/denominator and percentages.

Differences in continuous variables (including our primary endpoint: number of oocytes) between patients' 2nd IVF cycle and their preceding cycle are calculated via dependent-sample *t*-tests or Wilcoxon signed-rank tests, as appropriate. Categorical variables are analyzed via chi-square with Fisher exact test, as appropriate.

We also performed regression models with estimation by generalized estimating equations (GEE) to assess the effect of dose adjustments in the number of oocytes and number of good quality Day 3 embryos, after accounting for several confounders. The candidate confounders were age, BMI, cause of infertility and AFC. GEE were used to account for the within subject correlation in outcomes for repeated treatments. Results are presented with adjusted odds ratios (ORs) and 95% confidence intervals (CIs). All statistical tests used a two-tailed α of 0.05. All analyses performed using STATA 13.0.

TABLE 1 | Baseline characteristics of suboptimal responders.

AGE (YEARS)	
Mean (SD)	32 (4.5)
Median (IQR)	32 (29–35)
BMI (kg/m^2)	
Mean (SD)	23.5 (4.4)
Median (IQR)	22.4 (20–26)
INFERTILITY CAUSE, n(%)	
Male	87 (54.4)
Endometriosis	2 (1.25)
PCOS	7 (4.4)
Ovulatory	7 (4.4)
Tubal	10 (6.25)
Unexplained	47 (29.4)
FSH	
Mean (SD)	7.4 (2.5)
Median (IQR)	7 (6–8.8)
AMH	
Mean (SD)	3 (2)
Median (IQR)	2.8 (1.9–4)
AFC	
Mean (SD)	15 (7.8)
Median (IQR)	14 (10–18)
TIME INTERVAL BETWEEN ORs (DAYS)	
Mean (SD)	132 (68)
Median (IQR)	115 (82–174)

AFC, antral follicle count.
OR, oocyte retrieval.

RESULTS

Patient Characteristics According to Number of Oocytes Retrieved in the Two IVF Cycles

Overall, our longitudinal analysis included 160 suboptimal responders treated with a fixed GnRH antagonist in their second IVF cycle with the same [53 (33.1%)] or higher [107 (66.9%)] starting dose of rFSH.

The patients' baseline characteristics are presented in **Table 1**. Comparisons between the two IVF cycles revealed significant differences in the initial and total rFSH dose (**Table 2**). However, the duration of stimulation was comparable between the two cycles.

The number of oocytes retrieved and good quality embryos were significantly higher in the second IVF cycle [6 (5–8) vs. 9 (6–12) and 3(2–4) vs. 4(2–5), respectively, $p < 0.001$].

Generalized Estimating Equation (GEE) Regression Analysis for Number of Oocytes Retrieved and Good Quality Embryos in the 2nd IVF Cycle

A dose increment of rFSH remained the only significant predictor of the number of oocytes retrieved in the subsequent IVF cycle (coefficient 0.02, p-value = 0.007) of suboptimal responders after conducting GEE multivariate regression, while adjusting for relevant confounders (**Table 3**). Age, BMI, cause of infertility and AFC were not significantly associated with the oocyte yield of the 2nd IVF cycle. **Figure 2** represents the mean number of oocytes according to the dose of rFSH given, adjusting for the clustering among patients. Similarly, the dose increment had a positive (although non-significant) effect in the number of good quality Day 3 embryos (Supplementary Table I).

DISCUSSION

To the best of our knowledge, this is the first study to examine the effect of dose adjustments in a subsequent IVF cycle, using the same stimulation protocol and type of gonadotropin in suboptimal responders. Our study demonstrated that an increase in the dose of rFSH in women with a previous suboptimal response may significantly increase the number of oocytes retrieved in the following IVF cycle. Based on our results, a regression coefficient of 0.02 for the starting stimulation dose implies that an increase of 50 IU of the initial rFSH dose would lead to 1 more oocyte. This average increase of one oocyte by 50 IU increment of rFSH dose may be clinically relevant for women who fail an initial IVF attempt, given the delivery rate of 5% per oocyte with IVF (3, 7, 8). The increase in the oocyte yield was also translated to a higher number of good quality cleavage

TABLE 2 | Ovarian stimulation outcomes.

Same dose (150 IU) of rFSH in the 2nd cycle ($n = 53$) Increased dose (226525IU) of rFSH in the 2nd cycle ($n = 107$)	1st IVF/ICSI cycle $n = 160$	2nd IVF/ICSI cycle $n = 160$
INITIAL DOSE (IU)* **		
Mean (SD)	150	194(42)
Median (IQR)	150	200(150–200)
STIMULATION UNITS (TOTAL IU)* **		
Mean (SD)	1,434(493.7)	1,775(589)
Median (IQR)	1,350(1,200–1,500)	1,668(1,350–2,000)
DURATION OF STIMULATION (DAYS)*		
Mean (SD)	9.6 (2.4)	9.5 (1.8)
Median (IQR)	9 (8–11)	9 (8–11)
NUMBER OF OOCYTES* ** §		
Mean (SD)	6.5(1.6)	9.3(4.8)
Median (IQR)	6(5–8)	9(6–12)
NUMBER OF GOOD QUALITY DAY3 EMBRYOS* **		
Mean (SD)	2.9(1.6)	4(3)
Median (IQR)	3(2–4)	4(2–5)

*Wilcoxon signed-rank test significant at *** < 0.01, not significant at *>0.5.*

§For patients having the same dose (150IU) in the 2nd IVF cycle, the mean difference in the number of oocytes between cycles was 2.1 ($p < 0.05$), while for patients having a higher dose of rFSH, the mean difference in the number of oocytes between cycles was 3.2 ($p < 0.05$).

TABLE 3 | Generalized estimating equation (GEE) regression analysis for number of oocytes retrieved in the 2nd IVF cycle.

Number of oocytes in the 2nd IVF cycle	Coefficient	95% CI	*P*-value
Dose increment	0.02	0.005 to 0.04	0.009
Age	−0.05	−0.18 to 0.07	0.4
BMI	−0.09	−0.22 to 0.05	0.19
CAUSE OF INFERTILITY			
PCOS	–	–	
Tubal	1.5	-2.2 to 5.3	
Endometriosis	−1.9	−8.6 to 4.8	0.77
Male	1.03	−2.1 to 4.23	
Ovarian	0.7	−3.6 to 5.06	
Unexplained	1.5	1.79 to 4.7	
AFC	0.07	−0.008 to 0.16	0.08

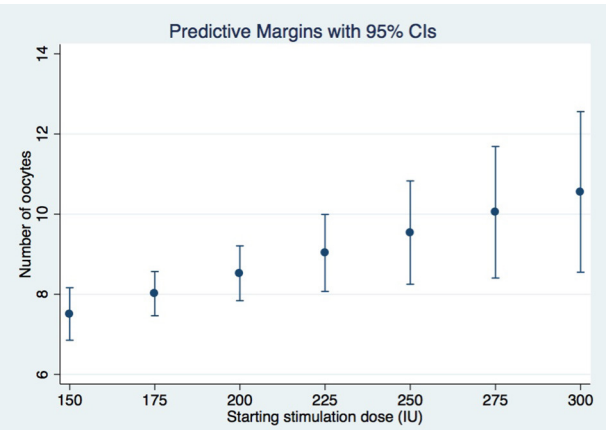

FIGURE 2 | Mean number of oocytes according to the dose of rFSH.

stage embryos; albeit the positive effect of dose increase was not statistically significant after adjustment for confounders.

Although many theories have been investigated for the nature of suboptimal response, it seems that a decreased sensitivity- or insensitivity of follicles to FSH (9) may be the most likely explanation. In fact, there is evidence that genetic variations of FSH receptor (FSHR) influence serum FSH levels and the physiological responsiveness of the target organ to FSH stimulation (10). If we further consider that no significant associations between FSHR polymorphisms and ovarian reserve markers have been found (11), women with suboptimal response may belong to this group and, therefore, require higher gonadotropin doses, contrary to their predicted response based on ovarian reserve markers.

Our study is one of the largest evaluating the variability of ovarian response in subsequent ovarian stimulation cycles, given that each patient served as her own control. Although several previous studies have investigated the ovarian response by comparing outcomes of subsequent cycles in a period of several years (12, 13), firm conclusions about the effect of ovarian stimulation can only be drawn if repetitive cycles are performed ideally within a short time frame, using the same stimulation strategy (e.g., type of gonadotropin, GnRH analog protocol, and decisions on patient management). In this regard, evidence derived from oocyte donation cycles has shown that the ovarian response is not altered in case a subsequent IVF cycle is started in a short period after the first attempt (14, 15). However, these studies evaluated the effect of stimulation on reproductive outcomes without taking into account the ovarian stimulation regimen, which is the most important parameter for decision-making. Our study differs significantly from those available in the literature, since all patients were infertile, had the same stimulation protocol with rFSH and used the same or higher initial dose of stimulation after their first failed suboptimal response. Our results correlate with a previous retrospective cohort study demonstrating that an increase in the average daily dose of gonadotropins was the only variable significantly associated with a higher oocyte yield in women with normal ovarian reserve, undergoing two IVF cycles (16).

Another point of discussion is that according to our results, the variability in ovarian response was, however, not strongly linked to individual patient demographics or baseline predictors. This is in agreement with two previous studies showing that neither basal FSH nor AFC could significantly predict transition in ovarian response following consecutive IVF cycles (16, 17).

One of the major strengths of our retrospective longitudinal study is that we included a large homogeneous group of women

who had the same stimulation protocol and the same type of gonadotropin in their second treatment cycle, performed in a short time interval. The rationale for such a study design was to take into account the individual variability by repeated measurements, eliminate potential confounders and be able to evaluate the dose adjustment "*per se*." Our study design reflects evidence based clinical practice given that all women had their first stimulation cycle with 150 IU of rFSH, based on the fact that they were predicted as normal responders (18).

However, caution is needed owing to limitations that do exist and need to be highlighted. First of all, the retrospective study design is per definition associated with inherent biases that may affect our results. Although the relatively greater oocytes yield with dose increment, this could represent a regression to the mean (17). Secondly, we excluded women who had a second IVF cycle in more than 12 months after their first egg retrieval. Nevertheless, our strategy was to decrease as much as possible the confounding effects, especially of age, by choosing a time interval in which the predictive ability of ovarian reserve markers has been shown to be the same (19). Thirdly, the adjustment in the starting stimulation dose of the subsequent cycle was based on the clinicians' discretion, with approximately one third of the patients keeping the same rFSH dose and two thirds having an increase in their initial dose. However, such an approach reflects current clinical practice and the regression analysis allowed to adjust for confounders and methodologically corroborate that the common strategy of dose increase in case of suboptimal response could be beneficial. Fourthly, patients were categorized as normal responders based on ovarian reserve biomarkers. Even if comparisons of AFC and AMH levels have generally yielded similar predictive value for ovarian response in 3 meta-analyses (20–22), limitations do exist. The major disadvantages of AFC are the sonographer dependent variability and problems related to technical aspects of ultrasound equipment (23), while the main limitations of the AMH test relate to assay variability and lack of standardized international assay (24). Finally, although the number of oocytes was found to increase with a higher starting dose, our design cannot allow evaluating the effect on fresh and cumulative live birth rates. The fact that the stimulation initial dose increase was related to a significant higher number of oocytes, but not good quality embryos, may be due to a Type 2 error.

In conclusion, after a failed cycle with suboptimal response, physicians review the cycle and often change stimulation protocol or gonadotropin dosing in an attempt to improve the outcome. By using a robust methodological approach, we answered one of the main queries, namely that an increase in the initial stimulation dose may significantly increase the oocyte yield in suboptimal responders. Our study could generate a hypothesis for a prospective randomized trial, in which suboptimal responders would be allocated to two different groups:one group with the same starting dose and a second group with a higher starting dose. The primary endpoint could be the oocyte yield and the follicular output ratio (FORT), as a qualitative marker of ovarian response (25). If we further consider that several suboptimal responders may have a variant of the β subunit of luteinizing hormone (LH) (v-LH) affecting FSH sensitivity (26, 27), the co-administration or rLH may also represent a valid option (28). However, further studies are urgently needed, in order to evaluate these promising concepts.

AUTHOR CONTRIBUTIONS

PD and NP contributed to the concept and the design of the study. PD was responsible for the data management, interpretation of the results, statistical analysis and he drafted the manuscript. All authors contributed to the interpretation of the results and editing of the manuscript. All authors approved 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/fendo.2018.00361/full#supplementary-material

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MINI REVIEW
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Defining Low Prognosis Patients Undergoing Assisted Reproductive Technology: POSEIDON Criteria—The Why

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Women with impaired ovarian reserve or poor ovarian response (POR) to exogenous gonadotropin stimulation present a challenge for reproductive specialists. The primary reasons relate to the still limited knowledge about the POR pathophysiology and the lack of practical solutions for the management of these conditions. Indeed, clinical trials using the current standards to define POR failed to show evidence in favor of a particular treatment modality. Furthermore, critical factors for reproductive success, such as the age-dependent embryo aneuploidy rates and the intrinsic ovarian resistance to gonadotropin stimulation, are not taken into consideration by the current POR criteria. As a result, the accepted definitions for POR have been criticized for their inadequacy concerning the proper patient characterization and for not providing clinicians a guide for therapeutic management. A novel system to classify infertility patients with "expected" or "unexpected" inappropriate ovarian response to exogenous gonadotropins—the POSEIDON criteria—was developed to provide a more nuanced picture of POR and to guide physicians in the management of such patients. The new standards are provoking as they challenge the current terminology of POR in favor of the newly defined concept of "low prognosis." This article provides readers a critical appraisal of the existing criteria that standardize the definition of POR and explains the primary reasons for the development of the POSEIDON criteria.

Keywords: assisted reproductive technology, hypo-responder, low responder, ovarian stimulation, poor ovarian response, poor ovarian reserve, POSEIDON criteria

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INTRODUCTION

The primary goal of assisted reproductive technology (ART) is the birth of a healthy child. This outcome depends on a multitude of non-mutual independent factors, including female age and the effect of ovarian stimulation (OS) (1, 2). Nowadays, clinicians rely on patient characteristics, ovarian reserve markers, and treatment history—if available—for clinical decision-making concerning OS strategy, aiming at securing the shortest time to live birth as well as the lowest risk of complications (3, 4).

The number of oocytes retrieved after OS represents a critical cornerstone of ART since it is an independent predictor of the likelihood of pregnancy (5–7). Although the ideal number of oocytes collected after ovum pickup has been a matter of debate in recent years, it seems reasonable to define a typical ovarian response as the retrieval of 10–15 oocytes after conventional OS (5). However, a significant proportion of patients who undergo OS has either a poor (<4 oocytes) or suboptimal (4–9 oocytes) number of oocytes retrieved (3–9). As a consequence, the number of resulting embryos available for transfer or cryopreservation is reduced, thus jeopardizing treatment success (3, 4, 10–12). The cost of *in vitro* fertilization (IVF) tends to be higher in poor and suboptimal responders than in normal responders because different strategies or repeat treatment cycles might be required. Altogether, these factors cause emotional, physical, and financial distress for the couple, particularly when multiple treatment cycles are required.

The standards that define poor ovarian response (POR) vary widely as several factors either isolated or in combination are used for identification of such patients (13). Not surprisingly, the reported prevalence of POR fluctuates markedly between 5.6 and 35.1% (14, 15). Regardless of the chosen definition, it is clear that the POR population accounts for a substantial subset of women treated in IVF clinics nowadays (16). Driven by socioeconomic and other issues, many women are currently postponing motherhood which results in a higher number of patients seeking ART treatments in their late thirties and early forties. Women in this age range are more likely to have a diminished ovarian response due to natural aging of the ovaries, highlighting the need for particular attention to this group of women undergoing ART (17).

The central element in the pathophysiology of low ovarian response is the presence of a reduced number of follicles responsive to FSH. This phenomenon is most often found in women of advanced maternal age, mainly because of reduced ovarian reserve caused by accelerated follicular loss (18). In some cases, however, a low ovarian response might be seen in good ovarian reserve patients caused by a suboptimal gonadotropin dosage used for OS, for example in obese women (19), or due to the presence of genetic polymorphisms affecting endogenous gonadotrophins or their receptors (20–22). Both conditions ultimately alter the response of recruitable follicles to exogenous gonadotrophins (23–25). It is, therefore, clear that the so-called POR does not have a single cause. Indeed, the population with a diminished ovarian response is heterogeneous and sometimes difficult to characterize (14).

Most women diagnosed as poor responders are less likely to conceive or might even have their IVF cycle canceled due to lack of embryos for transfer (26). Nonetheless, some studies evaluating this patient population report reasonable cumulative pregnancy rates, ranging from 6 to 47% after three cycles, according to patient's age (27). Moreover, up to 40% of women who respond poorly in their first IVF cycle, as defined by the number of oocytes collected, have been reported to end up as normal responders in the second cycle (11, 16, 26). These figures indicate that not all women diagnosed with low ovarian response are similar regarding the likelihood of pregnancy. The optimal portrayal of this group of women with a low ovarian response is essential for proper counseling regarding the chances of pregnancy and the use of individualized strategies to increase IVF success (3, 4). Nevertheless, the current definitions for POR have been criticized for their inadequacy concerning a proper characterization of the POR population and for not providing clinicians a guide for therapeutic management (3, 4, 9, 14, 15). In this review, we provide an overview of existing criteria utilized to define the POR population, along with their advantages and shortcomings. Subsequently, we discuss the issues of ovarian resistance to gonadotropin stimulation and the importance of balancing quantity and quality with regard to oocytes retrieved. Lastly, we explain why a novel system for the identification and classification of low prognosis patients undergoing ART—the so-called POSEIDON criteria—was developed.

CRITERIA FOR THE DEFINITION OF POOR OVARIAN RESPONSE TO OVARIAN STIMULATION

Several standards have been developed for the definition of POR. Parameters related to patient demographics, ovarian reserve tests, and outcomes of previous IVF cycles—alone or combined—are used to define the POR population (**Table 1**) (28–49). The numerous existing definitions differ concerning the parameters utilized and the threshold values established for each criterion. In a 2011 systematic review of 47 randomized clinical trials involving women with POR, 41 different definitions were used to define this group of patients (13). Notably, different definitions were used even in trials by the same group of researchers and no more than three trials use the same definition. In this review, the authors observed that the age criterion—considered essential by some investigators for the description of POR—was used in only 9% of studies (13). The disparity in POR definition renders the interpretation of trial results challenging. At the very least, conclusions about the different interventions tested must be interpreted with caution as regards their application in clinical practice.

Various terminologies utilized to define this group of patients further reflect the discrepancy of the definition of the POR patient. Researchers and clinicians often use ambiguous terms as POR, low ovarian response (47, 50, 51), hypo-response (20, 21), and diminished ovarian reserve (52–54). According to a 2015 survey study among reproductive specialists, the most used criterion to define POR was "the number of follicles produced" (14), unlike the POR criteria used in research studies. To complicate matters further, a not-for-profit patient organization dedicated to providing education to couples suffering from infertility (https://resolve.org/) defines POR as those women who require large doses of medication and who make less than an optimal number of oocytes, meaning that patients themselves have introduced a new element into the already complicated POR equation, namely, the suboptimal response to ovarian stimulation.

TABLE 1 | Parameters used isolated or in combination to define the poor ovarian response patient.

Characteristics	Parameter	References
Demographics	Female age	(28)
Ovarian reserve markers	Antral follicle count	(29, 30)
	Basal serum FSH levels	(31–33)
	Serum anti-Müllerian hormone levels	(30)
Previous IVF cycle outcomes	History of cycle cancelation	(34, 35)
	Number of preovulatory follicles on day of trigger	(28, 33, 35–41)
	Serum estradiol levels on day of trigger	(32, 37, 39, 42, 43)
	Number of oocytes retrieved	(34, 37, 43)
	Number of mature oocytes retrieved	(44, 45)
	Number of good quality embryos	(46)
	Daily and total gonadotropin consumption	(47–49)

THE BOLOGNA CRITERIA

In 2011, the European Society of Human Reproduction and Embryology (ESHRE) carried out the first systematic effort to define women with inadequate response to OS (55). This consensus definition—known as the Bologna criteria—was initially introduced with the primary objective of standardizing the definition of the POR patient based on oocyte quantity for use in research studies. The authors made specific recommendations for investigators to avoid use of random definitions in prospective clinical trials or conduct meta-analyses including studies with distinct POR definitions (55).

According to Bologna criteria, at least two of the following three criteria must be present to classify a patient as poor responder, namely, (i) Advanced maternal age, (ii) Previous POR after OS, and (iii) Abnormal ovarian reserve tests (**Table 2**). The age of 40 years and retrieval of three or fewer oocytes were adopted as the cutoffs to discriminate women with and without POR. Ovarian reserve tests, namely antral follicle count (AFC) and anti-Müllerian hormone (AMH) levels were also included, with variable ranges of <5–7 follicles or <0.5–1.1 ng/ml, respectively.

The Bologna criteria were partially successful in its intended primary goal. Among 51 POR interventional trials registered in *clinicaltrials.gov* from July 2011 to March 2017, 23 (45%) adopted the Bologna criteria. The number of subjects enrolled in such trials varied markedly from 23 to 939, but the vast majority of trials were not powered to detect differences in pregnancy rates. In fact, a sample size of ∼1,000 subjects would be required in binary outcome superiority trials to have a 90% chance of detecting, as significant at the level of 5%, a 20% increase in pregnancy rates between the control group and experimental group (https://www.sealedenvelope.com/power/binary-superiority/). Among the published trials with an adequate sample size to avoid a type II error (https://clinicaltrials.gov), only two reported a potential benefit of a given intervention with regard to pregnancy (56, 57).

TABLE 2 | ESHRE Bologna criteria.

PARAMETERS INCLUDED
• Advanced maternal age (≥ 40 years) or any other POR risk factor
• A previous incident of POR (cycles canceled or ≤ 3 oocytes with a conventional ovarian stimulation protocol)
• A low ovarian reserve test (AFC <5–7 follicles or AMH <0.5–1.1 ng/ml)

Two of these three criteria are required for a POR diagnosis. In addition, two previous episodes of POR after maximal stimulation are sufficient to classify a patient as POR even in the absence of the other criteria mentioned.

POR, poor ovarian response; AFC, antral follicle count; AMH, anti-Müllerian hormone.

A few retrospective cohort studies were also published using the Bologna criteria. On average, a live birth rate (LBR) of 10% or less was observed in women diagnosed with POR (58–60), therefore, suggesting that the Bologna criteria might be able to select a homogeneous population with poorer reproductive outcomes during ART. The correct identification of the subset of women with poor prognosis in IVF, apart from its usefulness in terms of clinical management and counseling, would be necessary from a public health perspective, particularly in countries with governmental treatment reimbursement (58).

LIMITATIONS OF THE EXISTING POR CRITERIA

A review from 2016 accumulating the evidence of interventional clinical trials in POR revealed that over 90% trials were unable to detect meaningful differences in pregnancy rates (61). These disappointing results might be caused by the fact that the available studies used various POR definitions and suboptimal study designs, thus, making it difficult to draw valid conclusions for any given treatment strategy (62, 63).

Patient heterogeneity is deemed to be a significant shortcoming in studies evaluating strategies for POR, including those in which the Bologna criteria were applied (64). In a 2013 study, different LBRs were reported for Bologna POR aged ≤35 (12%), 36–39 (8%), and ≥40 (6%) (59). Likewise, Hu et al. retrospectively evaluated 592 IVF cycles in Bologna criteria PORs and reported that pregnancy outcomes varied according to age group (65). The authors showed that implantation rates ranged from 15.3 to 29.4% in patients under 35 years. By contrast, it ranged from 6.3 to 24.1% in patients ≥35 years. Along the same lines, Cohen and colleagues retrospectively assessed live birth rates in a large Bologna POR patient cohort aged 40 years or greater (16). The live birth per cycle was 3.3 times higher (11.61 vs. 3.54%, $P < 0.001$) in patients aged 40–43 with more than three oocytes compared to counterparts with less than three oocytes. Furthermore, a 2017 RCT evaluating the use of recombinant LH supplementation in Bologna criteria POR showed that—in a post-hoc analysis—the subset of patients classified as moderate or severe poor responders who received

LH supplementation had higher LBR and lower pregnancy loss than the general population of POR patients (57).

Although the ESHRE consensus established the minimum criteria for the definition of POR, numerous patient categories with potentially different prognosis might be generated by using the criteria mentioned above (**Table 3**). Notably, studies explicitly evaluating pregnancy outcomes according to these subgroups of patients yielded conflicting results (58, 66, 67) (**Table 4**). Whereas reproductive success was similar among Bologna subgroups in the studies of Busnelli et al. (58) and La Marca et al. (66), the results differed according to the subset evaluated in the series of Bozdag et al. (67). In the latter study, which to our knowledge included the largest retrospective analysis of POR patients undergoing ART to date, the likelihood of pregnancy varied significantly according to the subgroups of POR evaluated (**Table 4**).

Lastly, another limitation of the Bologna criteria relates to the biomarkers cut-offs used to classify POR patients. The ranges of 5–7 for AFC and, more importantly, 0.5–1.1 ng/ml for AMH seems quite wide. In fact, little information was provided by the authors of the ESHRE consensus about the accuracy of such ranges in predicting POR (55). Since the attributed importance of ovarian biomarkers is high, technical and performance characteristics should be considered when applying cut-off ranges, in particular, the lack of standardized methods for the assessment of ovarian reserve markers among centers (68).

OVARIAN RESISTANCE TO EXOGENOUS GONADOTROPINS: A PREVIOUSLY NEGLECTED ASPECT

Ovarian stimulation is a crucial element of most IVF programs. The use of GnRH analogs in association with exogenous gonadotropins promote adequate follicular growth and steroidogenesis in the majority of normogonadotropic women who undergo ART. In the modern ART era, ovarian biomarkers, including AFC, and AMH have been used with fair accuracy to predict ovarian response to gonadotropin stimulation, thus, allowing clinicians to individualize OS (69). However, AFC and AMH cannot predict an unexpectedly poor or suboptimal response to gonadotropin therapy in women with adequate pre-stimulation parameters. Indeed, patients with adequate ovarian reserve might show hypo-responsiveness to gonadotropin stimulation (70, 71). The reasons for ovarian resistance to gonadotropin stimulation are not entirely understood. However, increasing evidence indicates that women with the so-called "hypo-response" to OS might harbor genetic mutations or single nucleotide polymorphisms (SNPs) of gonadotropins and their receptors that influence ovarian sensitivity to gonadotropin stimulation despite an apparently good prognosis (21, 25, 72–74).

Despite broadly categorized as PORs, the fate of women with hypo-response to OS differs from the classic POR patient. The results of a 2014 meta-analysis compiling 1129 IVF/ICSI cycles in POR patients supplemented or not with recombinant human LH (rec-hLH) illustrate this phenomenon (27). In this aforementioned review, the definition of POR to gonadotropin stimulation was based on the criteria utilized by each included study. It was noted that significantly more oocytes were retrieved in rec-LH supplemented cycles than in recombinant human FSH (rec-hFSH) monotherapy cycles (12 studies, $n = 1077$; weighted mean difference +0.75 oocytes; 95 % CI 0.14–1.36). The use of rec-hLH supplementation also improved clinical pregnancy rates by 30% overall (14 studies, $n = 1179$; relative risk [RR] 1.30; 95 % confidence interval [CI] 1.01–1.67; intention-to-treat population [ITT] population). Nevertheless, a careful examination of the included studies reveals that the beneficial effect of rec-hLH was more pronounced in studies involving hypo-responders rather than in those with classic POR. The inclusion of studies involving hypo-responders in that review explains the overall favorable results observed with rec-LH supplementation in the POR patient. Indeed, a 2018 systematic review carried out by the International Collaborative Group for the Study of rec-hLH (iCOS-LH) showed that a clear distinction between hypo-responders and classic PORs is paramount since the clinical relevance of adding rec-LH to OS was only evident in hypo-responders (75). Researchers have rightfully argued that critical methodological issues like the one discussed above should be taken into account when designing studies on poor responders (64, 76, 77).

From a clinical perspective, hypo-responders represent a patient category that differs from both normal responders and the classic POR. The hypo-responder is a patient with a normal ovarian reserve who ends up having an unexpected suboptimal or poor response to OS, usually manifested by a low follicular output rate (FORT), use of increased total dosages of gonadotropin, or lower than expected number of oocytes retrieved (9, 21, 25, 72). Management of hypo-responders might be associated with increased treatment costs, decreased cumulative live birth rates, and increased time to live birth. Until now, however, none of the POR criteria have taken into account this group of hypo-responders to ovarian stimulation.

TABLE 3 | Different patient categories generated by combining the parameters used to define the poor ovarian response patient according to Bologna criteria.

Criteria	Combined with
≥ 40 years	• One previous POR episode • Abnormal ORT
Other risk factor	• One previous POR episode • Abnormal ORT
One previous POR	• ≥40 years • Other risk factor • Abnormal ORT
Abnormal ORT	• ≥40 years • Other risk factor • Previous POR episode
2 previous episodes of POR after maximal stimulation	• Alone • Or with any other criteria

POR, poor ovarian response (cycles canceled or ≤3 oocytes with the use of conventional ovarian stimulation); ORT, ovarian reserve tests (AFC <5–7 follicles or AMH <0.5–1.1 ng/mL); Other risk factor: genetic or acquired conditions possibly linked to a reduced number of resting follicles.

TABLE 4 | Clinical studies evaluating IVF outcomes in different subgroups of poor ovarian responders according to the Bologna criteria.

Study	Number of patients (IVF/ICSI cycles) included	Subgroups included	Live birth rate/cycle (number of cycles)	Ability of Bologna criteria to identify homogeneous patient populations with similar pregnancy outcomes
Busnelli et al. (58)	362 (362)	Group 1: anamnestic risk factors for POR and one episode of POR; Group 2: one previous episode of POR and abnormal ORT; Group 3: anamnestic risk factors for POR and abnormal ORT; Group 4: anamnestic risk factors for POR, one previous POR cycle and abnormal ORT Group 5: two episodes of POR after maximal stimulation	Group 1: 10% (40) Group 2: 4% (52) Group 3: 6% (190) Group 4: 8% (73) Group 5: 0% (7) *P*-values did not differ among subgroups (P=0.65)	Yes; The study suffered from a type II error due to small patient cohort included in each subgroup.
La Marca et al. (66)	210 (452)	Group 1: ≥ 40 years-old + previous POR; Group 2: previous POR and abnormal ORT; Group 3: ≥ 40 years-old + abnormal ORT; Group 4: previous POR + ≥ 40 years-old + abnormal ORT; Group 5: two previous POR episodes	Group 1: 7.4% (76) Group 2: 6.6% (91) Group 3: 5.9% (76) Group 4: 6.7% (136) Group 5: 5.5% (73) *P*-values not provided	Yes; The study suffered from a type II error due to small patient cohort included in each subgroup.
Bozdag et al. (67)	821 (1257)	Group 1: ≥40 years-old + previous POR episode; Group 2: ≥40 years-old + AFC<7; Group 3: AFC<7 + previous POR episode; Group 4: ≥40y + AFC <7 + previous POR episode	Group 1: 3.3% (123) Group 2: 6.3% (253) Group 3: 8.7% (575) ($P = 0.001$; statistically different from all other groups) Group 4: 2.3% (306) ($P = 0.002$; statistically different from all other groups)	No; The number of subjects in each group was adequate to avoid a type II error.

ORT, ovarian reserve test; Anamnestic risk factors: advanced maternal age (≥40years), evidence of ovarian endometrioma at the basal ultrasound, previous ovarian surgery, previous chemotherapy, genetic abnormalities, shortening of the menstrual cycle.

OOCYTE QUANTITY VERSUS QUALITY

The decline in fertility with aging is caused by both a progressive reduction in the primordial follicle number across the woman's lifespan as well as an increased rate of oocyte chromosomal abnormalities and cytoplasmic dysfunctions (18). These phenomena ultimately result in a reduction of oocyte quantity and quality, thus, explaining the poorer IVF outcomes in older women when compared to younger counterparts.

Data from large databases unequivocally show that IVF success depends on both the number of oocytes retrieved and the women's age (5, 6). The critical role of female age on oocyte quality is easily illustrated by comparing delivery rates according to age in women with similar oocyte yield (5, 6); in this scenario, the older the patient the lower the delivery rates. This effect is noted not only in the general infertile population, but also in poor responders (15).

Despite the overall notion that the prognosis of a patient undergoing IVF can be measured by the number of oocytes retrieved, a valid critique of Bologna criteria and other classification systems for POR is that these standards fail to identify young women with expected POR due to abnormal ovarian biomarkers; i.e., women below 35 years-old who have not undergone OS (78, 79). Preimplantation genetic studies using microarray-based comparative genomic hybridization and next-generation sequencing (NGS) show that embryo euploidy rates are markedly higher in women younger than 35 years of age than older counterparts (80, 81). In fact, embryo ploidy is probably the leading factor explaining the differences in success rates between younger and older women who undergo IVF (82).

The probability of achieving at least one euploid blastocyst for transfer in patients undergoing IVF increases as a function of blastocyst cohort size in all age categories (80, 81). Since blastocyst euploidy rates are independent of cohort sizes, the higher the number of oocytes retrieved the higher the probability of having an embryo cohort with at least one euploid embryo (80, 81). Therefore, oocyte quantity and the age-related embryo euploidy rate are essential aspects to consider for both counseling purposes and treatment planning in women with POR. Failure to include these aspects in clinical studies might result in stratification of women with distinct biological characteristics, a bias that could dilute the magnitude of the effect concerning the intervention studied.

A PLEA FOR A MORE OPTIMAL DEFINITION AND STRATIFICATION OF THE LOW RESPONDER PATIENT UNDERGOING ART: THE POSEIDON CRITERIA

Despite the advancement toward a better definition of the POR patient with the publication of the Bologna criteria in 2011 (55), little has been achieved in terms of clinical

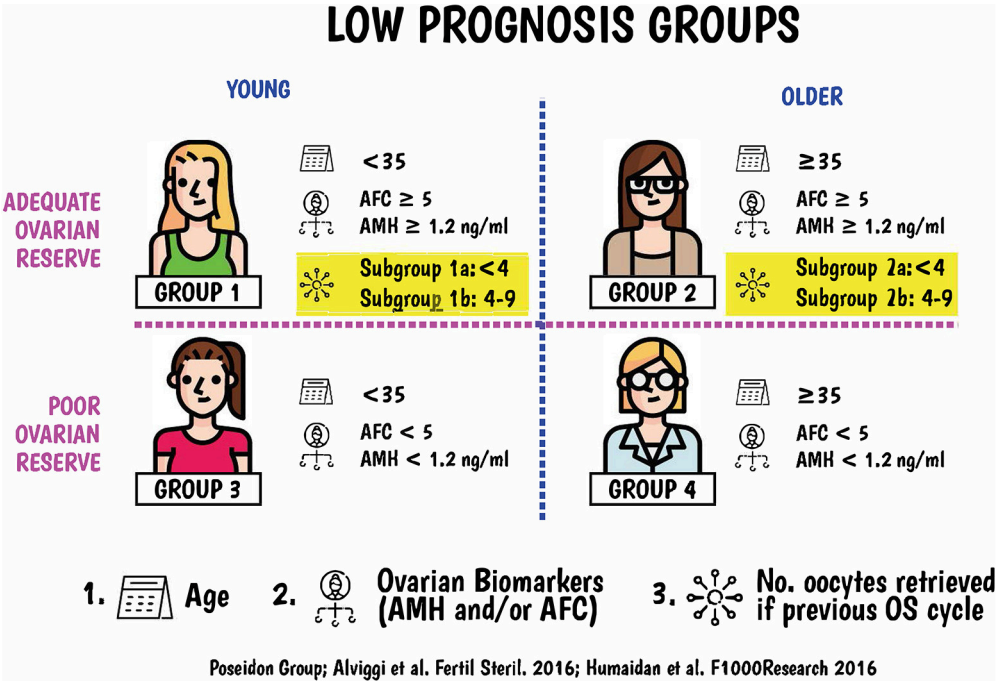

FIGURE 1 | The new Poseidon criteria to identify and stratify infertility patients with "expected" or "unexpected" impaired ovarian response to exogenous gonadotropins undergoing ART. Four distinct groups of low prognosis patients can be established based on quantitative and qualitative parameters, namely: 1. The age of the patient and the expected embryo aneuploidy rate; 2. Ovarian biomarkers [antral follicle count [AFC] and/or anti-Müllerian hormone [AMH]], and 3. The ovarian response of the patient in terms of oocyte quantity provided a previous cycle of stimulation was carried out. Art drawing by Chloé Xilinas, EXCEMED, Rome, Italy.

guidance concerning management. To date, clinicians remain without evidence-based guidance for therapeutic management of the POR patient and often rely on personal experience or anecdotal facts to handle such patients (14). Thus, development of criteria aiming at identifying and stratifying patients with low prognosis in ART is of utmost importance for clinical management. A correct stratification of homogeneous groups of low prognosis women could also help researchers identify treatment strategies best suited for each patient category.

The recently established POSEIDON (**P**atient-**O**riented **S**trategies **E**ncompassing **I**ndividualize**D** **O**ocyte **N**umber) Group, a collaborative effort among clinicians and researchers with a particular interest in reproductive endocrinology and ART, proposes a new and more detailed stratification of low prognosis patients who undergo OS for IVF (83, 84). A series of articles within this research topic of Frontiers in Endocrinology will discuss in great detail the newly launched POSEIDON criteria. In brief, this new system aims to introduce a fine-tuning of POR, using clinically relevant criteria to guide the physician (**Figure 1**). Essentially, the POSEIDON group proposes a change in the definition of POR from quite heterogeneous criteria to the concept of low prognosis, which better reflects the reproductive potential of these patients.

"Low Prognosis" seems to be the ideal terminology because it allows not only to identify patients who have a reduced probability of pregnancy in ART, but also to stratify the low prognosis patients into distinct categories based on quantitative and qualitative parameters, namely: (i) The age of the patient and the expected embryo aneuploidy rate; (ii) Ovarian biomarkers, and (iii) The ovarian response of the patient provided a previous cycle of stimulation was carried out (83). In addition to providing a system for the identification and classification of low prognosis patients undergoing ART, the group introduced a new measure of clinical success, namely, the ability to retrieve the number of oocytes needed to obtain at least one euploid blastocyst for transfer in each patient (84).

Notably, the POSEIDON group does not advocate trial-and-error to identify patients classified as groups 1 and 2. Other published algorithms might be considered as a means to optimize oocytes yield on the first cycle (85). However, the information from a previous cycle should be used wisely, whenever available, to most optimally plan the next ovarian stimulation strategy.

The POSEIDON criteria allow the clinician to first of all classify patients who have low prognosis in ART and secondly to prepare a stimulation plan aiming at reaching the number of oocytes needed to obtain at least one euploid blastocyst for transfer (4, 86). It is anticipated that the new concept of low prognosis will help improve the management of patients undergoing ART, promote a tailored approach to

patient handling, and identify more homogeneous populations for clinical trials, thereby, providing better tools with which to maximize IVF success rates.

CONCLUSIONS

Management of patients with an impaired ovarian reserve or POR to exogenous gonadotropin stimulation has challenged reproductive specialists for several decades. Apart from our limited understanding of its pathophysiology, wide heterogeneity exists in the definition of POR. A critical shortcoming of the existing POR criteria, which is largely based on ovarian biomarkers and numbers of oocytes retrieved after OS, is that they group women with distinct clinically relevant characteristics. This could explain the lack of scientific evidence to support any effective intervention for POR patients. As a result, practitioners have utilized different strategies in clinical management—often not evidence-based—since none of the existing POR criteria provide a clear path for management. In practical terms, counting the number of oocytes retrieved or estimating such numbers using ovarian biomarkers is not enough for clinical management. Equally important is the ability to determine the ovarian sensitivity to gonadotropins, which is modulated by genetic factors involving both gonadotropins and their receptors, and the age-related decrease in oocyte quality which largely depends on chromosomal abnormalities occurring before meiosis II.

The POSEIDON (**P**atient-**O**riented **S**trategies **E**ncompassing **I**ndividualize**D** **O**ocyte **N**umber) group—founded in 2015-introduced a new system to stratify infertility patients with "expected" or "unexpected" impaired ovarian response to exogenous gonadotropins. Furthermore, the group proposed a new measure for successful ART treatment, namely, the ability to retrieve the number of oocytes necessary to obtain at least one euploid embryo for transfer in each patient. This new stratification aims at providing a more nuanced picture of POR using clinically relevant criteria to guide the physician in the management of this increasing group of patients. Thus, the POSEIDON group proposes a change in the definition of POR, with sub-grouping, resulting in more homogenous populations. Hopefully, this new classification system will prove to be of daily help for clinicians as well as for patients, ultimately facilitating treatment and resulting in a shorter time to pregnancy and live birth.

AUTHOR CONTRIBUTIONS

SE designed the manuscript. All authors contributed to drafting and critical discussions. GB and MR scrutinized the literature and developed the Tables. All authors contributed to revised and accepted the final manuscript.

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Understanding Ovarian Hypo-Response to Exogenous Gonadotropin in Ovarian Stimulation and Its New Proposed Marker—The Follicle-To-Oocyte (FOI) Index

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Hypo-responsiveness to controlled ovarian stimulation is an undervalued topic in reproductive medicine. This phenomenon manifests as a low follicles output rate (FORT) with a discrepancy between the relatively low number of pre-ovulatory follicles which develop following ovarian stimulation as compared to the number of antral follicles available at the start of stimulation. The pathophysiology mechanisms explaining the ovarian resistance to gonadotropin stimulation are not fully understood, but the fact that both hypo-responders and normal responders share similar phenotypic characteristics suggests a genotype-based mechanism. Indeed, existing evidence supports the association between specific gonadotropin and their receptor polymorphisms and ovarian hypo-response. Apart from genotypic trait, environmental contaminants and oxidative stress might also be involved in the hypo-response pathogenesis. The ratio between the number of oocytes collected at the ovum pick up and the number of antral follicles at the beginning of OS [Follicle to oocyte index (FOI)] is proposed as a novel parameter to assess the hypo-response. Compared with traditional ovarian reserve markers, FOI might reflect most optimally the dynamic nature of follicular growth in response to exogenous gonadotropin. In this review, we contextualize the role of FOI as a parameter to identify this condition, discuss the underlying mechanisms potentially implicated in the pathogenesis of hypo-response, and appraise possible the treatment strategies to overcome hyper-responsiveness to gonadotropin stimulation.

Keywords: hypo-response, ovarian stimulation, ovulation induction, assisted reproductive technology, *in vitro* fertilization, follicle to oocyte index, follicle output rate, POSEIDON criteria

INTRODUCTION

Ovarian stimulation (OS) is an essential step in assisted reproductive technology (ART). The conventional OS approaches lead to sufficient follicular growth and proper estrogen levels in the majority of women. In this regard, the number of mature oocytes retrieved is the parameter most often used to assess ovarian response to exogenous gonadotropin, as oocyte number is closely related to the likelihood of achieving a live birth in ART (1). Based on oocyte number, women are usually classified as poor, suboptimal, normal or hyper-responders (2, 3). Outside these categories, a subgroup of women with impaired response to gonadotropin, termed "hypo-responders," also exists. An unexpected ovarian resistance to OS with use of standard age- and BMI-matched doses of exogenous FSH characterizes these patients (4–6). The clinical manifestation of ovarian resistance includes either an "initial slow response" to FSH stimulation concerning estradiol levels rise and follicle growth (7, 8) or can be retrospectively diagnosed in women who require higher-than-expected doses of gonadotropins considering their age, BMI, and ovarian reserve (9). In contrast to "suboptimal response," which is based essentially on the number of oocytes retrieved (between 4 and 9) (10), the hypo-response profile refers to those patients who show a resistance to gonadotropin stimulation and in which the number of oocytes retrieved at the end of stimulation is not consistent with the number of antral follicle count (AFC) available at the beginning of OS. In this review, we (1) illustrate how to identify patients with ovarian resistance to exogenous gonadotropins who undergo ART by use of a new marker named follicle-to-oocyte index (FOI), (2) discuss the underlying pathogenetic mechanisms associated with hypo-response, and lastly, (3) critically appraise possible treatment strategies to overcome this condition.

ASSESSMENT OF HYPO-RESPONSE

The prediction of ovarian response is crucial for an optimal and individualized management in the context of OS. It also allows clinicians to better counsel women about the risk of adverse events following OS, such as protracted cycles, cycle cancellation due to poor ovarian response, or ovarian hyperstimulation syndrome (OHSS). Generally, the ovarian response to gonadotropin stimulation can be explained by the interplay between demographic and anthropometric characteristics, and the individual's ovarian reserve. In this regard, biological (AFC) and biochemical [Anti-Müllerian hormone (AMH)] markers have been introduced to predict both the poor and hyper response with fairly good accuracy (3).

In our opinion, these biomarkers represent a "static" snapshot of the individual ovarian reserve which do not properly reflect the "dynamic" nature of follicular growth in response to exogenous OS. An interesting model to assess hypo-responsiveness during OS is the follicle output rate (FORT) introduced by Genro et al. in 2011 (11). This index is calculated as the ratio between the number of pre-ovulatory follicles obtained in response to OS with FSH administration and the pre-existing pool of small antral follicles (11, 12). A low FORT (e.g., 30%) indicates hypo-response, due to the discrepancy between the relatively low number of pre-ovulatory follicles which develop following OS as compared to the number of antral follicles available at the start of stimulation (**Figure 1**). Notably, low FORT indices are not associated with reduced ovarian markers, thereby suggesting that patients undergoing OS can present with a low FORT despite the presence of adequate ovarian markers (11).

Another parameter that might be used to assess hypo-responsiveness is the ovarian sensitivity index (OSI) (13). OSI is calculated by dividing the total administered FSH dose and the number of retrieved oocytes. A high OSI index reflects ovarian resistance to OS thereby suggesting a hypo-response profile.

Although both methods seem to be useful in the evaluation of hypo-response, some drawbacks should be considered. The FORT does not assess the actual number of oocytes retrieved, which is the parameter more strictly associated with live birth rates (1). On the other hand, OSI does not take into account the type of gonadotropin adopted (recombinant or urinary) nor does it consider the gonadotropin regimen utilized. In fact, recent evidence indicates that the use of luteinizing hormone (LH) or LH-like activity during OS improves follicle development in specific subgroups of women, including hypo-responders (14, 15). Along the same lines, OSI indices might be misleading if inappropriate low starting doses of exogenous gonadotrophins are given. Lastly, it has been suggested that OSI results are associated with AMH levels in women undergoing IVF (13), thus making it a less robust index to assess the dynamical aspect of the follicular response to OS.

FOLLICLE-TO-OOCYTE INDEX (FOI)

We propose an alternative approach to address the ovarian resistance to gonadotropin stimulation (or hypo-responsiveness) based on the concept of FORT, namely, the ratio between the total number of oocytes collected at the end of OS, and the number of antral follicles available at the start of stimulation (Follicle-to-Oocyte Index [FOI]) (**Figures 2**, **3**). **Figure 2** illustrates the difference between hypo-response to OS and suboptimal

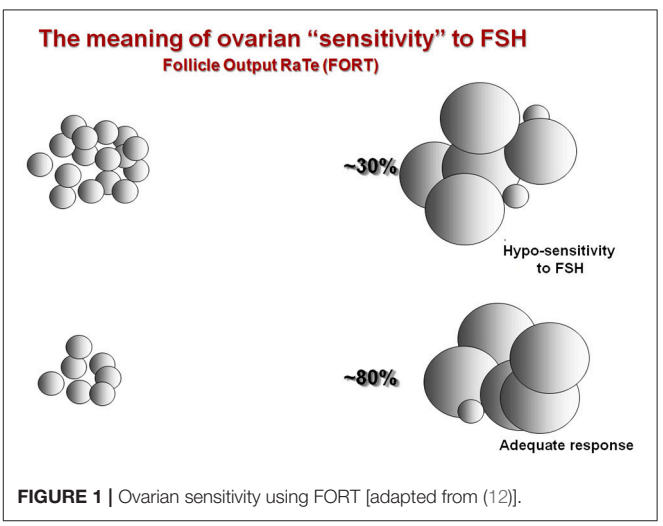

FIGURE 1 | Ovarian sensitivity using FORT [adapted from (12)].

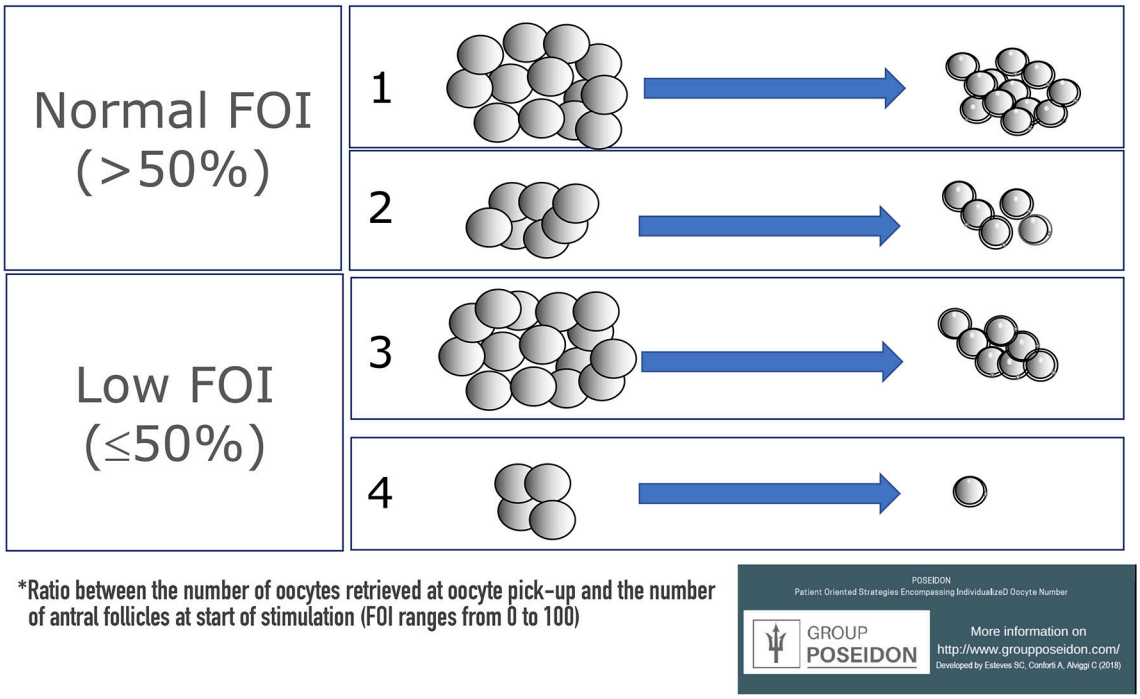

FIGURE 2 | Ovarian sensitivity using the Follicle-to-Oocyte Index (FOI). Case number 1 depicts a patient with normal FOI, in whom the number of oocytes retrieved was consistent with the AFC at the start of stimulation. Case number 2 illustrates a patient with suboptimal number of oocytes retrieved (between 4 and 9), but with a normal Follicle-to-Oocyte index (FOI >50%). Case number 3 shows a patient with both hypo-response and suboptimal oocyte number. This patient had only 7 oocytes collected despite an AFC of 15 at the beginning of stimulation (FOI ≤ 50%). Case number 4 depicts a patient with both hypo-response and poor response.

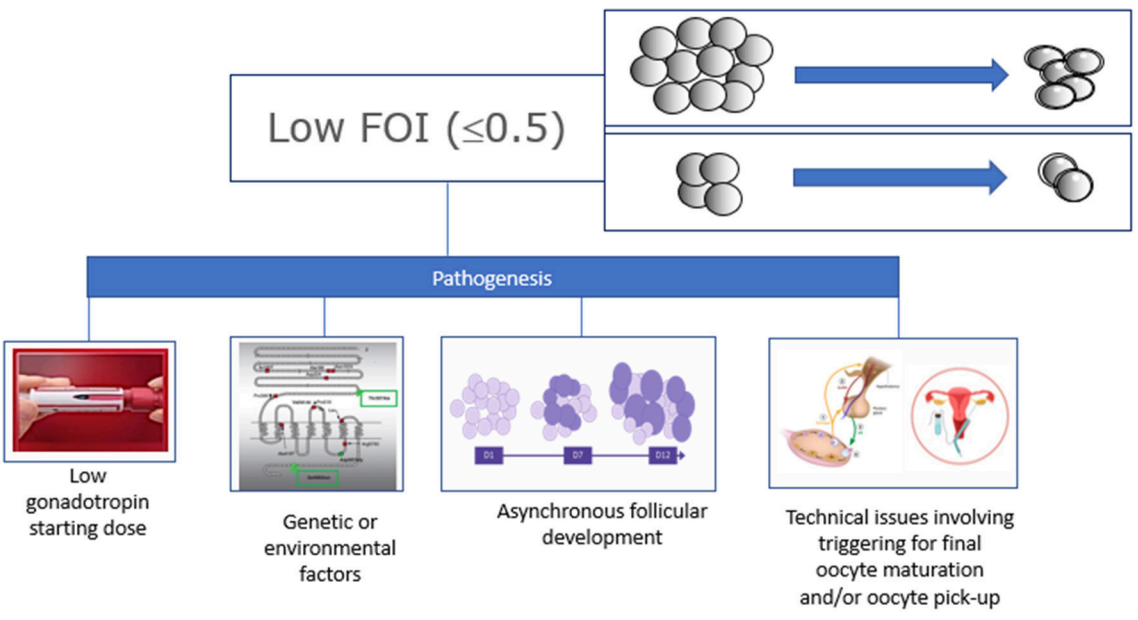

FIGURE 3 | Possible causes of low Follicle-to-Oocyte Indeces.

response. In detail, both cases 2 and 3 show a suboptimal response with the number of oocytes retrieved between 4 and 9. However, only case 3 illustrates a hypo-response profile in which just 7 oocytes were collected despite an AFC of 15 at the beginning of stimulation (FOI < 50%). On the other hand, in case 2, despite the low oocyte number, this hypothetical woman had 5 oocytes retrieved from an AFC of 7, thus illustrating a normal follicle-to-oocyte index (FOI >50%). Lastly, case number 4 depicts a patient with both hypo-response and poor response. Based on the examples above it is therefore clear that hypo-responsiveness and suboptimal/poor response are not synonymous.

FOI may be used alone or combined with FORT to most optimally reflect the ovarian resistance to OS. The results of FOI can also help to understand whether it is possible to exploit the ovarian reserve further using pharmacologic interventions. Lastly, FOI could be useful to predict the likelihood of success in ART, both concerning the chances of achieving at least one euploid blastocyst for transfer in each patient–the so-called POSEIDON marker of successful outcome-, (4, 6) as well as pregnancy success. Thus, low FOI values imply that only a fraction of available antral follicles was exploited during OS, suggesting that there might be therapeutic opportunities to change the fate of these women in a subsequent OS. Naturally, technical aspects related to oocyte retrieval and triggering for final oocyte maturation, both of which can influence FOI results, should be taken into account in patients with low FOI. The FOI is under evaluation by an ongoing multicenter Italian study (Impact of **G**onadotropin **GEN**etics Profile and **O**v**A**rian **R**eserve on **C**ontrolled **O**varian Stimulation, the **GENACOS** study). In future, we envision refining FOI by including the amount of gonadotropin used during OS. Additionally, a prediction model can be developed to estimate the likely number of oocytes to be retrieved at the end of OS by computing the results of AFC, polymorphisms of gonadotrophins and their receptors, and FSH starting dose.

PATHOGENESIS OF HYPO-RESPONSE

The pathophysiology mechanisms explaining hypo-responsiveness to OS are not fully understood. However, the link between ovarian response and individual genotype has been postulated by several authors (16–22). Furthermore, the fact that both hypo-responders and normal responders share similar phenotypic characteristics suggests a genotype-based mechanism (23–27). In other words, hypo-responders might have a particular genotype profile which influences their response to OS (28). Indeed, several studies support this concept. In a 2013 sizeable longitudinal study, we found that a common LH beta subunit variant was associated with increased FSH consumption during OS (29). In another study, we found that the prevalence of hypo-response was higher in G allele carriers of a common FSH receptor (FSHR) polymorphism (p.N680SA > G, rs6166) than in wild-type haplotypes (30). Furthermore, *in vitro* studies using human granulosa cells demonstrated that p.N680SA>G G homozygous showed higher resistance to FSH stimulation than p.N680SA>G A homozygous carriers at the FSH receptor level (31, 32). Along the same lines, it has been shown that N680SA>G G homozygous display increased basal endogenous FSH levels (33) compared to p.N680SA>G A homozygous, thus corroborating the hypothesis of an impaired FSHR function in carries of G allele. Added to this, *in vivo* studies have shown that carriers of another FSHR polymorphism, namely, A allele, have ovarian resistance to OS as expressed by a higher consumption of exogenous gonadotropin than G allele carriers (34, 35). It is out of the scope of our paper to provide readers a comprehensive review of the impact of gonadotropin receptors polymorphisms in OS, but a recently published systematic review and meta-analysis by our group confirmed that polymorphism of FSHR could impair ovarian response to exogenous gonadotropin (28).

Apart from the genotypic trait, there is also evidence that environmental contaminants might influence ovarian response to gonadotropin stimulation. In a 2014 retrospective study, we showed that elevated intra-follicular levels of benzene were associated with a reduced number of oocytes retrieved and embryos available for transfer in women who underwent IVF (36) (**Figure 4**). The mechanism underlying this phenomenon is not clear, but the authors hypothesized that the toxic effect of benzene leads to a transduction deficiency of the FSHR. In fact, this hypothesis is supported by the fact that basal FSH levels were significantly higher in women with higher intra-follicular benzene levels than in women with low intra-follicular benzene levels (36). Other pollutants were also associated with an impaired ovarian response in IVF (37). In a 2017 retrospective study, a significant inverse association was found between the levels of polychlorinated biphenyl congeners (PBC) in follicular fluid of women undergoing ART and the ovarian response to gonadotropins measured by both the number of oocyte retrieved and estradiol levels (38). Notably, the number of antral follicles also seem to be affected by the levels of PCB congeners in follicular fluid (38).

Lastly, accumulating evidence indicates that oxidative stress might also affect both folliculogenesis and spermatogenesis (39, 40). In detail, it was hypothesized that oxidative stress

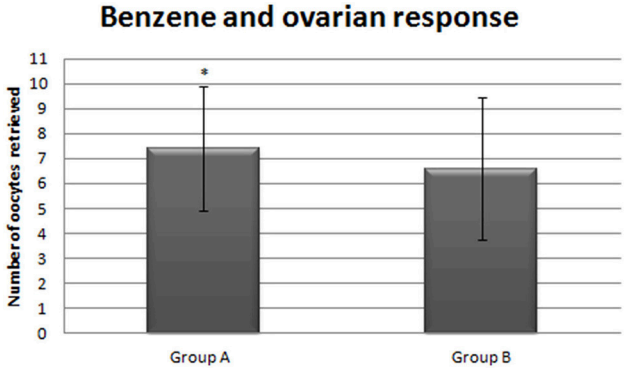

FIGURE 4 | Number of oocytes collected form patients with low (Group A) vs. high intrafollicular benzene levels [adapted from (36)]. *stand for statistically significant.

and excessive free radicals such as reactive oxygen species (ROS) might influence the quality of oocytes, spermatozoa, and embryos, as well as their environments (41), thus negatively affecting the outcome of IVF (39, 42). In a 2016 pilot study involving women with polycystic ovarian syndrome (PCOS) (43), in whom oxidative stress seemed to be a relevant pathogenetic factor, we demonstrated that myo-inositol plus active antioxidants (glutathione, selenium, vitamins C and E, and zinc), given twice a day for 5 months preceding OS, had a favorable effect on the outcome of IVF by increasing the number of mature oocytes (44). Recently, in 2018, Xu et al. reported the results of a randomized controlled trial with the use of coenzyme Q10 as a pretreatment to OS in patients with low prognosis in ART (45). The authors utilized the POSEIDON criteria to enroll young patients (<35 years-old; POSEIDON group 3) with poor ovarian reserve parameters (4, 6). In their study, the use of coenzyme Q10 (200 mg thrice daily for 60 days preceding the IVF cycle) was associated with an increased number of retrieved oocytes, fertilization rate, and high-quality embryos than in non-treated women. Lastly, a 2017 Cochrane meta-analysis supports the above observations by showing that antioxidant intake might provide a benefit for subfertile women who undergo ART (42). Whether oxidative stress has a role in the pathogenesis of hypo-response deserves further investigation.

CLINICAL MANAGEMENT OF PATIENTS WITH HYPO-RESPONSIVENESS TO OVARIAN STIMULATION

In clinical practice, ovarian resistance to gonadotropin stimulation is still a largely undervalued issue. Overall, clinicians do not ask themselves whether or not the number of oocytes retrieved after OS was consistent with the patient's potential based on the results of AFC at the start of stimulation. In our opinion, the number of oocytes retrieved should be interpreted in the light of an individual ovarian reserve. For example, in a woman with an AFC of 12, recruitment of 7 oocytes, which is far above the adopted POR threshold (4, 46), might still denote an inappropriate ovarian response to stimulation. Nevertheless, very few trials have investigated the role of interventions in women with ovarian resistance (i.e., hypo-responsiveness) to OS and until recently no practical guidelines were available.

An increase in the daily dose of exogenous FSH represents the intuitive approach to overcome ovarian resistance to exogenous FSH, and it was indeed adopted by several investigators. This strategy might be applied to rescue an ongoing OS cycle in women with an initial slow ("steady") response to gonadotropin stimulation (7, 8, 47). Increased FSH dosages has been mostly utilized in women treated with GnRH-a long protocols, where follicle "stagnation" during the first days of OS is more frequently detected. The increase in the FSH starting dose might be also an option in women who show hypo-sensitivity to gonadotropin stimulation in a previous cycle. In the latter, use of higher dosages of recombinant FSH might mitigate the negative effect of FSHR polymorphisms on ovarian response. In one study, Behre et al. demonstrated that increasing the daily FSH dose might counteract the negative effect of FSHR polymorphisms in normogonadotropic women with p.N680SA > G, rs6166 haplotype. In their study, the recombinant FSH dose of 225 IU/day was able to prevent low estradiol levels achieved at the end of OS in p.N680SA>G G homozygous stimulated with 150 IU/day (16). These results were corroborated by a 2012 study conducted by Genro et al. The authors reported that the FORT was not significantly influenced by the presence of FSHR p.N680SA > G, rs6166 polymorphism when a high FSH dose (300 IU per day) was given during OS (48).

Based on the aforementioned observations, one could argue that a starting FSH dose between 225–300 IU should be considered for all good prognosis patients undergoing ART, independently of genotype characterization. Although this approach might counteract the vast majority of the polymorphisms of gonadotrophins and their receptors, it is clearly not cost-effective. The study by Behre et al. (16) mentioned above indicates that a remarkable proportion of untested women would achieve an optimal FORT with a lower FSH starting dose. In addition, the MERIT study demonstrated that the indiscriminate use of a 225 IU/day FSH starting dose led to progesterone rise in a relevant percentage of women with good ovarian reserve (49). In this study, progesterone elevation was not observed if a starting dose of 150 IU/day had been adopted (50). Hence, FSHR genotype testing before OS might be clinically useful and cost-effective to identify those women who benefit from an increment in the FSH starting dose from those who do not (28).

The use of recombinant LH (r-hLH) supplementation has also been investigated as a means to overcome hypo-responsiveness to gonadotropin stimulation (51–53). Recently, a systematic review compiled the evidence concerning the use of r-hLH supplementation in hypo-responder women undergoing IVF (15). From the analysis of RCTs, the authors concluded that addition of rLH might be more advantageous than increasing rFSH dosage. Notwithstanding the promising results with the use of r-LH in hypo-responders, the existing literature is limited by the availability of only few reports, thus indicating the need of further research. Likewise, the use antioxidant supplementation as a means of alleviating the plausible negative effects of ROS on the follicular environment and ovarian resistance to gonadotropin stimulation is open for research.

CONCLUSION

Several non-mutually exclusive factors seem to influence ovarian resistance to gonadotropin stimulation. The driving theory explaining its pathophysiology relies on the genotypic profile of gonadotropins and their receptors. Genetic phenotyping of relevant polymorphisms seems to be the optimal method to identify these patients. Until genotyping testing becomes widely available, other indices such as the FORT and FOI can be used as surrogate measures to identify women with ovarian resistance (hypo-responsiveness) to gonadotropin stimulation. Particularly, FOI, assessing the actual number of oocytes retrieved could represent a better tool to determine whether the ovarian reserve

was adequately exploited during stimulation. Guidance on how to most optimally manage patients with hypo-response to OS is lacking, but limited evidence indicates that the use of higher FSH daily doses alone or combined with recombinant LH supplementation are the most effective ways to counteract the negative effects of hypo-responsiveness to exogenous gonadotropin administration. Further research is warranted to fully unravel the underlying mechanisms leading to ovarian resistance to gonadotropin stimulation and to determine the most prevalent polymorphisms associated with this condition. Additionally, the impact of different pharmacological regimens as a means of overcoming ovarian resistance to gonadotropin stimulation needs to be investigated in more detail.

AUTHOR CONTRIBUTIONS

CA, AC, SE, CYA, and GD idealized the paper and wrote the first draft. RVa, RVe, SS, and EC participated in literature research and paper writing. All author listed have made intellectual contribution to the work and approved the final version.

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A Novel Predictive Model to Estimate the Number of Mature Oocytes Required for Obtaining at Least One Euploid Blastocyst for Transfer in Couples Undergoing *in vitro* Fertilization/Intracytoplasmic Sperm Injection: The ART Calculator

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The POSEIDON group (**P**atient-**O**riented **S**trategies **E**ncompassing **I**ndividualize**D** **O**ocyte **N**umber) has introduced "the ability to retrieve the number of oocytes needed to achieve at least one euploid embryo for transfer" as an intermediate marker of successful outcome in IVF/ICSI cycles. This study aimed to develop a novel calculator to predict the POSEIDON marker. We analyzed clinical and embryonic data of infertile couples who underwent IVF/ICSI with the intention to have trophectoderm biopsy for preimplantation genetic testing for aneuploidy. We used the negative binomial distribution to model the number of euploid blastocysts and the adaptive LASSO (Least Absolute Shrinkage and Selection Operator) method for variable selection. The fitted model selected female age, sperm source used for ICSI, and the number of mature (metaphase II) oocytes as predictors ($p < 0.0001$). Female age was the most important factor for predicting the probability of a blastocyst being euploid given each mature oocyte (loglikelihood of age [adjusted for sperm source]: 30.9; $df = 2$; $p < 0.0001$). The final predictive model was developed using logistic regression analysis, and internally validated by the holdout method. The predictive ability of the model was assessed by the ROC curve, which resulted in an area under the curve of 0.716. Using the final model and mathematical equations, we calculated the individualized probability of blastocyst euploidy per mature retrieved oocyte and the minimum number of mature oocytes required to obtain ≥1 euploid blastocyst—with their 95% confidence interval [CI]—for different probabilities of success. The estimated predicted probabilities of a mature oocyte turn into a euploid blastocyst decreased progressively with female age and was negatively modulated overall by use of testicular sperm across age ($p < 0.001$). A calculator was developed to make two types of predictions automatically, one using pretreatment information to estimate the minimum number of mature oocytes to achieve ≥1 euploid blastocyst,

and another based on the actual number of mature oocytes collected/accumulated to estimate the chances of having a euploid blastocyst using that oocyte cohort for IVF/ICSI. The new ART calculator may assist in clinical counseling and individualized treatment planning regarding the number of oocytes required for at least one euploid blastocyst in IVF/ICSI procedures.

Keywords: assisted reproductive technology, ART calculator, intracytoplasmic sperm injection, blastocyst, preimplantation genetic testing for aneuploidy, female age, decision support models, POSEIDON criteria

INTRODUCTION

Globally approximately 10% of the couples have difficulties to conceive, with the highest prevalence in Eastern Europe, North Africa, Middle East, and Oceania (1). Female factors, alone or combined with male factors, contribute to ∼70% of infertility cases. Assisted reproductive technology (ART) has become an essential element of care for many couples suffering from infertility (2). The International Committee for Monitoring Assisted Reproductive Technologies (ICMART) reported over four million ART treatments worldwide between 2008 and 2010 (3), most of which using ICSI as the fertilization method (4). In Europe and the United States, over 2% of all infants born result from ART treatments (5), and over 8 million babies were born from ART worldwide (6).

Despite the notable developments in ART over the last decades, which improved live birth rates from 26% in the 90's to about 40% nowadays (7), the incidence of male infertility has increased, in parallel with a decline in semen quality (8, 9). The etiology and severity of male infertility seem to independently affect reproductive outcomes even under ART settings (7, 10). Moreover, the age of the population seeking ART is increasing steadily as both women and men are postponing childbearing. Aging couples, in turn, poses enormous challenges for clinicians and researchers alike as female age seems to be the central factor for pregnancy success (11).

The success of ART has traditionally been reported as the live birth rate (3). However, widespread use of preimplantation genetic testing (PGT) and embryo cryopreservation in the past two decades has allowed the introduction of alternative metrics of effectiveness. In 2016, the POSEIDON (**P**atient-**O**riented **S**trategies **E**ncompassing **I**ndividualize**D** **O**ocyte **N**umber) collaborative group proposed a new metric of success in ART, namely, the ability to obtain the number of oocytes needed to achieve at least one euploid blastocyst for transfer (12). Indeed, transfer of euploid embryos markedly reduces the age-related decrease in implantation rates (13–15), thus making the POSEIDON's marker a pragmatic endpoint for clinicians providing care to infertility patients (16).

A clinical predictive model to estimate the number of oocytes needed to achieve at least one euploid embryo for transfer -and that provides a revised estimate of the probability of achieving this outcome when fewer than the predicted number of oocytes are obtained after one or more oocyte retrieval cycles- would be invaluable for both patient counseling and establishment of a working plan with a clear goal for management. We, therefore, assessed the factors influencing embryo ploidy and estimated the predicted probability of blastocyst euploidy as a function of each mature oocyte retrieved. We used mature oocytes in preference over all oocytes as the former are the gametes with the capacity to support embryo development to the blastocyst stage. Then, we developed an integrative predictive model composed of pretreatment risk factors to estimate the minimum number of mature oocytes needed to achieve at least one euploid blastocyst for transfer, with the aim of offering clinicians and patients a counseling tool at the point of care.

MATERIALS AND METHODS

This cohort study included consecutive infertile couples attending ANDROFERT Fertility Center in Campinas, Brazil, from February 2016 to June 2017. The Ethics Committee of *Instituto Investiga* approved the study (Approval number 1.913.076; CAAE 64291417.0.0000.5599).

Study Population and Patients' Eligibility Criteria

We queried our ART database (ClinisysIVF®) for infertile couples who underwent *in vitro* fertilization/intracytoplasmic sperm injection (IVF-ICSI) treatment with the intention to have trophectoderm biopsy for preimplantation genetic testing for aneuploidy (PGT-A). PGT-A was used for reasons of advanced maternal age, severe male factor infertility, recurrent miscarriage, repeated implantation failure, as well as for patients who were concerned about the euploidy status of their embryos. Eligible patients were all consecutive couples undergoing their first treatment cycle in our Clinic irrespective of the protocol used for ovarian stimulation. We only included patients with a complete IVF/ICSI record. Furthermore, the included patients had at least one mature oocyte retrieved. The mature (metaphase II [MII]) oocytes were inseminated for own use and all resulting viable blastocysts were biopsied.

Women who underwent PGT for balanced translocations or single-gene diseases, polar body biopsy, and PGT on day 3 embryos were excluded. Patients who had treatment involving oocyte donation were excluded. We also excluded patients who had PGT-A on frozen-thawed blastocysts and those whose cycles involved insemination using sperm from different sources (e.g., ejaculated and surgically retrieved sperm) or the use of both fresh and frozen-thawed gametes (e.g., fresh and frozen-thawed sperm or fresh and frozen-thawed oocytes).

Baseline characteristics of couples included female and male age, body mass indexes (BMI), infertility duration,

infertility factor, presence and type of azoospermia, antral follicle count (AFC), anti-Müllerian hormone (AMH) levels, the presence of poor ovarian reserve (POR), and semen parameters. Treatment characteristics included the type of ovarian stimulation, gonadotropin regimen, total gonadotropin dose, sperm source for ICSI, and gamete status for ICSI. Treatment outcomes included the number of oocytes retrieved, number of mature (MII) oocytes retrieved, number of two-pronuclei (2PN) zygotes, number of blastocysts, and number of euploid blastocysts (**Supplementary Table 1**).

Assessment of Infertility Factors and Ovarian Reserve

All included couples were evaluated by both a reproductive endocrinologist and an andrologist as per our institution's protocol. Ovarian reserve was determined by antral follicle count (AFC), which was carried out on the early follicular phase (17), and AMH levels using the modified Beckman Coulter AMH generation II assay (18). A POR was defined according to the Poseidon criteria as AFC < 5 and/or AMH < 1.2 ng/ml (12). Male partners underwent a thorough evaluation, including history, physical examination, semen analysis, hormone profile (serum FSH, LH, and total testosterone), and genetic testing (Yq microdeletions and karyotyping) as appropriate (19). Semen analysis was carried out at our institution's andrology laboratory according to the 2010 World Health Organization manual for the examination of human semen (20, 21). Additionally, assessment of sperm DNA fragmentation (SDF) in fresh ejaculates was carried out in all males, using the sperm chromatin dispersion assay (SCD; Halosperm®; Spain) (22), unless the sperm count was too low for an accurate determination of DNA fragmentation levels. The type of azoospermia was determined by a combination of clinical and laboratory data and confirmed by histological evaluation of testicular biopsy specimens taken during sperm retrieval (23, 24).

Ovarian Stimulation Protocol

Both the conventional antagonist and minimal stimulation protocols were used for ovarian stimulation (OS). In brief, the antagonist protocol involved subcutaneous (SC) administration of recombinant FSH monotherapy (rec-FSH; Gonal-F®, Merck) or rec-FSH combined with recombinant LH (2:1 ratio rec-FSH and rec-LH; Pergoveris®, Merck). Gonadotropin administration started either on day 2 or day 3 of the cycle after confirmation of absence of ovarian cysts by ultrasound scanning, and a flexible GnRH antagonist regimen was initiated by daily SC administration of 0.25 mg cetrorelix (Cetrotide®, Merck) when the leading follicles achieved 12–14 mm in mean diameter, including the day of trigger (25). The minimal stimulation protocol involved the use of either clomiphene citrate or letrozole early in the cycle followed by a low dose of injectable recombinant gonadotropin. The choice of OS regimen and gonadotropin dosage was based on the clinician's assessment of ovarian reserve, female age, and history of previous response to OS. At our institution, minimal ovarian stimulation is reserved for selected POR patients.

Trigger and Oocyte Retrieval

Final oocyte maturation was achieved by SC administration of triptorelin 0.2 mg (Decapeptyl®, Ferring) or recombinant hCG (Ovidrel®, Merck). In general, the criterion for trigger included the presence of two follicles of 17 mm or greater. Oocyte retrieval was carried out under transvaginal ultrasound guidance and intravenous sedation with propofol 35–36 h after triggering.

Laboratory Procedures

The cumulus-corona-oocytes complexes were stripped after exposure to hyaluronidase, classified according to nuclear maturity, and kept in culture at 37°C and 5.5% CO_2 until sperm microinjection (26). The injected oocytes were incubated for 16–18 h at 37°C under 5.5% CO_2 and 5% O_2 until fertilization was confirmed by visualization of 2PN and two polar bodies 16–18 h after insemination. Zygotes were kept in culture to reach the blastocyst stage, and embryo quality was scored according to the criteria described by Gardner (27). Oocyte retrieval, sperm processing, and ICSI were carried out in clean room environments (28).

Trophectoderm Biopsy and Preimplantation Genetic Testing

PGT-A was performed using trophectoderm cells, which were subjected to next-generation sequencing (NGS) analysis of 24 chromosome copy numbers with the purpose of transferring only euploid embryos. In brief, biopsies were performed on embryos that reached the blastocyst stage on days 5–7 by cutting a small piece of trophectoderm (5–10 cells) with the aid of non-contact diode laser (Octax™, MTG, Germany), as previously described (29). The biopsied fragments were immersed into 0.2 mL PCR tubes in a total volume of 2.5 uL of Tris-EDTA Buffer pH 8.0 (ThermoFisher Scientific Baltics, Vilnius, Lithuania), frozen at −20 Celsius degrees, and shipped to Chromosome laboratory (São Paulo, Brazil) for analysis. Specimens were subjected to cell lysis, whole genome amplification (WGA), and construction of libraries using the Ion Reproseq kit (ThermoFisher Scientific, Germany). The DNA quantity was estimated using StepOne (ThermoFisher Scientific, Germany) following the manufacturer's protocol, and NGS was performed using the Ion Torrent PGM™ platform (ThermoFisher Scientific, Germany). Euploidy data analysis was carried out on the Ion Reporter software version 5.2 calibrated at medium sensitivity, using Low-Coverage Whole-Genome workflow. Copy numbers were measured quantitatively, and embryos were classified according to the PGDIS criteria for reporting embryo results (30). Embryos with <20% of abnormal cells were classified as euploids whereas embryos with >80% of abnormal cells were deemed aneuploid. Mosaic embryos were those with abnormal cells ranging from 20 and 80%.

Statistical Analysis

Descriptive statistics were calculated for patient demographics and treatment characteristics. We analyzed the distribution of the number of euploid blastocysts per patient to determine how to model our dataset. Then, we determined the influence of a total of 26 pretreatment and treatment predictors

on this distribution (**Supplementary Table 1**). For this, we used the adaptive LASSO (Least Absolute Shrinkage and Selection Operator) method [31, 32]. Once the predictors were selected, we utilized logistic regression to fit the final model. The binary response was euploidy (yes/no) for each mature oocyte. To assess the effect of predictors on critical intermediate embryonic stages, we conducted separate logistic regression analyses with the binary responses "2PN zygote (yes/no) for each mature oocyte," "blastocyst (yes/no) for each 2PN zygote," and "euploid blastocyst (yes/no) for each biopsied blastocyst."

We made the following assumptions: (i) the embryos are statistically independent concerning the ploidy status, and (ii) the probability of a mature oocyte to reach the blastocyst stage is constant across women, depending only on explanatory variables (predictors) that might affect the response. With these assumptions, the logistic model generates the probability, "p," as an output, where "p" is the probability that any mature oocyte would turn into a euploid blastocyst, given the relevant predictors. The final model was internally validated using the holdout method. The dataset was randomly partitioned in two, i.e., training and validation data sets. The training dataset size was 80% of the total and it was used for the calculations of the fitting; the validation data set was 20% of the total. The quality of the fit was evaluated by the area under the curve (AUC) of the ROC curve. The effect size of predictors on the blastocyst euploidy probability was calculated as the % decrease in blastocyst euploidy.

The probability of a mature oocyte to become a euploid blastocyst, p, was used to compute the minimum number of mature oocytes (n) needed to obtain ≥1 euploid blastocyst, using the formula $n \geq \frac{\log(1-\pi)}{\log(1-p)}$. The probability of success was denoted by π. Its complement, $1 - \pi$, is the risk, i.e., the probability of having no euploid blastocyst despite achieving the estimated number of mature oocytes. The 95% confidence intervals for "p" were obtained from the logistic regression. These limits were introduced in the formula for "n," to generate the corresponding limits of the confidence interval for the required number of mature oocytes. The mathematical operations are valid since the estimators are based on the maximum-likelihood and the functions are monotone. Lastly, we created an online calculator—named "ART Calculator"—to make two types of predictions automatically, using the formula and mathematical equations described above. The first is based on pretreatment predictors to estimate the minimum number of mature oocytes to achieve ≥1 euploid blastocyst for transfer in infertile couples undergoing IVF/ICSI. The second utilizes pretreatment information and the actual number of mature oocytes collected or accumulated to provide a revised estimate of the probability of achieving the aforesaid outcome when fewer than the predicted number of mature oocytes are obtained after one or more oocyte retrieval cycles. Computations were carried out using JMP® PRO 13 (SAS Institute, Cary, North Carolina, US). We adopted an alpha level of <0.05 as significant. The ART Calculator was programmed using Hypertext Preprocessor (PHP) language.

RESULTS

Population Characteristics

A total of 347 patients were included, and their demographics and treatment characteristics are reported in **Table 1**. The mean female age of our selected cohort was 38.9 years (95% confidence interval [CI]: 32.4–42.4 years) with a mean number of mature oocytes retrieved per patient of 6.3 (95% CI: 1.0–12.0). The mean number of blastocysts available for TE biopsy and NGS analysis per patient was 2.1 (95% CI: 0.0–5.0). A total of 2,520 mature oocytes were injected, resulting in 882 blastocysts that were subjected to PGT-A. Overall, the percentage of euploid embryos after NGS in our cohort was 34.8%. The mean number of euploid blastocysts per patient was 0.74 (95% CI: 0.0–2.0). The distribution of the number of euploid blastocysts per woman was found to be the negative binomial (**Supplementary Material**).

Development of Predictive Model

For the selection of variables, the stopping rule on the LASSO procedure was based on the adjusted Akaike Information Criteria (AIC). The model is a generalized linear model. The response is the number of euploid blastocysts. The negative binomial distribution was chosen for the fit. Accordingly, the link function is the logarithm. For the overdispersion, we chose the identity as the link function. The fitted model selected female age, sperm source used for ICSI—in particular, testicular sperm extracted from men with non-obstructive azoospermia (NOA)—, and the number of mature oocytes as predictors (**Supplementary Table 2**). Apart from these variables, no significant association was found between the response variable and all other pretreatment and treatment characteristics (**Supplementary Table 2**).

Female age was to a large extent the most relevant factor for predicting the probability of a blastocyst being euploid given each mature oocyte. The difference in the loglikelihood ascribed to age—adjusted for sperm source—was 30.9 ($df = 2$; $p < 0.0001$). The number of mature oocytes was also significantly associated with the response "≥1 euploid blastocyst," as expected, due to a positive cohort-size effect. This parameter was included in the final model as part of the response variable in association with blastocyst euploidy.

The final predictive model, based on female age and type of sperm used for ICSI, and its correspondent equation is presented in **Table 2**. The estimated predicted probabilities of a mature oocyte turning into a euploid blastocyst decreased progressively as a function of female age and were negatively modulated overall by use of testicular sperm from men with NOA across age (**Figure 1**). The effect size of female age on blastocyst euploidy probability per MII oocyte from year (t) to year (t+1) was defined as the ratio p(t+1)/p(t) × 100. There was a significant decrease ($p < 0.001$) in the probability of a MII oocyte become a euploid blastocyst. The overall yearly reduction in the blastocyst euploidy probability per MII oocyte using ejaculated and testicular sperm were 14.4 and 12.1%, respectively. The loss was progressive with every year of female age but the yearly reduction was not remarkably affected by sperm source (**Supplementary Table 3**).

TABLE 1 | Characteristics of 347 couples and their treatment at first cycle of intracytoplasmic sperm injection (ICSI) and trophectoderm biopsy for preimplantation genetic testing for aneuploidy (PGT-A).

Characteristics	Mean	95% CI
Infertility duration (years)	7	4–10
Female age (years)	38.9	32.4–42.4
Male age (years)	42.4	35.0–53.0
BMI, female (kg/m^2)	24.5	20.3–31.2
BMI, male (kg/m^2)	27.6	23.1–32.3
Infertility factor, *N* (%)		
Male factor	117 (33.8)	-
Unexplained	63 (18.2)	-
Endometriosis	33 (9.5)	-
Endocrine/Anovulatory	26 (7.5)	-
Anatomic/Tubal	10 (2.9)	-
>1 type	98 (28.1)	-
AFC (*n*)	6.7	3-12
AMH (ng/mL)	1.39	0.20-3.00
Semen parameters:		
Sperm count (M/mL)	30.5	0.0–79.8
Total motility (%)	63.4	43.6–76.0
Sperm morphology (%)	2.9	1.0–5.1
DFI (%)	21.6	10.0–43.0
Azoospermia, *N* (%)	65 (18.7)	–
Non-obstructive	44	–
Obstructive	21	
POR, *N* (%)	178 (51.3)	–
Conventional OS; *N* (%):	304 (87.6)	–
rFSH monotherapy	111	–
rFSH+rLH	193	–
Minimal stimulation, *N* (%)	43 (12.4)	–
Total gonadotropin dose (IU)		
Conventional	3,145	1,875–3,300
Minimal	525	315–795
Sperm source for ICSI; N (%):		
Ejaculate	391 (71.5)	–
Epididymis	27 (4.9)	–
Testicle	129 (23.6)	–
Gamete status for ICSI; N (%)		
Fresh, sperm [S] + oocyte [O]	301 (86.8)	–
Frozen-thawed, [S + O]	0 (0.0)	–
Combined, fresh [S] + frozen-thawed [O]	7 (2.0)	–
Combined, frozen-thawed [S] + fresh [O]	39 (11.2)	–
Oocyte and embryo parameters:		
No. Oocytes retrieved	8.2	2.0–16.2
No. Mature (MII) oocytes	6.3	1.0–12.0
%MII oocytes	78.1	50.0–100.0
Fertilized oocytes (2PN)	4.3	1.2–8.2
%2PN fertilization	67.3	33.3–100.0
No. Blastocysts	2.1	0.0–5.0
%Blastulation	48.9	0.0–100.0
No. Euploid blastocysts	0.74	0.0–2.0
%Euploid blastocysts	34.8	0.0–100.0

BMI, body mass index; OS, ovarian stimulation; AFC, antral follicle count; AMH, anti-Müllerian hormone; DFI, Sperm DNA fragmentation index; FSH, follicle stimulating hormone; POR, poor ovarian reserve according to POSEIDON criteria; 2PN, two pronuclei zygote; MII, metaphase II.

Results of logistic regression analyses assessing the effect of predictors on critical intermediate embryonic stages showed that the impact of testicular sperm on the final model depended primarily on its negative effect ($p < 0.0001$) on the probability of obtaining a 2PN zygote per mature oocyte. This effect was independent of female age (**Supplementary Material**). The overall geometric mean of the reduction in the probability of having a 2PN zygote per MII oocyte by use of testicular sperm over ejaculated sperm was 17%. By contrast, testicular sperm alone had no significant effect on the probability of a 2PN zygote turn into a blastocyst, and the effect was only marginal ($p = 0.07$) on the probability of having a euploid blastocyst per biopsied blastocyst. However, when associated with female age, testicular sperm had a significant negative effect on the probability of having a euploid blastocyst (per biopsied blastocyst) ($p < 0.0001$). In this case, the overall female age-adjusted geometric mean of the reduction in the probability of having a euploid blastocyst (per biopsied blastocyst) by using testicular compared with ejaculated sperm was 24%.

Unlike testicular sperm, female age had no significant effect on the probability of having a 2PN zygote per mature oocyte, but it affected the chances of having both a blastocyst per 2PN zygote ($p = 0.003$) and, more importantly, a euploid blastocyst per biopsied blastocyst ($p < 0.0001$) (**Supplementary Material**).

Model Validation and Performance

The model validation was carried out using the holdout sampling method. The AUCs obtained from the fitted model on both datasets—training and validation—were virtually identical, thus confirming that our model was internally validated (**Supplementary Material**). The predictive ability of the model assessed by the area under the ROC curve was 71.6%.

Development of Calculator

Using the probabilities generated by our model in conjunction with the formula $n > \frac{\log(1-\pi)}{\log(1-p)}$, we created an online calculator to compute the minimum number of mature oocytes needed to obtain ≥1 euploid blastocyst automatically, which can be used at the point of care as a counseling tool and potentially influence decision and management. The calculator computes the value of "p," given the female age and sperm source. Then, given the value of the accepted risk, that is, 1-π, it uses the formula to compute the minimum number of mature oocytes and its associated uncertainty (95% confidence interval). Pretreatment, the calculator allows the user to set the probability of success and generates the minimum number of mature oocytes needed for at least one euploid blastocyst accordingly. The higher the required probability of success (lower risk), the higher the number of mature oocytes needed to achieve the intended goal. Posttreatment, the calculator estimates the probability of achieving at least one euploid blastocyst when fewer than the predicted number of mature oocytes are obtained after one or more oocyte retrieval cycles. The online calculator is available at https://members.groupposeidon.com/Calculator/.

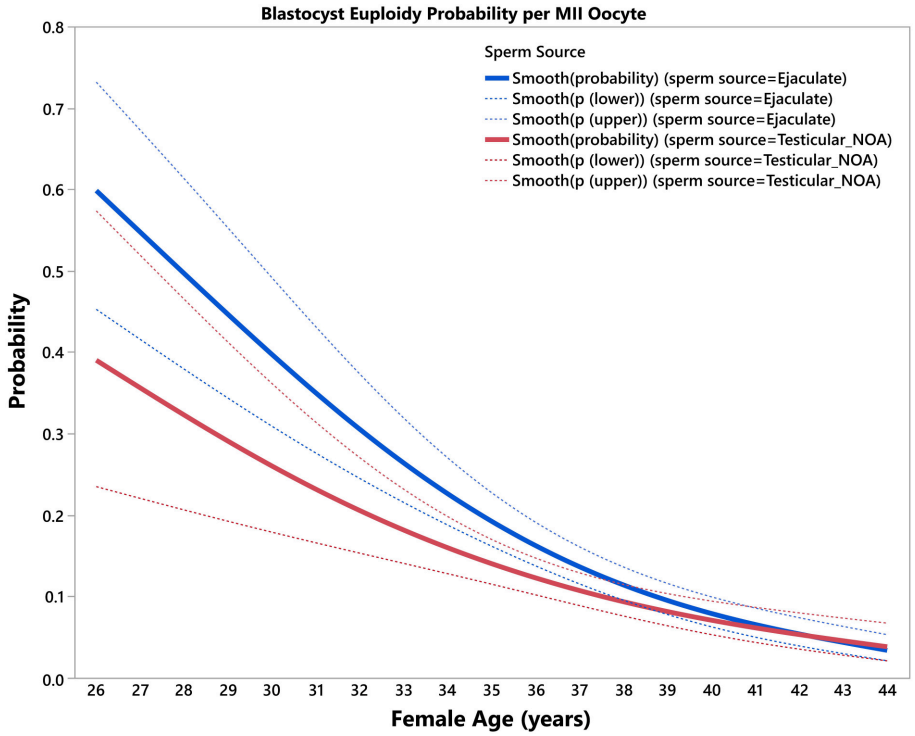

FIGURE 1 | Blastocyst euploidy probability per mature (MII) oocyte. The plots show the probability of a MII oocyte turn into a euploid blastocyst as a function of female age. The estimated probabilities (solid curves) and their 95% confidence interval (dotted curves) are presented according to sperm source to be used for IVF/ICSI, namely, ejaculated sperm (blue) and testicular sperm extracted from patients with non-obstructive azoospermia (NOA) (red). The relations are non-linear and characterized by a differential modulatory effect of sperm source across age (see text).

Examples of Predicting the Individualized Number of Mature Oocytes Needed for Achieving ≥1 Euploid Blastocyst for Transfer

As an example, for a probability of 80% of success set by the user, i.e., 20% risk of having zero euploid blastocyst, a patient of 37 years-old undergoing IVF/ICSI who will use ejaculated sperm from her partner needs a minimum of 11 (confidence interval: 9–13) mature oocytes to obtain at least one euploid blastocyst for transfer (screenshot, **Figure 2**). The computation means that this predicted number of mature oocytes has a chance of 80% of success (or 20% risk of failure) in achieving at least one euploid blastocyst. By contrast, if the same patient utilizes testicular sperm for ICSI from a partner with NOA, the minimum number of mature oocytes will be 14 (confidence interval: 11–17), assuming the same probability of success. If this hypothetical patient had seven mature oocytes collected, then the revised estimates concerning the probability of having at least one euploid blastocyst would be ∼64 and 55% for ejaculated and testicular sperm, respectively (screenshot; **Figure 3**).

Using another example, for a probability of 90% of success set by the user, i.e., 10% risk of zero euploid blastocyst, a patient of 30 years-old will need a minimum of 4 (band interval: 3–6) mature oocytes to obtain at least one blastocyst for transfer by use of ejaculated sperm for ICSI. The predicted number of mature oocytes will be 7 (confidence interval: 5–11) if testicular sperm is used. In this case, the prediction indicates a chance of 90% of success in achieving at least one euploid blastocyst. Like the previous case, the revised probability of having at least one euploid blastocyst can be obtained. If she then had 3 mature oocytes collected, the revised estimates concerning the probability of having at least one euploid blastocyst would be 78 and 55% for ejaculated and testicular sperm, respectively. **Figures 4**, **5** depict the probability curves to obtain at least one euploid blastocyst according to the number of mature oocytes for different age groups and sperm sources.

DISCUSSION

The primary goal of our prediction model was to allow the development of a calculator to provide individualized pretreatment estimates concerning the number of mature oocytes needed to achieve ≥1 euploid blastocyst for transfer in infertile couples undergoing IVF/ICSI treatment. We found that the age of the woman was by far the most critical predictor for the likelihood of achieving ≥1 euploid blastocyst for transfer. Aside from woman's age, sperm source for ICSI, in particular testicular sperm obtained from men with non-obstructive azoospermia, resulted in a response lower than with the use of ejaculated sperm for all female ages. Based on

TABLE 2 | Final model for pred (p) of euploid blastocyst per mature (MII) oocyte.

Equation
$Y = a + b$ [Sperm = "Ejaculate"] $+ c$ [Sperm = Ejaculate](FemaleAge-38.9066) $+ d$ [Sperm=Testicular_NOA](FemaleAge-38.9066),
where $p = \left(\frac{1}{1+e^{-y}}\right)$

Term	Estimate	SE	Wald ChiSquare	Prob >ChiSquare
(Intercept)	−2.6518	0.1174497	371.96	<0.0001
spermSource [EJACULATE]:(ageFemale-37.9384)	−0.2045457	0.0269435	57.63	<0.0001
spermSource [TESTICULAR_NOA]:(ageFemale-37.9384)	−0.1530924	0.0354465	18.65	<0.0001
spermSource [Ejaculate]	0.2231659	0.1174497	3.61	0.0574
Statistics: Response: euploid blastocyst given MII oocytes Distribution: binomial Estimation method: Nominal logistic fit Mean model link: Logit Area under the curve: 0.71589				

The Nominal Logistic Fit was the final model with the best prediction of the probability of ≥1 euploid blastocyst per mature (MII) oocyte. The full equation is written at the top of the table. Each particular characteristic is displayed with an associated P-value (Prob >ChiSquare) giving the indication of how much weight each variable will contribute to the predictive number of mature oocytes. a, intercept; b, spermSource [EJACULATE]; c_v, spermSource [EJACULATE]:(ageFemale-37.9384), d, spermSource [NOA]:(ageFemale-37.9384); SE, standard error.

these variables, we developed a predictive model to estimate the individualized probability of blastocyst euploidy per each mature oocyte retrieved. Our results indicate that the estimated probability of a mature oocyte turn into a euploid blastocyst decreases progressively with aging, and sperm source exerts a modulatory effect. Specifically, the use of testicular sperm from men with NOA negatively modulates the probability of a mature oocyte become a euploid blastocyst overall. After model internal validation, we developed a mathematical equation to compute the individualized minimum number of mature oocytes needed to achieve at least one euploid blastocyst for transfer based on the predicted probabilities. Lastly, we created a calculator to make these computations automatically.

Our foremost motivation for conducting this study was to develop a clinical tool to objectively estimate the POSEIDON's marker of success in ART, namely, "the ability to retrieve the number of oocytes needed to obtain at least one euploid embryo for transfer in each patient" (12, 31). The number of oocytes needed to achieve at least one euploid embryo is a logical endpoint that could help clinicians to both counsel their patients more effectively and plan treatment with the mindset to achieve the individualized oocyte number (16, 32). Although live birth rate (LBR) is the preferable endpoint in couples undergoing ART, it depends on a multitude of controlled and uncontrolled factors, thus making it challenging for individualized predictions about the number of oocytes needed to achieve the desired outcome.

Our model relied essentially on analysis of an ICSI dataset from infertile couples who have undergone PGT-A using NGS analysis. This design seems ideal as the outputs of the whole IVF process were obtained for analysis. We used ≥1 euploid blastocyst as a dependent variable due to the importance of such variable for ART success. Indeed, ∼50–60% of euploid blastocysts implant across all age categories, thus indicating that availability of a euploid blastocyst for transfer may offset to a great extent the adverse effect of increased female age on pregnancy success (14). Currently, analysis of embryo genetic status is carried out by a variety of methods using blastocyst trophectoderm cells, which largely replaced fluorescence *in situ* hybridization (FISH) analysis of cleavage-stage blastomere cells, as they provide reliable information on the copy numbers of all 24 chromosomes. Among the existing methods, recent reports suggest that next-generation sequencing (NGS) has the highest accuracy (13–15). Euploidy rates by NGS between trophectoderm cells (TE) and embryo inner cell mass (ICM) are similar, with a low (∼3%) rate of clinically relevant non-concordance between a mosaic TE and a euploid ICM (33).

Importantly, our prediction tool does not imply by any means that PGT-A should be carried out routinely. Naturally, we included only cycles with PGT-A because the model development was based on the dependent variable "euploid blastocyst ≥1." Therefore, information about blastocyst genetic status had to be available for calculating the probability that a mature oocyte would become a euploid blastocyst. Clinicians willing to use the ART Calculator do not have to provide PGT-A data nor do they need to offer PGT-A to their patients unless they wish to confirm the results of the ART Calculator in their settings.

We also used mature oocytes as a response variable since these are the gametes with the capacity to support embryo development to the blastocyst stage and live birth. In ART, ovarian stimulation using exogenous gonadotropins is routinely applied to promote the growth of multiple follicles. Human chorionic gonadotropin (hCG) or GnRHa are the commonly used agents for triggering final oocyte maturation, which can be administered alone or combined in different dose schemes (34). Following trigger, immature "metaphase I" oocytes progress to the mature "metaphase II" stage of development (35). During this process, the first polar body is extruded, thus allowing diploid cells to turn into haploid gametes that attain competence for fertilization by spermatozoa. After the trigger, oocyte retrieval should be precisely timed to enable the effective retrieval of mature oocytes. However, several issues might affect the proportion of mature oocytes available for fertilization. As

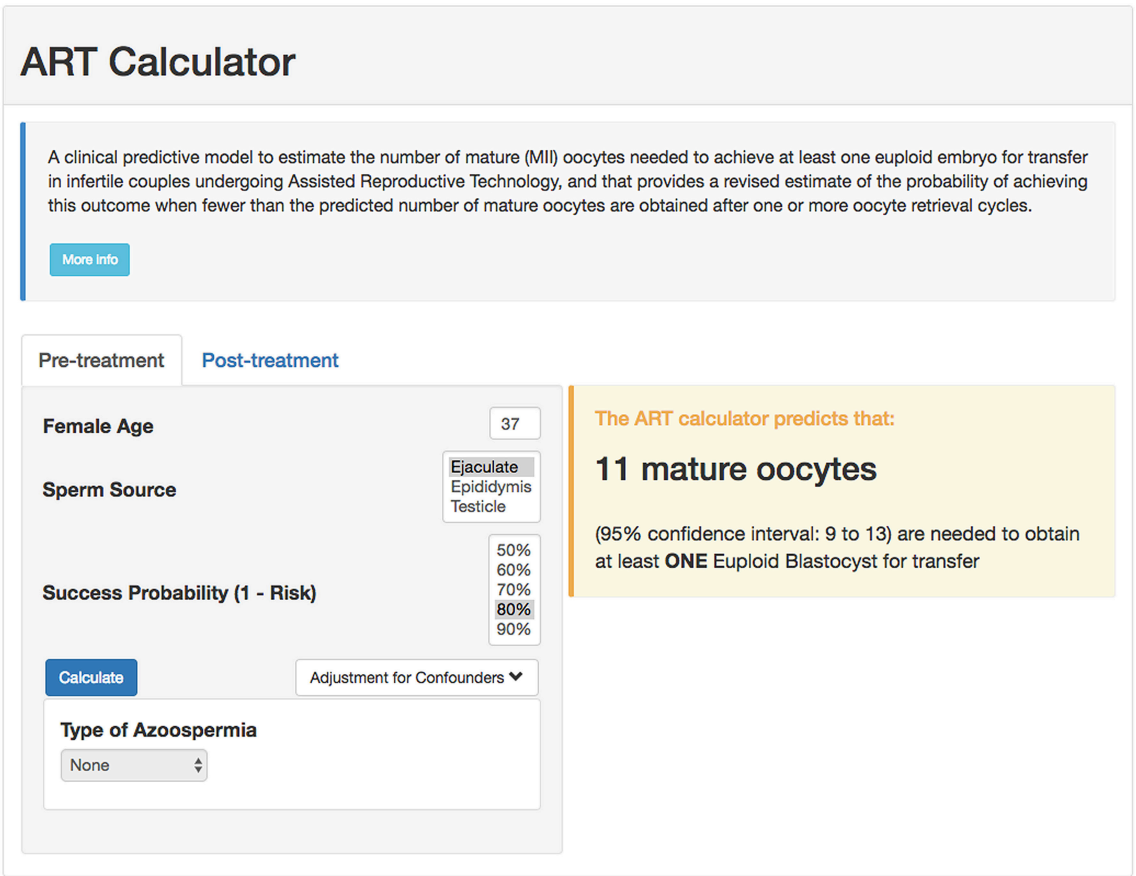

FIGURE 2 | Online calculator to determine the minimum number of mature oocytes required to obtain at least one euploid blastocyst for transfer in infertile patients undergoing IVF/ICSI cycles. The figure shows how the online calculator can be used in an office-based setting. Pretreatment, clinicians should input the patient age and the sperm source to be used for IVF/ICSI. If the option "Testicle" is marked, then the type of azoospermia should be also defined. The probability of success is set by the user and indicates the chance of having ≥1 euploid blastocyst when the predicted number of mature oocytes is achieved. Its complement is the risk, that is, the chance of having no (zero) euploid blastocysts when the predicted number of oocytes is achieved. Once the button "calculate" is pressed, a text box will pop-up on the right side of the screen, indicating the predicted minimum number of mature oocytes needed for obtaining at least one euploid blastocyst, with its 95% confidence interval.

examples, short duration of OS, reduced follicle size on day of trigger, short time interval between trigger and oocyte retrieval, and patient errors in timing or injection technique, as well as problems in absorption, can contribute, alone or combined, to reduced mature oocyte output (36, 37). In our study, we avoided these confounding factors by modeling predictors as a function of mature oocytes to increase the generalizability of our prediction model.

Interpretation

Not surprisingly, we found that female age was the most important variable to predict the likelihood of embryo euploidy, thus corroborating previous reports (29, 38, 39). In a recent study, we estimated the age-related decrease in the probability of blastocyst euploidy—calculated per biopsied blastocyst—using NGS data from fresh trophectoderm human cells (29). We observed that the geometric mean of the yearly decrease variation in the probability of a blastocyst being euploid was 13.6%, but the effect was progressive with every year of female age, varying from 1.2% in women below the age of 30 to over 15% in those older than 39 years. In the study mentioned above, we also found that blastocyst cohort size had an impact on the likelihood of having at least one euploid embryo for transfer across all age groups. The present study confirms these findings using mature oocytes. Indeed, with aging, there is an increase in both oocyte chromosomal abnormalities and cytoplasmic dysfunctions, as well as a progressive reduction in the number of primordial follicles (40). As a result, both embryo quantity and quality are reduced, thus explaining the reasons why IVF success is lower in older women than in younger counterparts (41).

The source of sperm used for ICSI also affected the chances of achieving ≥1 euploid blastocyst for transfer in infertile couples undergoing ART. In particular, we found that use of testicular sperm extracted from men with NOA had a negative modulatory effect. However, the effect of sperm source on the blastocyst euploidy probability per mature oocyte was markedly dependent on female age. Despite significant in younger patients, the impact of testicular sperm from men with NOA was virtually offset in

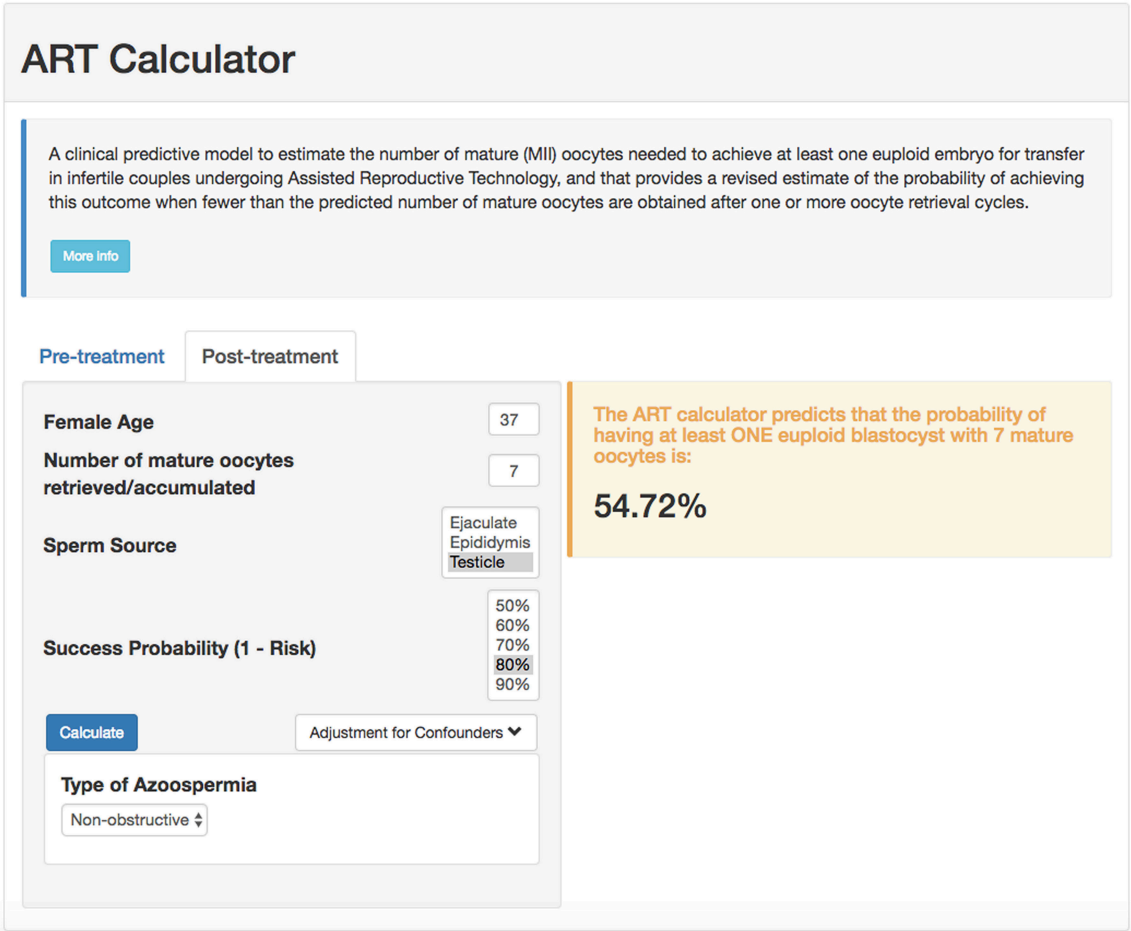

FIGURE 3 | ART online calculator. The figure shows how the online calculator can be used posttreatment, i.e., when fewer than the predicted number of mature oocytes are obtained after one or more oocyte retrieval cycles. Clinicians should input the pretreatment information and the actual number of mature oocytes collected or accumulated. The probability of success is set by the user; it reflects the chance that the estimation is correct given the number of oocytes input. Once the button "calculate" is pressed, a text box will pop-up on the right side of the screen, indicating the predicted probability of achieving ≥1 euploid blastocyst with the number of mature oocytes available.

women of 40 years and over. According to our results, the chances of mature oocytes turning into euploid blastocysts are below 8% in such patients and are negligible after the age of 44. These observations indicate that in these patients the negative influence of age on embryo quality is so dramatic that it cannot be changed further by any factor, including the sperm source. By contrast, ejaculated sperm, epididymal or testicular sperm from men with obstructive azoospermia (OA), and testicular sperm from non-azoospermic men with high sperm DNA fragmentation had no apparent adverse effect on the number of euploid blastocysts. Our results are consistent with previous reports which showed that pregnancy success by ICSI is differentially affected by both sperm source and type of azoospermia (23, 26). In general, men with NOA who have their sperm used for ICSI are at a reproductive disadvantage (23). The reasons are not entirely known but might be related to the fact that testicular specimens from NOA men have higher rates of DNA fragmentation and aneuploidy than both ejaculated and epididymal/testicular counterparts from other male infertility categories (42, 43). Hence, critical embryonic stages might be affected by using such sperm for ICSI, including zygote and embryo development, thus decreasing both the number and genetic quality of resulting blastocysts (44).

In the present study, we showed that the likelihood of obtaining ≥1 euploid blastocyst depended on the number of retrieved mature oocytes. These findings confirm previous observations showing that the proportion of IVF/ICSI patients with at least one euploid blastocyst for transfer depends on female age and blastocyst cohort size (29, 38, 39). Moreover, they are consistent with the overall positive association between oocyte number and delivery rates (45, 46), especially when the cumulative live birth rates are computed (47). Our results indicate that for any given probability of blastocyst euploidy, the higher the number of MII oocytes the higher the chances of having at least one euploid blastocyst within the patient embryo cohort, an effect that was modulated by female age and sperm source used for ICSI. Measuring the effect size of predictors, we

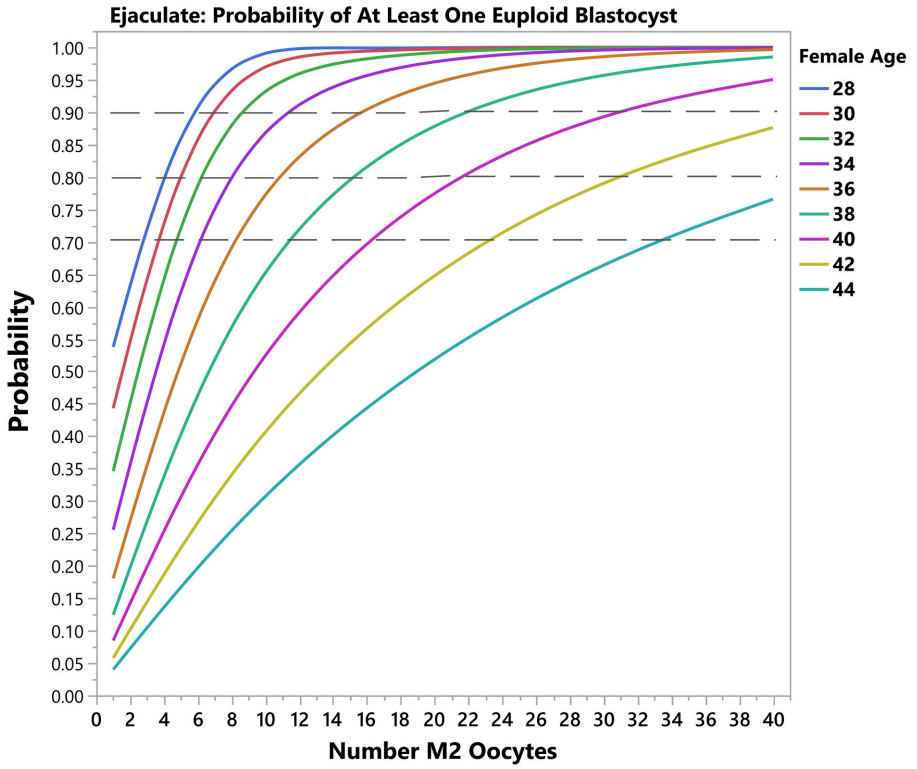

FIGURE 4 | Predictive model output (Ejaculated sperm). The plots show the predicted probability of having ≥1 euploid blastocyst oocyte according to the number of mature oocytes. Each solid curve represents a female age category. The dotted reference lines indicate the 70, 80, and 90% bands for achieving the desired outcome.

found that the blastocyst euploidy probability was reduced by approximately 14% and 12% for every year of female age overall when ejaculated and testicular sperm were used, respectively, but the magnitude of loss was differentially affected by age (**Supplementary Table 3**). A 30-year-old patient will lose about 10% in this probability in a year, whereas the loss is about 1.5x higher in a patient aged 40. In mathematical terms, although a euploid blastocyst may be achieved in women older than 40 at the expense of high oocyte numbers (**Figures 3**, **4**), this may be unrealistic in clinical practice as well as prohibitively costly. Indeed, it has been suggested that the added benefit of increasing the number of oocytes in women older than 41 using current therapeutic strategies is limited, and should be discouraged in women older than 43 years (48).

Clinical Importance

To our knowledge, this is the first pretreatment model to estimate the individualized number of oocytes needed to obtain at least one euploid blastocyst for transfer in infertile couples undergoing IVF/ICSI. By converting our model into a calculator, healthcare providers can estimate such numbers automatically. Our model is primarily intended to be a counseling tool for shaping expectations of couples before embarking on ART. However, it may also be used to help clinicians design individualized patient-oriented treatment strategies aiming at obtaining the number of mature oocytes needed for achieving ≥1 euploid blastocyst for transfer. For example, assuming a risk of 20%, the minimum number of mature oocytes for at least one euploid blastocyst in a couple whose woman is aged 37 and the male partner has viable sperm in his ejaculate varies from 9 to 13. This goal is feasible to achieve using individualized conventional OS in women with normal or high ovarian reserve, unlike in poor ovarian reserve patients (32, 49, 50). In the latter, the clinician might consider alternative OS protocols involving oocyte or embryo accumulation (51, 52). By contrast, given the conditions as above, the predicted number of mature oocytes varies from 2 to 4 in a young patient of 30 years-old. In such a case, even in the presence of low ovarian reserve, the clinician might achieve the intended goal using a single OS cycle and thus advise the patient accordingly. Along the same lines, in patients with adequate pre-stimulation ovarian parameters who had a suboptimal ovarian response in a previous cycle of conventional OS (e.g., Poseidon's groups 1 and 2), estimating the individualized oocyte number might help clinicians to explore pharmacological interventions aimed at increasing the oocyte yield (49, 50, 53). In older patients with low ovarian reserve, the predicted oocyte number might be tough to achieve even after using the best OS protocol and multiple oocyte retrievals, especially when the partners have NOA and testicular sperm are to be used for ICSI. In such cases, our model allows patients and clinicians to make informed decisions based on the predicted number of oocytes needed to obtain at least one euploid blastocyst for transfer. Along the

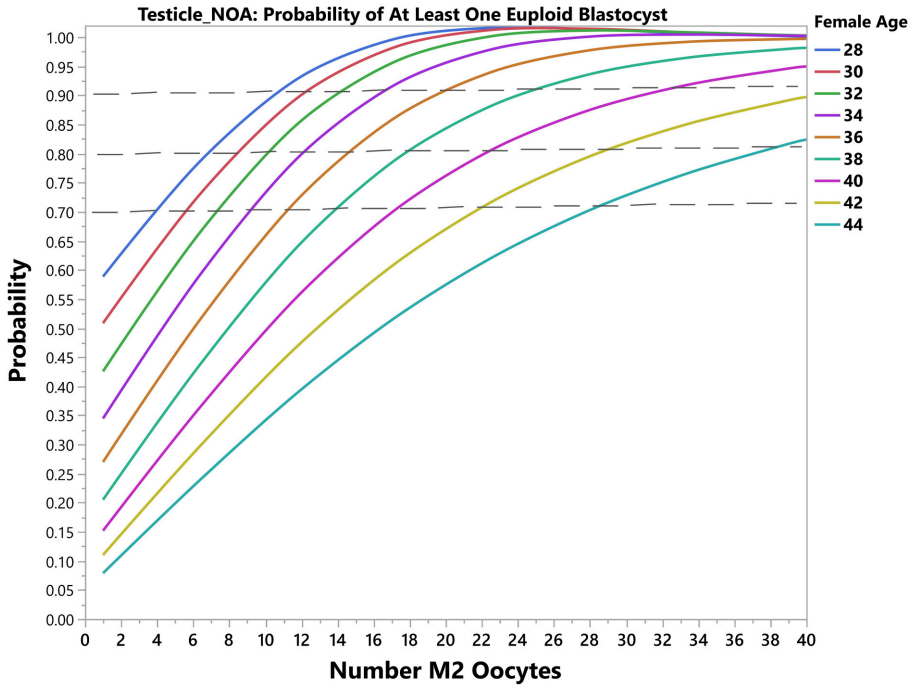

FIGURE 5 | Predictive model output (Testicular sperm from non-obstructive azoospermic [NOA] men). The plots show the predicted probability of having ≥1 euploid blastocyst oocyte according to the number of mature oocytes. Each solid curve represents a female age category. The dotted reference lines indicate the 70, 80, and 90% bands for achieving the desired outcome.

same lines, posttreatment, i.e., after the retrieval of less than the predicted number of mature oocytes, the ART calculator provides invaluable information about the likelihood of achieving a euploid blastocyst, thus allowing transparent discussion and shared-decision making.

According to our model, in women of the same age, the probability of a mature oocyte turn into a euploid blastocyst is reduced if testicular sperm from a partner with NOA were used for ICSI. The aforesaid negative effect of testicular sperm was also noted when intermediate responses were analyzed separately, in particular, the "2PN zygote probability per mature oocyte," and to a lesser extent the "blastocyst euploid probability per biopsied blastocyst." This means that the observed effect of testicular sperm on blastocyst euploidy is due to a combined adverse effect across critical embryonic steps, mainly the fertilization stage. As a result, the final number of blastocysts available for transfer is reduced, thus affecting the likelihood of having at least one euploid blastocyst within the patient embryo cohort. Thus, in such cases, the number of mature oocytes has to be adjusted to account for the loss during the IVF process. Notably, our data indicate that the negative effect of testicular sperm was only remarkable in ICSI cycles involving men with NOA, corroborating other reports (44). By contrast, the use of testicular sperm from men with obstructive azoospermia or non-azoospermic patients with high DFI was not associated with the probability of blastocyst euploidy per mature oocyte. Indeed, previous reports indicate that in these cases testicular sperm perform optimally for ICSI (54–56). The possible reasons for lack of any detrimental effect by use of testicular sperm from men with high DFI and OA are that these cells have lower sperm DNA fragmentation rates than ejaculated counterparts (55–57). Moreover, unlike NOA, spermatogenesis in men with OA is not disrupted (24, 54).

On the other hand, female age had no significant effect on the probability of a MII oocyte turn into a zygote. However, the age of the woman markedly affected the subsequent embryonic stages, in particular, the probability of a blastocyst turning into a euploid blastocyst, thus indicating that the age-related decrease in the probability of each mature oocyte turning into a euploid blastocyst is intrinsically related to both oocyte and embryo quality (40). In women aged 40 years and over, in whom the impact of age on oocyte and embryo quality is so remarkable, the negative effect of testicular sperm on the blastocyst euploidy probability per mature oocyte is virtually lost (**Figure 1**).

Strengths and Limitations

Many studies produced models to predict live birth after a single or multiple IVF/ICSI cycles (11, 58–60). However, no model like ours exists to predict the minimum number of mature oocytes needed to achieve at least one euploid blastocyst for transfer. Although models predicting live birth are useful for counseling purposes, they do not provide a target goal for clinical management. In contrast, our predictive model is intended to serve both as a useful clinical tool for counseling infertile couples and to guiding clinicians to most optimally treat the patient with the mindset to achieve the individualized oocyte number.

Another study has suggested that in addition to female age, ovarian biomarkers, in particular, AMH, could influence the chances of obtaining euploid embryos (39). In this study, the authors used a univariate regression analysis to identify variables with a tendency of association with the primary outcome. Then, these variables were included in the multivariate analysis, which showed that female age and AMH were independently associated with the rate of euploid blastocysts. However, information about how regression analyses were modeled concerning the distribution of the number of euploid blastocysts and the impact of the source of sperm and type of azoospermia were not available.

A critical question when developing predictive models is to determine the variables that best describe the response variable. We have chosen the LASSO statistical method because the procedure allows for simultaneous estimation and variable selection by applying a shrinking (regularization) process that penalizes the coefficients of the regression variables (61). As a result, it removes not only redundant variables but also discovers relevant predictive variables, thus minimizing prediction error. Internal validation showed that the predictive ability of our model was accurate, thus confirming previous observations that the LASSO method is a powerful tool for selecting a reduced number of explanatory variables to describe a response variable (62, 63). The method is, therefore, advantageous as it not only makes the model easier to interpret but also enables algorithms to work faster and reduce overfitting. Furthermore, we assessed the distribution of the number of euploid blastocysts, and here we report for the first time that this distribution follows a negative binomial. Applying the correct distribution is critical to most optimally select the model for statistical analysis; if a wrong assumption concerning the response variable is taken, the generalizability of the prediction model is undermined (64).

Since our model was developed using retrospective data from a single ART Clinic, there is a need to validate its prediction ability externally to confirm generalizability. Along these lines, our estimations cannot be generalized to IVF patients undergoing cleavage-stage embryo transfer as our study is based on blastocyst biopsies and NGS analysis. Also, we did not assess the accuracy of our estimations using other genetic analysis platforms. Lastly, the effect of cycle number and other OS regimens were not analyzed. Our model should be used with caution to decide whether a patient should undergo fertility treatment.

Future Research

External multi-center validation is currently ongoing using suitable ART datasets from different countries. If required, model calibration using external datasets will be carried out as a means to increase performance and generalizability.

CONCLUSION

We developed an internally validated pretreatment model to predict the minimum number of mature oocytes needed to obtain at least one euploid blastocyst for transfer in infertile couples undergoing IVF/ICSI. The model was used to create a novel calculator to make the predictions automatically. This tool will help healthcare providers to counsel infertility patients concerning the individualized oocyte number needed to optimize the chances of having a euploid blastocyst for transfer, thus shaping patients' expectations. Also, the model may have utility to guide clinicians on a risk-shared decision analysis about ART treatment options aimed at achieving the individualized oocyte number, although further external validation is required.

AUTHOR CONTRIBUTIONS

SE designed and coordinated the study. JC carried out the statistical analyses and helped with data interpretation. FB coordinated data collection and extraction, helped with the study design and data interpretation. JS coordinated the development of the ART Calculator prototype and its hosting platform. All authors contributed to drafting and critical discussions, revised, and accepted the final 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/fendo.2019.00099/full#supplementary-material

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Commentary: A Novel Predictive Model to Estimate the Number of Mature Oocytes Required for Obtaining at Least One Euploid Blastocyst for Transfer in Couples Undergoing *in vitro* Fertilization/Intracytoplasmic Sperm Injection: The ART Calculator

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Keywords: mathematical model, age effect, mature oocytes, blastocyst transfer, euploidy

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A Commentary on

A Novel Predictive Model to Estimate the Number of Mature Oocytes Required for Obtaining at Least One Euploid Blastocyst for Transfer in Couples Undergoing *in vitro* Fertilization/Intracytoplasmic Sperm Injection: The ART Calculator
by Esteves, S. C., Carvalho, J. F., Bento, F. C., and Santos, J. (2019). Front. Endocrinol. 10:99. doi: 10.3389/fendo.2019.00099

As elaborated in the excellent paper by Haahr et al. (1) the POSEIDON group 3 (POR patients below the age of 35 years) represent a much easier to treat entity than their older counterparts. In general their chance of producing aneuploid embryos is considerably lower than in women of older age. According to Franasiak et al. (2) the aneuploidy rate identified on the basis of 221 trophectoderm biopsies is 31.3% at the age of 34 years, increasing steadily to over 80% at age 43 and onwards. Therefore, the likelihood of transferring a euploid embryo is high in POSEIDON group 3 patients, even in cases where only two embryos develop from fertilized oocytes. This nevertheless underlines the importance of maximizing the number of good quality mature oocytes by choosing the best individual stimulation approach possible (3). Because there may be considerable high individual variation in the rate of oocyte aneuploidy and resulting embryos even in young patients as has been shown by Minasi et al. (4) it may be worth to clarify the situation of chromosomal problems in the oocytes at an early stage of treatment—i.e., during the first treatment cycle. In countries where embryo biopsy is legally not permitted (like Germany) this can be achieved by performing biopsies on the two polar bodies from normally fertilized oocytes. This will cover only the maternal contribution to chromosomal mal-distribution which nevertheless represents the vast majority of these problems. The high concordance rate of polar body results and the chromosomal constitution of the corresponding oocytes has been well-documented (5). If the results for an individual patient show normal-for-age aneuploidy rates subsequent therapies can focus on optimization of oocyte yield while PGT-A may be added as an adjunct technology for cases identified to have higher rates to spare the patient unnecessary transfers or spontaneous abortions. Application of polar body genetic

analysis in patients of POSEIDON groups 3 and 4 with high aneuploidy rates has the additional advantage to allow for fresh transfer of identified euploid embryos in the same cycle thus avoiding the risk of losing precious material during freezing and thawing procedures, and also avoiding the need for prolonged culture to the blastocyst. This may facilitate even POR patients of younger age to shorten the time-to-pregnancy or rather time-to-Live-Birth.

The paper by Haahr et al. (1) is presently the best available guidance for the clinician faced with patients presenting with reduced ovarian reserve to individually tailor the approach to therapy to offer the maximum chance for pregnancy and birth. The additional detailed presentation of information on adjuvant therapies opens the path for further clinical research about their relevance in improving the perspective for all POR patients. Especially for women meeting the POSEIDON group 3 criteria this is the perfect assistance to enable the achievement of live birth rates above 20% by taking the best possible path from the very beginning of the treatment.

AUTHOR CONTRIBUTIONS

VB preparation of manuscript. RF revision and completion. All authors contributed to the article and approved the submitted version.

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Response: Commentary: A Novel Predictive Model to Estimate the Number of Mature Oocytes Required for Obtaining at Least One Euploid Blastocyst for Transfer in Couples Undergoing In Vitro Fertilization/ Intracytoplasmic Sperm Injection: The ART Calculator

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A Commentary on:

Commentary: A Novel Predictive Model to Estimate the Number of Mature Oocytes Required for Obtaining at Least One Euploid Blastocyst for Transfer in Couples Undergoing *in vitro* Fertilization/Intracytoplasmic Sperm Injection: The ART Calculator
By Fischer R and Baukloh V (2020). Front. Endocrinol. 11:618. doi: 10.3389/fendo.2020.00618

We thank Fischer and Baukloh for their commentary (1) on the subject matter of our article concerning a novel predictive model (ART Calculator) to estimate the minimum number of metaphase II (MII) oocytes required to obtain at least one euploid blastocyst for transfer in couples undergoing assisted reproductive technology (ART) (2). We developed the ART calculator to help clinicians objectively estimate the POSEIDON's group metric of success in ART (3, 4). With the ART calculator, the POSEIDON's metric can be estimated without preimplantation genetic testing for aneuploidy (PGT-A).

The model provides two types of predictions. Pre-treatment, it estimates the minimum number of MII oocytes to achieve ≥1 euploid blastocyst for transfer, and post-treatment, it provides a revised estimate of the probability of achieving ≥1 euploid blastocyst when fewer than the predicted number of MII oocytes are obtained (1). In practical terms, the ART calculator's main output is the average minimum number of MII oocytes required for at least one euploid blastocyst, which increases progressively with the female age and is magnified further by the use of testicular sperm from patients with nonobstructive azoospermia. Accordingly, patient-oriented strategies to achieve the

number of oocytes needed to obtain one euploid embryo for transfer may be elaborated to potentially increase success prospects (5, 6).

Following its publication, the ART calculator was validated in a multicenter study involving approximately 1,500 infertile patients subjected to IVF-ICSI and PGT-A (7). The validation study showed that the estimations provided by the ART calculator were strongly correlated with the actual probability of blastocyst euploidy per MII oocyte (r = 0.91) and the minimum number of MII oocytes required to obtain at least one euploid blastocyst (r = 0.88). In both the original and validation studies, female age was the primary factor affecting blastocyst euploidy among infertile couples undergoing ART (2, 7).

Fischer and Baukloh, in their analysis of our study, raised two valid points that warrant further elaboration. First, given the overall concordance of trophectoderm biopsy and polar body biopsy, the ART calculator's estimation might be somewhat extrapolated for determining the number of MII oocytes needed to have at least one euploid MII oocyte; an intriguing hypothesis that warrants further evaluation.

Secondly, and more importantly, the authors discuss embryo ploidy's intraindividual variation, which has relevance for patient counseling and treatment. Indeed, our studies show that there is variation in the probability of blastocyst euploidy within same-age women (2, 7, 8). To account for this variability, the ART calculator provides—in addition to the average minimum number of MII oocytes required for at least one euploid blastocyst—the 95% confidence interval of each estimation. Due to the intrinsic uncertainties in estimations of biological phenomena like embryo ploidy status, the ART calculator also allows users to set the desired probability of success (e.g., 70, 80, 90%) for predicting the number of MII oocytes. For example, according to the ART calculator applying a user-defined 80% probability of success, a patient of 39 years-old undergoing IVF-ICSI with ejaculated sperm will require 16 MII oocytes (95% CI 13–20) to obtain at least one euploid blastocyst for transfer (www.grouposeidon.com). This computation means that the predicted number of MII oocytes has an 80% chance of achieving at least one euploid blastocyst.

Along these lines, a novel finding from our research relates to the hypothesis of statistical independence of embryos concerning ploidy status. It implies that the ploidy status of a given embryo does not affect the probability of another embryo from the same cohort being euploid or aneuploid. In our validation study's dataset, the negative binomial distribution fits the number of euploid blastocyst optimally (goodness-of-fit test: Pearson Chi-square = 4.577; Prob>Chi-square = 0.59; **Supplementary Figure (7)**). In our case, since the P < X2 = 0.59, the null hypothesis—the data come from a binomial distribution—is accepted. This type of distribution is consistent with the hypothesis of statistical independence. Therefore, we suggest that the negative binomial distribution is used for modeling in studies applying logistic regression analysis for assessing the effect of predictors on blastocyst ploidy status.

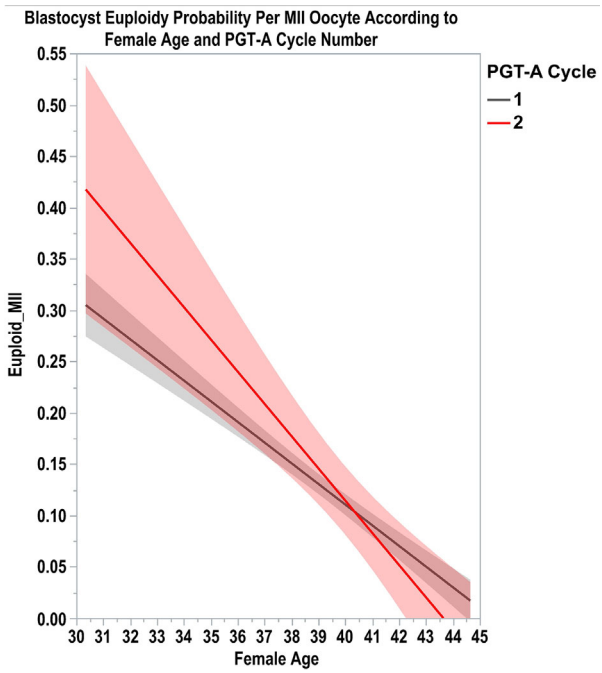

FIGURE 1 | Lines of fit show a linear regression with 95% confidence intervals for continuous X (female age) and Y (euploid blastocyst per MII oocyte) probability according to PGT-A cycle number.

Lastly, Fischer and Baukloh shared their oocyte polar body biopsy and NGS analysis data, which indicate little difference in the number of euploid oocytes among women who had two consecutive PGT-A treatments. We also looked at whether these findings hold for blastocysts. For this, we examined our dataset of 747 consecutive patients subjected to IVF-ICSI with ejaculated sperm and PGT-A by NGS over a two-year period. Logistic regression analysis for the binary response 'euploid blastocyst = yes/no', accounting for age, cycle number, sperm source, paternal age, infertility factor, maternal BMI, and ovarian reserve markers (AFC and AMH) revealed that only female age ($p < 0.0001$) is a relevant predictor of blastocyst euploidy. The probability of blastocyst euploid per MII oocyte progressively decreased with the female age, but it was not affected by whether the patient was on the first or second PGT-A cycle (L-R Chi-square 1.307; $p = 0.52$) (**Figure 1**). A total of 88 patients had two PGT-A cycles within a 6-month period. In this cohort, the PGT-A cycle number did not materially affect euploidy rates and the number of euploid blastocysts (mean ± SE): 34.9% and 0.77 ± 0.05 (cycle 1), and 36.2% and 0.69 ± 0.10 (cycle 2). Our data support the notion that the probability of an MII oocyte becomes a euploid blastocyst is reasonably constant across women, depending only on explanatory variables (predictors) that might affect the response. Moreover, our findings indicate that blastocyst euploidy rates are relatively stable across cycles of the same women within a short time frame.

AUTHOR CONTRIBUTIONS

SE collected the data and drafted the commentary. JC analyzed the data and drafted the commentary. All authors contributed to the article and approved the submitted version.

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Understanding Follicular Output Rate (FORT) and its Implications for POSEIDON Criteria

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The management of low prognosis patients in ART represents a challenge for reproductive specialists. Different profiles and biologic characteristics have been identified among these patients. Indeed, while poor ovarian response can be seen in patients with impaired ovarian reserve, others, identified as hypo-responders, show unexpected poor or suboptimal response to controlled ovarian stimulation despite satisfying ovarian parameters. These hypo-responders are associated during FSH stimulation to slow initial responses in terms of estradiol levels and follicle growth, longer stimulations, and/or greater cumulative FSH doses. Hence, it appears that ovarian sensitivity to gonadotropins differs from a patient to another, and plays a determinant role on ovarian response to stimulation. Although precise mechanisms remain to be elucidated, increasing evidence suggests that ovarian sensitivity to FSH could be influenced by the presence of genetic mutations or single nucleotide polymorphisms of gonadotropins and their receptors. Evaluating ovarian sensitivity to FSH therefore appears as a key element to improve IVF success rates in these low prognosis patients and open new treatment perspectives. Since the traditional ovarian markers currently used are not sufficient to accurately reflect ovarian response to FSH, a tool to assess ovarian sensitivity to gonadotropin stimulation was required. The present review aims to present Follicular Output Rate (FORT) as an efficient quantitative and qualitative marker of ovarian responsiveness to gonadotropins, discuss the underlying mechanisms of impaired sensitivity to FSH and the possible FORT implications for Poseidon criteria.

Keywords: follicular output rate, FORT, POSEIDON criteria, hypo-response, controlled ovarian stimulation, FSH receptor polymorphism

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INTRODUCTION

Mechanisms underlying poor ovarian response (POR) in assisted reproductive technology (ART) remain unclear. As no consensus on the management of poor responders exists, these low prognosis patients represent a challenge for reproductive specialists (1). The Bologna Criteria (2), established in 2011, defined poor responders to controlled ovarian stimulation (COS) by the presence of at least two of the following characteristics: advanced maternal age (≥40 years), a previous incident of POR (cycles canceled or ≤3 oocytes with a conventional ovarian stimulation protocol), and/or a low ovarian reserve tests [antral follicle count (AFC) <5–7 follicles or serum anti-Müllerian hormone (AMH) levels <0.5–1.1 ng/mL]. Although successful in reducing the variability of POR definitions

(3), the Bologna criteria failed to reflect the very different profiles and significantly variable biologic characteristics of these patients (4, 5). Notably, whereas POR can be observed in patients with impaired ovarian reserve, others show an "unexpected" poor or suboptimal response to COS despite satisfying ovarian parameters.

Consequently, the Poseidon classification (6) (with as endpoint the number of oocytes required to obtain at least one euploid embryo), distinguishes patients of low prognosis despite an adequate ovarian reserve (Groups 1 and 2: AFC >5 and AMH >1.2 ng/mL) from those with poor ovarian features (Groups 3 and 4: AFC <5 and AMH <1.2 ng/mL) (7). Patients of Poseidon Groups 1 and 2 show an initial slow response to FSH stimulation in terms of estradiol levels and follicle growth, require longer stimulations, and/or greater cumulative FSH doses despite their correct ovarian parameters (8, 9). Hence, markers currently used (such as AFC and AMH) are not sufficient to predict ovarian response accurately, notably for these "hypo-responders" who raise the question of ovarian sensitivity to FSH (10–12). Other methods are needed to enable identification and optimal counseling for these patients.

The present review aims to present Follicular Output Rate (FORT) as a tool to assess ovarian responsiveness to gonadotropins, discuss the underlying mechanisms of impaired sensitivity to FSH and the possible FORT implications for Poseidon criteria.

MATERIALS AND METHODS

A systematic search was led using the MEDLINE (PubMed), SCOPUS, EMBASE, and Cochrane Library databases, using the following keywords and MESH search terms: "follicular output rate," "FORT," "poor ovarian responder," "poor ovarian response," "POR," "hypo-response," "hypo-responder," "ovarian sensitivity," "Poseidon," "assisted reproductive technology," "ART," "controlled ovarian stimulation," "COS," "COH," "IVF," "ICSI." "FSH receptor," "FSHR," "polymorphism," "LH receptor," "LHR," "pollution AND ovarian sensitivity." All relevant studies (limited to human studies) published before October 2018 were considered, without language restrictions. The reference lists of relevant reviews and articles were also hand-searched.

FOLLICULAR OUTPUT RATE (FORT)

So far, the strength of ovarian response to ovarian stimulation had been analyzed by considering the number of pre-ovulatory follicles obtained at the end of COS (13–16). However, the number of pre-ovulatory follicles obtained does not reliably reflect antral follicle responsiveness to FSH since it is greatly dependent on the number of pre-treatment small antral follicles (17). Similarly, the quantitative relationship between AMH levels and the number of mature follicles and fertilizable eggs observed in certain studies (18–22) may merely result from the positive correlation between AMH levels and pre-treatment number of small antral follicles (23–27), and does not itself attest of the sensitivity to FSH treatment (28). Therefore, identifying an index that considered the number of small antral pre-treatment follicles appeared crucial.

Genro et al. (28) were the first to introduce the concept of FORT in a prospective study of 162 patients. FORT was defined as the ratio of pre-ovulatory follicle (16–22 mm in diameter) count (PFC) on hCG day × 100/small antral follicle (3–8 mm in diameter) count at baseline. Patients (mean age of 34.6 ± 0.3 years) were undergoing COS protocol with a single-dose of time-release GnRH agonist on cycle days 1–3 (3 mg, IM, Decapeptyl, Ipsen Pharma, Paris, France), followed after complete pituitary desensitization had been confirmed, by daily recombinant FSH injections (Gonal-F, Serono Pharmaceuticals, Lyon, France) at a dosage of 300 IU/day for at least 5 days, and continued until the day of hCG. At baseline, women had 14.8 ± 0.3 antral follicles. After treatment, the total number of pre-ovulatory follicles obtained was 6.9 ± 0.2, with a corresponding FORT of 47.5 ± 1.4%. A positive relationship between serum AMH levels and the number of small antral ($p < 0.0001$) and PFC ($p < 0.04$) was observed. PFC tended to be lower in the low-AMH group when compared to the other groups ($p = 0.246$). Interestingly, FORT was negatively and significantly correlated with serum AMH levels, both in univariate and after stepwise regression analysis ($p < 0.001$). FORT values significantly and progressively decreased from the low (AMH < 1.69 ng/mL; $n = 41$), average (AMH 1.69–3.20 ng/mL; $n = 82$), to high (>3.20 ng/mL; $n = 39$) AMH groups. Whereas, FORT was positively associated to total recombinant FSH dose ($p < 0.006$) and duration of COS ($p < 0.001$), it was not significantly associated to age, body mass index, nor to basal estradiol or FSH levels.

Since then, FORT has been confirmed as an efficient quantitative, as well as qualitative, marker of ovarian response during COS. Gallot et al. (17) prospectively analyzed 322 patients who underwent the same protocol as that of Genro et al. (28). Patients were classified into three distinct FORT groups, arbitrarily chosen according to whether FORT values were under the 33th percentile (<42%, low FORT group; $n = 102$), between the 33th and the 67th percentile (42–58%, average FORT group; $n = 123$), or above the 67th percentile (>58%, high FORT group; $n = 97$) of distribution. Similarly, sets of three different groups according to ages, AFC, and PFC values were formed. Initial AFC was of 15.2 ± 0.2 follicles. Overall, FORT was of 50.6% (range, 16.7–100.0%). Coherently with previous results regarding the negative association between AMH levels and FORT (28), most patients (41%) of the low AFC group had a high FORT, while a minority of patients of the high AFC group (20%) belonged to the high FORT group. The number of oocytes and embryos obtained increased progressively from the low to the high FORT groups ($p < 0.001$), irrespective of age and absolute pre-COS AFC and post-COS PFC (**Table 1**). Furthermore, FORT levels were significantly correlated with the percentage of top-morphology embryos ($r = 0.14$; $p < 0.02$). Patients with a larger proportion of FSH-responsive antral follicles had better outcomes after IVF-ET, supporting the hypothesis that scant responsiveness of antral follicles to exogenous FSH reveals some degree of follicle/oocyte dysfunction (31, 32).

Consistently, Zhang et al. (29) observed in a larger cohort of 1,503 non-PCOS patients that the number of retrieved oocytes

TABLE 1 | Results of IVF-embryo transfer and outcome in the low, average, and high FORT groups.

	Low FORT (<33th percentile)	Average FORT (33–67th percentile)	High FORT (>67th percentile)	*p*-value
Gallot et al. (17)				
Antral follicle count	16.6 ± 0.3	15.1 ± 0.4	14.0 ± 0.4	< 0.001
Pre-ovulatory follicle count	5.4 ± 0.1	7.5 ± 0.2	9.7 ± 0.3	< 0.001
Retrieved oocytes	8.6 ± 0.4	10.3 ± 0.4	11.8 ± 0.6	< 0.001
Number of metaphase II oocytes	7.4 ± 0.4	8.7 ± 0.3	10.0 ± 0.6	< 0.001
Total embryos	5.7 ± 0.3	5.9 ± 0.3	7.4 ± 0.4	< 0.002
Implantation rate (%)	23.3	34.4	37.7	< 0.004
Clinical pregnancies/oocyte retrieval (%)	33.3	51.2	55.7	< 0.004
Ongoing pregnancies/oocyte retrieval (%)	23.5	43.9	43.3	< 0.003
Zhang et al. (29)				
Antral follicle count	14.51 ± 6.33	14.00 ± 5.37	12.32 ± 4.28	< 0.001
Pre-ovulatory follicle count	5.49 ± 2.85	8.41 ± 3.27	11.54 ± 4.33	< 0.001
Retrieved oocytes	8.45 ± 5.64	11.52 ± 6.37	13.30 ± 6.34	< 0.001
Total embryos	4.94 ± 3.22	6.37 ± 3.69	7.33 ± 3.89	< 0.001
Good-quality embryo rate (%)	65.98	66.48	68.91	0.033
Implantation rate (%)	29.71	33.80	35.08	0.031
Clinical pregnancy rate (%)	46.27	53.80	55.86	0.012
Hassan et al. (30)				
Antral follicle count	16.7 ± 3.4	14.4 ± 4.5	11.4 ± 3.2	< 0.001
Pre-ovulatory follicle count	6.1 ± 1.6	7.6 ± 2.3	8.9 ± 2.4	< 0.001
Retrieved oocytes	5.4 ± 1.5	6.8 ± 2.3	7.4 ± 2.1	< 0.001
Number of metaphase II oocytes	4.5 ± 1.7	5.6 ± 2.4	6.2 ± 2.0	< 0.001
Fertilized oocytes	2.7 ± 1.5	3.9 ± 2.2	4.4 ± 1.9	< 0.001
Fertilization rate	48.4 ± 21.8	55.3 ± 20.3	57.4 ± 19.2	0.006
Total embryos	2.1 ± 1.3	3.2 ± 2.2	3.6 ± 1.8	< 0.001
Number of good-quality embryos	1.0 ± 1.0	2.0 ± 1.7	1.8 ± 1.4	< 0.001
Transferred embryos	1.4 ± 0.5	1.7 ± 0.4	1.8 ± 0.4	< 0.001
Clinical pregnancy rate (%)	29.9	43.3	57.8	< 0.001

and total number of embryos progressively increased from the low to high FORT groups ($p < 0.001$; **Table 1**). Mean FORT was of 65%. Moreover, Rehman et al. (33) found in a prospective study of 282 patients that an increase in FORT value by one unit was associated to increased mean numbers of oocytes retrieved (β coefficient: 0.135), metaphase II oocytes obtained (β coefficient: 0.128), and fertilized oocytes (β coefficient: 0.089). There was a positive relationship between FORT and clinical pregnancy rates (35.8%), and FORT values were higher in pregnant compared to non-pregnant patients (64.2 vs. 49.3%, respectively, $p = 0.0001$). Hassan et al. (30) reported similar results in a prospective study on 303 women undergoing IVF/ICSI for unexplained infertility. Patients were divided into three groups according to FORT: low FORT ($n = 97$), below the 33rd percentile, moderate FORT ($n = 104$) with values between the 33rd and the 67th percentiles, and high FORT ($n = 102$), above the 67th percentile. There was a progressive and significant increase from low to high FORT groups regarding number of retrieved oocytes (5.4 ± 1.5, 6.8 ± 2.8, and 7.4 ± 2.1, respectively; $p < 0.001$), clinical pregnancy rates (29.9, 43.3, and 57.8%, respectively; $p < 0.001$), and fertilization rates (48.4% ± 21.8 vs. 55.3% ± 20.3 and 57.4% ± 19.2, respectively; $p = 0.006$; **Table 1**). Multivariate logistic regression analysis revealed that the correlation between FORT and pregnancy was independent of potential confounding factors ($p = 0.008$).

MECHANISMS OF HYPO-RESPONSE

Although ovarian hypo-response in ART remains to be elucidated, increasing evidence suggests that the presence of genetic mutations or single nucleotide polymorphisms (SNPs) of gonadotropins and their receptors could influence ovarian sensitivity to gonadotropin stimulation (8, 34, 35).

FSH receptor (FSH-R) is a type of G-protein-coupled receptor that mediates FSH intracellular signals through cyclic adenosine monophosphate pathways (8). Two polymorphisms of FSH-R (Thr307/Asn680 and Ala307/Ser680) have been associated to a higher requirement of exogenous gonadotrophins during COS (36, 37). Perez et al. (36) showed that basal FSH levels were significantly different according to FSH genotype (6.4 ± 0.4 IU/L, 7.9 ± 0.3 IU/L, and 8.3 ± 0.6 IU/L for Asn/Asn, Asn/Ser, and Ser/Ser groups, respectively, $p < 0.01$). The number of FSH ampoules required for successful stimulation was also

significantly different among the three groups (31.8 ± 2.4, 40.7 ± 2.3, and 46.8 ± 5.0 for the Asn/Asn, Asn/Ser, and Ser/Ser groups, respectively, $p < 0.05$).

To better illustrate the relationship between FSH-R and FSH doses, Alviggi et al. (8) conducted a retrospective randomized study in which 17 patients requiring a cumulative dose of recombinant FSH (rFSH) >2,500 UI were compared to 25 patients requiring <2,500 UI. Women requiring more than 2,500 UI of rFSH had significantly longer stimulations ($p = 0.03$), lower serum estradiol levels on hCG day ($p = 0.001$), a lower number of oocytes retrieved ($p = 0.0005$), and a lower number of transferred embryos ($p = 0.001$). Interestingly, the incidence of Ser/Ser genotype was higher in patients requiring greater doses of rFSH ($p = 0.02$). Also, patients with higher rFSH consumption and FSH-R Ser680 variant carriers had a longer infertility condition. Hence, FSH-R Ser680 may affect female fertility and delay pregnancy occurrence (8).

Moreover, the role of FSH polymorphisms on responsiveness to COS treatments was described in a meta-analysis of 33 studies lead by Alviggi et al. (38). Notably, the AA genotype of the FSH-R gene at position−29 has been reported to be associated with poor ovarian response. Achrekar et al. (39) showed in a retrospective analysis that subjects with AA genotype at the−29 position required higher amounts of exogenous FSH ($p = 0.001$), had significantly lower oestradiol concentrations before HCG day compared with the GA genotype ($p = 0.015$), and had a lower number of pre-ovulatory follicles ($p = 0.001$), and a lower number of retrieved oocytes ($p = 0.003$). Additionally, Desai et al. (40) observed that these AA genotype patients significantly expressed lower amounts of FSH-R protein, and that the relative mRNA expression of FSH-R was significantly decreased compared to GG genotype patients ($p = 0.027$). These results could be explained by the fact that DNA with the A allele might be less accessible for binding of transcription factors compared to the G allele.

However, polymorphisms of FSH-R do not seem to influence antral follicle responsiveness to strong FSH doses, as far as it is measurable by the FORT. Genro et al. (41) observed in 124 patients undergoing COS that FORT index were comparable between Thr307Ala and Asn680Ser carriers or non-carriers when stimulated with an initial dose of 300UI. Further studies using lower, yet more discriminating, FSH doses are required to determine whether this lack of difference is due to the intensity of the FSH signal or to a lack of functional relationship between these SNPs of FSHR and follicle reactivity to FSH.

The genotypic profile of LH receptors may also play a role in ovarian hypo-response. In the comparison of three groups undergoing a gonadotrophin-releasing hormone analog long protocol followed by stimulation with rFSH (Group A: 22 women requiring a cumulative dose of rFSH >3,500 IU; Group B: 15 patients requiring 2000–3500 IU; Group C: 23 women requiring <2,000 IU), Alviggi et al. (42) showed that Group A had significantly lower estradiol peaks ($p < 0.05$) and a lower number of oocytes retrieved ($p < 0.05$) (7.3 ± 1.5, 11.7 ± 2.4, and 14.7 ± 4.1 in the three groups, respectively). Seven carriers (31.8%) of v-betaLH were found in Group A, whereas only one variant (6.7%) was observed in Group B and no variant was detected in Group C.

These results were confirmed in a larger series of 220 patients stimulated by rFSH (34). V-betaLH was present in 11% of patients. The study population was divided into two groups according to their LH genotype (wt/wt, $n = 196$; v-betaLH, $n = 24$). Patients with v-betaLH received a significantly higher cumulative-dose of r-hFSH ($p = 0.048$). LH genotype had a statistically significant effect on the cumulative dose of rFSH ($p < 0.01$), showing a progressive increase from wt/wt to v-betaLH heterozygotic and homozygotic women.

As few data exist on the potential influence of pollution on ovarian sensitivity, the role of environmental factors on ovarian response to COS remains to be elucidated. A Chinese study exploring the influence of fluoride exposure on FSH-R gene polymorphism in 679 women suggested that fluoride exposure was associated to lower GnRH serum levels, but no correlation with the FSH-R polymorphism AA genotype at position−29 was observed (43).

CLINICAL IMPLICATIONS

Efficient as a quantitative and qualitative marker of ovarian sensitivity to FSH, FORT index should be used in everyday practice. Considering that only follicles between 16 and 22 mm on hCG day effectively respond to FSH may be a possible limitation of FORT. Smaller follicles might also present some degree of FSH responsiveness. However, as it is also possible that very small follicles, which could not be counted by ultrasound at baseline, may also have begun FSH-driven maturation after the start of COS and reached intermediate sizes on hCG day, the inclusion of average-sized follicles on hCG day into the calculation of FORT could confuse interpretation of the results. FORT is also limited by the technical impossibility to track the development of each follicle individually, and therefore cannot assess the possible differences in the FSH-driven growth of follicles (28).

Other tools such as Ovarian sensitivity index (OSI) (44) have also been suggested as a surrogate of AMH assay in predicting ovarian responsiveness to FSH in IVF. OSI corresponds to the total FSH dose administered divided by the number of retrieved oocytes. However, the interpretation of OSI is limited by the fact that it was obtained using a GnRH-agonist buserelin plus rFSH in a classical long protocol; hence, the correlation between OSI and AMH observed could differ in case of a different stimulation schedule or different drugs. More recently, authors introduced Follicle to Oocyte Index (FOI) (9), defined by the ratio between the total number of oocytes collected at the end of ovarian stimulation and the number of antral follicles available at the start of stimulation. FOI $\leq$ 50 was considered low. Trigger type and efficiency may influence FOI, and further studies are warranted to confirm the use of FOI as a marker of ovarian response.

Assessing ovarian sensitivity to FSH with FORT and understanding mechanisms behind hypo-response in ART opens new possibilities in the treatment of hypo-responders.

Increasing FSH doses has been proposed, notably by Behre et al. (45), who evaluated its effect in patients with Ser680 polymorphism. Patients were randomly assigned to an FSH dose of 150 UI/day or 225 UI/day. The control group (Asn/Asn, $n = 44$) received a dose of 150 UI/day. Peak estradiol levels on hCG day were significantly lower for patients stimulated with 150 UI/day ($p = 0.028$). Increasing the FSH dose from 150 to 225 UI/day overcame the lower oestradiol response in women with Ser/Ser.

The benefit of adding LH in hypo-responders has also been explored (46, 47). Ferraretti et al. (48) randomized women showing a hyporesponsiveness to FSH into three groups, one receiving an increased dosage of FSH ($n = 54$), one receiving administered recombinant LH (rLH) in addition to the increased dose of FSH ($n = 54$), and another was given additional FSH and LH using hMG as a combined drug ($n = 22$). Addition of rLH significantly improved pregnancy, implantation, and live birth rates. Regarding LH doses, the randomization of 46 patients undergoing ovarian stimulation in two groups (supplementation with a daily rLH dose of 75 UI or 150 UI) showed a significant advantage for patients receiving 150 UI in terms of mean number of oocytes retrieved and percentage of mature oocytes, whereas these patients received a significantly lower mean number of rFSH vials (44.6 ± 7.4 vs. 36.1 ± 3.8) (49).

Moreover, older patients in ART may notably benefit from additional LH. On the one hand, the endocrine changes occurring with ovarian aging include an increase of serum FSH levels in the early follicular phase, which are not accompanied by an LH increase but by a progressive decrease of basal androgen levels. Follicular capacity to induce androstenedione synthesis after rFSH administration is reduced in older patients compared with younger reproductive-aged patients, whereas E_2 secretion is preserved by increased aromatase function (50). In a study lead by Bosch et al. (50), whereas patients up to 35 years old ($n = 380$) did not appear to benefit from rLH, patients aged 36 to 39 years ($n = 340$) had significantly higher implantation rates (95%CI[1.04–2.33]) when rLH was added. Clinically higher although not significant ongoing pregnancy rates per started cycle (95% CI[0.93–2.38]) were observed. Consistently, Humaidan et al. (51) showed a significant benefit of exogenous LH supplementation for women aged above 35 years old in terms of implantation rates and significantly reduced total FSH consumption.

CONCLUSION

Considering the lack of efficient tool to accurately evaluate ovarian hypo-response, FORT proves to be a relevant and crucial quantitative, and qualitative index that should be used in everyday practice for the care and management of hypo-responders in ART. Impaired sensitivity to FSH revealed by FORT should be considered in the decision of treatment protocol, gonadotropin, and stimulation doses to be used for hypo-responders. Improving follicular responsiveness to FSH may also be a key to ameliorate prognosis of POSEIDON groups 3 and 4 "expected" poor responders. Reconsidering criteria for COH cancellation based on the output of follicle response to exogenous FSH rather than on the absolute counting of follicles recruited by treatment should be discussed. It is expected that a better understanding of low prognosis patients undergoing ART will help improve individualized ovarian stimulation management and identify more homogeneous populations for clinical trials, thereby, providing better tools with which to maximize IVF success rates.

AUTHOR CONTRIBUTIONS

All authors listed have contributed to the work and approved the final version. JL and MG performed the literature research, wrote the paper and proofread it.

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Ovarian Reserve Markers to Identify Poor Responders in the Context of Poseidon Classification

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It is well-known that poor ovarian reserve is a cause of infertility, poor response to gonadotrophin stimulation and poor success rate after *in vitro* fertilization (IVF) cycles. Some years ago a consensus was elaborated on precise criteria which can lead to a correct identification of poor responders (the Bologna criteria). More recently, the POSEIDON group has proposed a new stratified classification of patients with low prognosis, also with the aim of providing clinical indications for the management of these patients. A literature search was carried out for studies that investigated the ability of ovarian reserve markers, in particular AMH and AFC, to predict poor ovarian response in IVF cycles; secondly, studies regarding the Bologna criteria and their prognostic value were analyzed and available literature on POSEIDON classification was reported. The most recent markers of ovarian reserve (serum AMH and ultrasound AFC) have shown to provide a direct and accurate measurement of ovarian follicle pool. These markers have generally shown comparable predictive power for ovarian response and a number of retrieved oocytes in IVF cycles. "Abnormal ovarian reserve test" is a very important parameter both in the Bologna criteria and in the POSEIDON classification. Several studies have already been published about the reproductive outcome of patients defined as poor responders according to the ESHRE Bologna criteria: all of them agree on the poor IVF outcome and low pregnancy rate of these patients. Instead, being the POSEIDON classification of very recent publication, the efficacy of the POSEIDON approach in improving management and outcomes of POR patients has yet to be tested and validated with future prospective clinical trials. Prediction of poor response may help clinicians choose the stimulation protocol with the aim of gaining patient compliance and cost reduction, and many efforts have been made by researchers in this sense, including the formulation of the Bologna criteria and of the POSEIDON classification, in which the ovarian reserve markers (AMH and AFC) play a fundamental role.

Keywords: IVF, poor response, ovarian reserve markers, AMH, AFC, Bologna criteria, live birth rate, POSEIDON classification

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INTRODUCTION

In the last decades, a high number of studies has been carried out on the possibility of measuring ovarian reserve through ovarian reserve markers. In reproductive medicine this is a leading field of research, as ovarian reserve markers hold an important diagnostic and prognostic value. It is well-known that a low ovarian reserve may be an important cause of infertility. Moreover, knowing

the ovarian reserve of a single woman allows clinicians to predict individual response to controlled ovarian stimulation (COS) in IVF cycles: if a patient has, for instance, a low ovarian reserve, she will probably achieve a poor ovarian response after COS. This condition is characterized by a low number of growing follicles and low serum estradiol levels after exogenous gonadotropin stimulation, resulting in a poor oocytes retrieval and, often, in a poor reproductive outcome (1–5). However, among poor responder patients the prognosis may be influenced by other parameters, such as patient's age and the outcome of previous IVF cycles.

For this reason, the European Society of Human Reproduction and Embryology (ESHRE) consensus has recently established that a response can be defined as poor (POR) when at least two of the following three criteria are present: (i) advanced female age (ii) a previous POR (iii) an abnormal ovarian reserve test (ORT) or in the absence of the above criteria two previous POR following maximal stimulation (6).

In literature, several studies have already been published on IVF outcome in poor responder patients defined according to the Bologna criteria. They all confirm a low live birth rate in these patients (7–9).

Nevertheless, since a certain heterogeneity concerning some biological and clinical features among patients included in the definition of POR still persists, a new classification stratified in subgroups based on these characteristics as well, that is the POSEIDON classification, was proposed in order to improve the performance of tailored therapies in the outcome of these patients and identify more homogeneous populations to be included in future clinical trials (10). This review aims at analyzing the role of ovarian reserve markers (AMH and antral follicle count) in predicting poor response after COS according to both classifications. Moreover, we will discuss the ability of the Bologna criteria to predict a poor reproductive prognosis in these patients as well as the innovations introduced by the POSEIDON classification.

DEFINITION OF OVARIAN RESERVE MARKERS

Over the years, numerous ovarian reserve markers have been proposed. Serum FSH, measured in early follicular phase (day 3–5 of the menstrual cycle) together with estradiol, has been widely applied in reproductive medicine, but it is only an indirect marker of ovarian reserve and its blood concentrations rise only when ovarian reserve is severely compromised (11). Similarly, literature consistently reports only a moderate sensitivity and specificity of this marker in predicting ovarian response to ovarian stimulation. Various cut-off values ranging from 10 to 15 IU/L have been recommended for predicting poor response in IVF (12–15), but only few patients meet this high threshold, limiting the usefulness of the marker.

The most recent markers of ovarian reserve, i.e., serum AMH and ultrasound antral follicle count (AFC), have shown to provide a direct and accurate measurement of ovarian follicle pool. AMH is produced only by small antral follicles until 6–8 mm diameter and secreted in serum. AFC is performed by ultrasound and counts all identifiable antral follicles of 2–10 mm present in both ovaries (16–18). As the pool of small antral follicles measured when performing AFC is the same that secretes AMH and it is proportional to the overall number of primordial follicles in the ovaries, AFC and AMH are highly correlated and show similar values in reflecting oocyte quantity (19).

Comparing AMH with AFC, AMH has the advantage of a very little intra- and inter-cycle variability (20, 21). On top of the well-known age-related decline in AMH, significant fluctuations have been reported for a number of conditions and this has to be taken into account when interpreting values in clinical practice (20).

Fluctuations in the menstrual cycle have also been reported (22, 23), questioning about using AMH as a single reliable marker in the clinical situation. But these fluctuations appear to be random and minor, and limited to high AMH values, therefore not causing changes in the management of the single patient. This suggests that in clinical practice AMH can be measured independently of the cycle phase. The exact role of patients' characteristics like ethnicity or lifestyle, for example habits like smoking, on intra- and inter-individual variability of AMH needs to be further investigated. Moreover, problems of low comparability of measured values among laboratories related to the old manual essays seem nowadays to be solved by the new recent automated essays, which should guarantee repeatable and comparable dosages (24). The new automated essays measure lower AMH concentrations than the old manual essays (−16% with Access AMH and −20% with Elecsys AMH), but AMH levels are still strongly correlated to AFC, especially in patients with low ovarian reserve (24, 25).

On the other hand, AFC exhibits some degree of intra- and inter-cycle and inter-observer variability (21, 26) that must be taken into account when considering this marker for diagnostic purpose. In order to reduce such variability, recommendations on the methodology and on the equipment setting have been given (18). The recent introduction of three-dimensional (3D) automated follicular tracking should decrease the above mentioned variability (27, 28), but it needs advanced ultrasound equipment to date, which is not yet largely available.

Direct comparisons between AFC and serum AMH in IVF cycles have generally shown a similar capacity in predicting ovarian response and the number of retrieved oocytes. Having failed in showing an independent relationship between AFC and oocyte yield, a few studies are in favor of AMH as the strongest predictor of ovarian response, while other studies have proven AFC to have a stronger predictive value (29–35). Anyway, these markers globally predict ovarian response in IVF better than all other known markers (17, 36–39)

When introducing ovarian reserve markers in clinical practice with the objective of predicting ovarian response, it is fundamental to establish acceptable cut-off levels for the markers themselves. AMH and AFC cut-off values reported in literature are very variable (4). Such variability could be explained by factors such as the low number of subjects included in some of these studies, the variability in the measuring methods used for the two markers and the different definitions of poor ovarian

response adopted by the various authors, consequently resulting in variations in the diagnostic performance of markers of ovarian reserve. Clinicians should therefore adopt cut-off values from the published study that may better reflect their clinical setting.

According to published studies, having good sensitivity and specificity, a cut-off value of AMH ranging between 0.7 and 1.3 ng/ml may be considered acceptable for the prediction of poor response in IVF (4, 37, 38). Using the most recent AMH automated essay (Access AMH), the cut-off point at 90% specificity and 74.1% sensitivity for poor response prediction was defined 0.93 ng/ml with ROC analysis (25).

AFC can be used to reliably predict ovarian response in IVF too, but literature shows a considerable variability in agreed AFC cut-off levels (17, 40). Inevitably, AFC thresholds for clinical practice depend on available ultrasound technology and resolution, and therefore need updating over time. Focusing on the most recent papers, generally based on modern technologies, the most frequently reported cut-off values for prediction of poor response range between AFC <5 and <7 (17). AFC thresholds calculated through modern 3D ultrasound technology haven't been published yet.

Thanks to their ability to predict ovarian response to stimulation, both markers are valid tools for the individualization of ovarian stimulation treatment and, in particular, for the choice of the starting dose of FSH (3, 4, 19, 32). A recent Cochrane review has confirmed that tailoring the FSH starting dose on ovarian reserve markers may reduce cases of OHSS, but it has not been able to demonstrate that it improves live birth rates compared to a policy of giving all women 150 IU (41). A recent multicenter prospective cohort study with two embedded RCTs, performed on 1,515 women randomized to an individualized or standard FSH dose (150 IU), reported the same conclusions: individualized dosing reduced the incidence of mild and moderate OHSS, but live births between the two groups were comparable (42).

Studies on the ability of AMH and AFC to predict oocytes quality and live births are controversial (32, 43–47). A recent study by La Marca et al. (48) reported a strong positive age-independent relationship between circulating AMH and the rate of euploidy in blastocysts after an IVF cycle (48). According to this observation, a large cohort study on 1230 IVF-ICSI cycles reported that AMH and age equivalently predict live birth (44). On the other hand, a large retrospective study performed on 69,336 fresh and 15,458 frozen embryo transfer cycles demonstrated that the areas under the curve (AUC) for AMH as predictor of live birth in fresh cycles and thawed cycles were, respectively, 0.631 and 0.540, suggesting that AMH alone is a weak, even if significant, age-independent predictor of live birth after ART (46).

OVARIAN RESPONSE PREDICTION AND MANAGEMENT

The occurrence of poor ovarian response in IVF ranges from 10 to 20% and it increases with female age. Once a patient at risk of poor ovarian response is identified, she should be informed by the clinician about dramatic prospects. First of all, she will probably have a poor response to COS, which means a low number of oocytes retrieved following a standard IVF protocol. This results in a poor reproductive prognosis, as the chance of an adequate number of good embryos to transfer is reduced. Moreover, the clinician should inform the patient that the scientific community is, so far, not aware of any valid solution, that is of any therapeutic protocol that could modify or improve the patient's ovarian response and prognosis.

This information may be quite stressful for the patient, so it is necessary to accurately identify poor responder patients through standardized criteria. However, literature highlights that this is far from easy: in 2011 a systematic review (49) revealed that among 47 randomized trials on poor ovarian responders, 41 different definitions for women with poor ovarian response have been used, with differences both in the criteria considered for the definition (including age, ovarian reserve markers, outcomes of a previous IVF cycle like number of follicles on the last day of ovarian stimulation or number of oocytes retrieved, etc…) and in the threshold values used for each criteria.

The lack of uniformity in the definition of poor responder patients has convinced The European Society of Human Reproduction and Embryology (ESHRE) to elaborate a consensus where precise criteria lead to the identification of different groups (or "phenotypes") of poor responders (6). In order to define a poor ovarian responder (POR), at least two of the following three features must be present: (i) Advanced maternal age (≥40 years) or any other risk factor for POR; (ii) A previous POR (≤3 oocytes with a conventional stimulation protocol);(iii) An abnormal ovarian reserve test (i.e., AFC <5–7 follicles or AMH< 0.5 −1.1 ng/ml). In the absence of advanced maternal age or abnormal ORT, two episodes of POR after maximal stimulation are sufficient to define a patient as a poor responder. In particular, different categories of POR may be defined from the different combinations of the Bologna criteria as follows: (i) one previous poor response and age ≥40 years, (ii) one previous poor response and abnormal markers of ovarian reserve, (iii) age≥40 years and abnormal markers of ovarian reserve (the so-called expected poor response category), (iv) one previous poor response in a woman aged≥40 years and with abnormal markers of ovarian reserve, v) two previous POR cycles following maximal stimulation.

This classification recognizes that the reproductive prognosis and therefore the definition of POR certainly depends on the ovarian reserve, measured by AMH or AFC, but also on other anamnestic and clinical factors, such as age and outcome of a previous IVF cycle or previous ovarian surgery, which are integrated into the definition.

Following the consensus, several studies have been published about the reproductive outcome of patients defined as poor responders according to the ESHRE Bologna criteria. All of these studies agree on the poor IVF outcome and low pregnancy rate of these patients (7–9, 50). In a study by La Marca (7) a database containing IVF reports from 210 women defined as POR was analyzed. The study demonstrated that the five different groups of POR had similar IVF outcomes, with a live birth rate between 5.5 and 7.4%. It was therefore concluded that poor responders in the five subgroups identified by the Bologna criteria represent

a homogenous population (7). The same results were reported in a retrospective study by Busnelli et al. (8), performed on 362 POR undergoing IVF: live birth rate was 6% (95% CI: 4–9%), not significantly different in the different subgroups of POR (8). In line with previous studies, in a large retrospective study by Bozdag et al. (9) performed on 821 patients fulfilling the Bologna criteria, the live birth rates per started IVF cycle ranged from 2.3 to 8.7%. In contrast with previous studies, the subgroup of POR presenting AFC<7 and a previous poor ovarian response, defined as "young proven" PORs subgroup, was found to be associated with the most favorable live birth and implantation rates, compared to other subgroups, characterized by patients' age >40 and a previous poor response and/or AFC < 7 (9).

By better identifying the perimeter of patients to be considered as poor responders, the Bologna Criteria certainly represent an important step in the definition of POR and for the prediction of an IVF cycle, thus allowing clinicians to provide patients with improved counseling.

A few authors (10, 51–53) have, however, focused on clinical and biological aspects that would deserve greater consideration in the classification of poor patients (including the age-related oocyte quality and the genetic profile that conditions the ovarian sensitivity to the stimulation with the gonadotropins). These authors have highlighted how, with regard to these aspects, there is a persistent heterogeneity among POR patients and have criticized the lack of indication of differentiated management strategies for the different subgroups of patients (**Table 1**). This means that the same type of treatment may not be optimal for all the patients defined as POR, even when having a similar prognosis. While this may have a logical basis, it is far from being proved on a solid scientific ground made of well-designed multicenter trials. Up to now, there is not sufficient evidence for clinicians to recommend a particular therapeutic strategy resulting in improved live birth rate for poor responder women.

Treatment with a GnRH antagonist protocol instead of a GnRH agonist protocol was proposed for these patients as it avoids the very deep suppression of endogenous FSH and LH concentrations in the early follicular phase at the stage of follicular recruitment, thus giving hope for a better egg retrieval. Some trials and meta-analyses actually showed that the GnRH agonist long protocol and the GnRH antagonist regimen are comparable in their efficacy for the outcome of IVF in poor responders (55–57).

In this regard, we think that if the standard agonist long protocol offers no benefits in poor reponder patients in terms of chance of pregnancy when compared to an antagonist protocol, the choice of the protocol should aim to improve patient compliance (58) in addition to cost reduction (59). This is possible with an antagonist protocol as it allows shorter duration of stimulation and reduced gonadotrophin consumption (4, 60).

In this context, a recent large randomized trial demonstrated the non-inferiority of a mild ovarian stimulation protocol with GnRH antagonist compared to a standard approach with the long GnRH agonist protocol. Ongoing pregnancy rate was 12.8% (25/195) for mild ovarian stimulation vs. 13.6% (27/199) for conventional ovarian stimulation (95% CI: 0.57–1.57), while the duration of ovarian stimulation and the amount of gonadotrophins used were significantly lower in the mild stimulation strategy (61).

Different studies performed on women predicted to be poor responders showed that increasing the FSH dose doesn't impact on the number of retrieved oocytes (62, 63). The maximum

TABLE 1 | Summary of the existing literature on Poseidon classification.

Author	Publication	Type of article	Main finding
Poseidon Group (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number), Alviggi et al. (10)	Fertil Steril. 2016; 105(6):1452–3	Commentary	Definition of Poseidon categories: Group 1: Patients < 35 years with sufficient prestimulation ovarian reserve parameters (AFC ≥5, AMH ≥1.2 ng/mL) and with an unexpected poor or suboptimal ovarian response. Group 2: Patients ≥ 35 years with sufficient prestimulation ovarian reserve parameters (AFC ≥5, AMH ≥1.2 ng/mL) and with an unexpected poor or suboptimal ovarian response. Group 3: Patients < 35 years with poor ovarian reserve prestimulation parameters (AFC <5, AMH <1.2 ng/mL). Group 4: Patients ≥ 35 years with poor ovarian reserve prestimulation parameters (AFC <5, AMH <1.2 ng/mL).
Humaidan et al. (54)	F1000Res. 2016; 5:2911	Commentary	Definition of Poseidon categories and discussion as to why the new concept has been proposed
Haahr et al. (52)	Reprod Biol Endocrinol. 2018; 16(1):20.	Review	Discussion on how the treatment of the expected poor ovarian response patient should be individualized in all steps of ART, including the choice of GnRH analog, the gonadotropin type and dose, ovulation trigger, and the possible use of adjuvant therapies.
Esteves et al. (53)	Front Endocrinol (Lausanne). 2018; 9:461.	Review	Critical appraisal of the existing criteria that standardize the definition of POR and explanation of reasons for the development of the POSEIDON criteria.

number of oocytes that can be retrieved in women is determined by the number of recruitable antral follicles in the ovaries and it is obvious that a dose of FSH higher than the maximal one will never compensate for the lack of substrate.

Recently, new ovarian stimulation protocols are under study for their application on poor responders. Among these, the double ovarian stimulation, that is the ovarian stimulation in follicular and luteal phases within a single ovarian cycle, seems capable of increasing the number of retrieved oocytes and available embryos for the single patient (64). The first data have been published on possible advantages of a GH co-treatment with the mild stimulation protocol or the GnRH antagonist protocol in poor responders (65–67). Some RCTs and meta-analysis have also been published regarding LH supplementation within the rFSH stimulation in IVF cycles: there is no agreement on the benefit of this therapy in the general population, while particularly in POR patients it showed an improvement of the clinical pregnancy rate and live birth rate (51, 52, 68, 69). Supplementation with androgens also seems to give some positive results in these patients, although the available trials are still too low in number to make recommending such a therapy possible (52). Finally, a few studies report that a dual trigger ovulation regimen with GnRH agonist plus hCG could significantly improve number and maturity of retrieved oocytes in poor responders, but more studies are needed to evaluate a possible positive effect of the dual trigger on the clinical pregnancy rate (70, 71).

In this context, in order to differentiate more homogeneous subgroups of patients that could benefit from a specific management, in 2016 Alviggi and the POSEIDON group attempted to develop a new classification in which patients defined as "low prognosis" were divided in four groups according to age, ovarian reserve and ovarian response (**Table 2**). In the POSEIDON classification a poor ovarian reserve pre-stimulation is defined on the basis of ovarian reserve markers, precisely AFC <5 or AMH <1.2 ng/ml. This identifies the so-called "expected poor ovarian responders": these patients belong to GROUP 3 if they are aged < 35 years or GROUP 4 if they are > 35 years old; the 2 groups with the same low ovarian reserve are thus differentiated for the oocyte quality and therefore for the expected aneuploidy rate of the oocytes taken. As a matter of fact, there is in literature a quite broad agreement that 35 years of age represent the beginning of age-related changes not only in oocytes quantity, but also in their quality (with an embryo euploidy rate that decreases by 2.4 percentage points for every year increase in female age and a blastocyst euploidy rate that drops from 60% before 35 years to 30% after 40 years, and a subsequent decline in implantation potential) (54, 72). In a subsequent review (52) the suggested recommendations for these categories of patients have been better explained: the number of oocytes necessary to obtain at least one euploid embryo for transfer in each patient is estimated between 4 and 7 for group 3 and 12 oocytes for group 4; suggested treatments in both groups include both the long GnRh agonist protocol and the GnRh antagonist protocol, ovarian stimulation with 300 IU/day of rFSH, with rLH adjuvant therapy for group 4, the possibility of performing a double stimulation with an oocyte/embryo accumulation and frozen embryo transfer. The use of an androgen adjuvant therapy (DHEA, testosterone) needs further investigation before it can be recommended whereas the possibility of oocyte donation is only considered in group 4.

TABLE 2 | Comparison between Bologna criteria and Poseidon's stratification.

Bologna criteria	Poseidon's stratification
1) Maternal age ≥40 years + A previous POR	GROUP 1
	Age <35 years + adequate ORT + An unexpected POR (<9 oocytes retrieved)
2) An abnormal ORT (AFC <5–7 follicles or AMH< 0.5 −1.1 ng/ml) + A previous POR	GROUP 2
	Age ≥35 years + adequate ORT + An unexpected POR (<9 oocytes retrieved)
3) Maternal age ≥40 years + An abnormal ORT (AFC <5–7 follicles or AMH< 0.5 −1.1 ng/ml)	GROUP 3
	Age <35 years + An abnormal ORT (AFC<5; AMH<1.2 ng/ml)
4) Maternal age ≥40 years + An abnormal ORT (AFC <5–7 follicles or AMH< 0.5 −1.1 ng/ml) + A previous POR	GROUP 4
	Age ≥35 years + An abnormal ORT (AFC<5; AMH<1.2 ng/ml)
5) 2 previous POR	

GROUPS 1 and 2 of the POSEIDON classification include instead the "unexpected poor ovarian response,"i.e., those patients who, despite having a good ovarian reserve based on the values of the ovarian reserve markers (AFC> 5, AMH> 1.2 mg / ml), obtained a low oocyte number: between 4 and 9 oocytes retrieved (subgroups 1-a and 2-a) or even fewer than 4 oocytes retrieved (subgroups 1-b and 2-b). Once again, given the same ovarian response, the two groups are distinguished according to the age of the patients (group 1 <35 years, group 2> 35 years). A poor response in these patients may be due to iatrogenic hypostimulation or also to genetic polymorphism in FSH receptors (FSHR), LH receptor (LHR) or LH. It is actually reported in the literature that some polymorphisms in the alleles coding for these molecules (such as the FSHR Ser680 allele, or the LHβ variant) are associated with higher FSH consumption, thus characterizing patients requiring higher doses of rFSH during the controlled ovarian stimulation for ART (52, 73, 74). At the current state of knowledge, no hormonal or ultrasound marker exists that allows for the early recognition of these polymorphisms in the single patient. Furthermore, an adjuvant therapy with rLH supplementation is suggested in this subgroup to increase the implantation rate and in group 2 to improve the oocyte quality also (68). Finally, it seems that genetic

polymorphisms in FSH receptor are not associated to significant variation in ovarian reserve markers (75, 76).

Obviously the efficacy of the POSEIDON approach in improving management and outcomes of POR patients will have to be tested and validated with future prospective clinical trials. Moreover, AMH and AFC cut-off values for poor and normal response prediction will probably be updated in the next years, together with the spread of new AMH automated essays end modern ultrasound technology. As a consequence, POSEIDON categories might undergo a revision in the inclusion criteria on the ground of ovarian reserve markers.

CONCLUSIONS

Serum AMH and ultrasound AFC have shown to provide a direct and accurate measurement of ovarian follicle pool. These markers have generally shown a similar capacity to predict ovarian response and number of retrieved oocytes in IVF cycles.

In spite of this, the definition of a patient as poor responder appears heterogeneous in literature, also for the fact that not only the ovarian reserve, but also other clinical-anamnestic factors are important in determining a poor response to COS. This is not irrelevant if we consider the clinical and psychological implications of a POR diagnosis. The development of the Bologna Criteria and of the POSEIDON classification, combining the ovarian reserve markers with age, with the previous response to COS and with other risk factors for POR, aims at providing a significant help in the standardization of the criteria used for this diagnosis and in the improvement of these patients' clinical management. While waiting for studies conducted on the new criteria, we can say that, according to the data currently available in literature, the prediction of poor response and the use of consequently tailored treatment can have positive results in terms of patient compliance and cost reduction, but it does not seem to involve a relevant improvement in IVF outcome (60, 77).

AUTHOR CONTRIBUTIONS

AL, VG, and EM contributed in literature search and in writing the review.

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REVIEW
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Management of Women With an Unexpected Low Ovarian Response to Gonadotropin

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POSEIDON groups 1 and 2 patients respond poorly (<4 oocytes retrieved) or sub-optimally (4–9 oocytes retrieved) to gonadotropin stimulation despite the presence of adequate ovarian parameters, which negatively affect their cumulative chances of delivering a baby using Assisted Reproductive Technology. A polygenic trait involving gonadotropins and/or their receptors seems to be the primary pathophysiology mechanism explaining this phenomenon. The clinical management is mainly focused on maximizing oocyte yield as to increase the likelihood of having at least one euploid embryo for transfer. Indices such as FORT (follicle output rate) and FOI (follicle-to-oocyte index) may be used to determine if the ovarian reserve was properly explored during a previous ovarian stimulation. Testing for the presence of common polymorphisms affecting gonadotropins and/or their receptors can also be considered to identify patients at risk of hypo-response. An individualized estimation of the minimum number of oocytes needed to obtain at least one euploid embryo can assist counseling and treatment planning. Among currently existing pharmacological interventions, use of recombinant FSH in preference over urinary gonadotropin preparations, FSH dosage increase, and use of rLH supplementation may be considered -alone or combined- for optimally managing POSEIDON's groups 1 and 2 patients. However, given the recent introduction of the POSEIDON criteria, there is still a lack of studies examining the role of interventions specifically to patients classified as groups 1 and 2, thus making it an area for open research.

Keywords: hypo-response, ovarian stimulation, Assisted Reproductive Technology, ovarian reserve, follicle-to-oocyte index, POSEIDON criteria, suboptimal response, ART calculator

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INTRODUCTION

The primary goal of Assisted Reproductive Technology (ART) is to provide effective and safe personalized solutions to help infertile couples obtain a live birth. This objective should be attained with the mindset of securing the shortest time to live birth while avoiding negative consequences for the mother and newborns. In this regard, the transfer of a single embryo at the blastocyst

stage provides a higher implantation rate than the transfer of a cleavage stage embryo and limits the occurrence of multiple pregnancies (1–4). ART failure is indeed a leading cause of treatment dropout and is associated with an impairment of the psychological wellness of treated couples (5–7). Furthermore, the higher the number of ovarian stimulation (OS) cycles the higher the financial burden on couples, with potential long-term effects on general well-being (8). Thus, ART programs should strive to obtain a single live-birth using the least number of OS cycles as possible.

The novel Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number (POSEIDON) criteria (9–11) were introduced to help clinicians explore the possibility of using patient-oriented strategies to obtain the number of oocytes needed to achieve at least one euploid embryo for transfer in low prognosis women undergoing ART, as these patients represent the most vulnerable group concerning treatment failure and treatment dropout. A clear definition of the low prognosis patient population is, therefore, essential to avoid heterogeneity and allow the use of personalized management to achieve the intended goal. In brief, POSEIDON patients are subdivided into four subgroups based on a combination of factors including (i) age, (ii) results of functional and biological ovarian reserve markers, such as Antral Follicle Count (AFC) and Anti-Müllerian Hormone (AMH), and (iii) ovarian response concerning the number of oocytes retrieved in previous OS if this information was available (**Figure 1**). In practical terms, the POSEIDON criteria stratify the low prognosis patients in two main categories based on oocyte yield, namely, the "expected" low ovarian response (Group 3 and 4) and the "unexpected" low ovarian response (Groups 1 and 2).

POSEIDON's groups 1 and 2 encompass women who had poor (<4) or suboptimal (4–9) number of oocytes retrieved after a conventional OS despite the presence of an adequate ovarian reserve, defined by an AFC of ≥5 and/or an AMH ≥1.2 ng/mL. Indeed, retrieval of fewer than 10 oocytes is associated with decreased cumulative live birth rates (CLBR) (12). Among women with normal ovarian reserve, 10–15 oocytes seem to be the optimal target for increasing the likelihood of live birth rate in fresh embryo transfer (ET) cycles (13). However, retrieval of more than 15 oocytes might be advantageous concerning CLBR, i.e., when all fresh and frozen-thawed ETs are considered (12, 14). Thus, given a patient who fits POSEIDON's groups 1 or 2, the final goal would be to find ways to maximize oocyte yield aiming at obtaining more than 9 oocytes at the end of stimulation.

In this paper, we review the pathophysiology and discuss the available treatment strategies of low prognosis women according to POSEIDON's groups 1 and 2.

UNEXPECTED SUBOPTIMAL OR LOW OOCYTE NUMBER AND ITS ASSOCIATION WITH OVARIAN HYPO-RESPONSE TO GONADOTROPIN STIMULATION

Patients who fit POSEIDON's groups 1 and 2 criteria should be critically assessed by looking at two indices, namely, the FORT (follicle output rate) and the FOI (follicle-to-oocyte index). The follicle output rate (FORT) measures the consistency between the pool of antral follicles at the beginning of OS and the number of pre-ovulatory follicles at the end of stimulation (15, 16). Along the same lines, the FOI assesses the consistency between the pool of antral follicles at the beginning of OS and the number of oocytes retrieved at oocyte pick-up (**Figure 2**). Thus, a discrepancy between the available antral follicle pool and the number of pre-ovulatory follicles at the end of stimulation (e.g., FORT<50%), or the number of retrieved oocytes (e.g., FOI <50%) is suggestive of hypo-response to gonadotropin stimulation, albeit other contributory causes might exist as depicted in **Figure 3** (17). The advantages and shortcomings of using FOI and FORT to identify hypo-responders to gonadotropin stimulation have been discussed in detail elsewhere (17).

The pathophysiology mechanisms explaining the hypo-response to gonadotropin stimulation, also known as "ovarian resistance" to gonadotropin stimulation, are not fully understood. However, environmental contaminants, as well as specific genotypic traits have been hypothesized as possible contributory factors (17–21) (**Figure 3**). In particular, genetic polymorphisms affecting the gonadotropins and their receptors might impact OS outcomes (22, 23). Such polymorphisms include those affecting the FSH receptor genes, such as FSHR c.2039 A>G (rs6166) (24, 25), FSH β chain [FSHB−211 G>T (rs10835638)] (26), and FSH promoter region [FSHR−29 G>A (rs1394205)] (27, 28). Of particular interest is the FSHR polymorphism (rs6166), which has been implicated in ovarian resistance to exogenous gonadotropin (24). The single nucleotide polymorphism SNP (rs6166), known as Serine680 (Ser680) variant, causes the replacement of Asn with Ser at the 680 position and is located in the intracellular domain of the FSH receptor protein (29, 30).

Interestingly, it has been reported that Ser680 carriers with polycystic ovarian syndrome show resistance to clomiphene citrate (31). Another variant in a promoter region of FSHR, namely FSHR, 29 G>A), was associated with negative effects during OS. In a study by Achrekar et al. involving patients undergoing OS for ART, women homozygous for the rs1394205 variant genotype AA had a lower number of oocytes and lower pregnancy rates than those with the GG genotype (32). This observation was confirmed in a more extensive study by Desai et al. (27). These authors retrospectively evaluated 100 normogonadotropic women with regular menses who were candidates for IVF. The carriers of AA genotype showed a lower number of oocytes retrieved and a higher consumption of exogenous gonadotropin than GG carriers. As for the FSH β chain polymorphism (rs10835638), a study involving 169 healthy women, and 186 infertile women suggested that this polymorphism is associated with significantly higher FSH and LH levels in both healthy and female infertility patients (33). In this report, the T-allele carriers were found more frequently among idiopathic infertility cases, a fact that could be explained by the influence of this particular polymorphism on FSHR function (26, 34, 35).

A systematic review and meta-analysis published in 2018 summarized the data of 33 studies regarding the clinical relevance

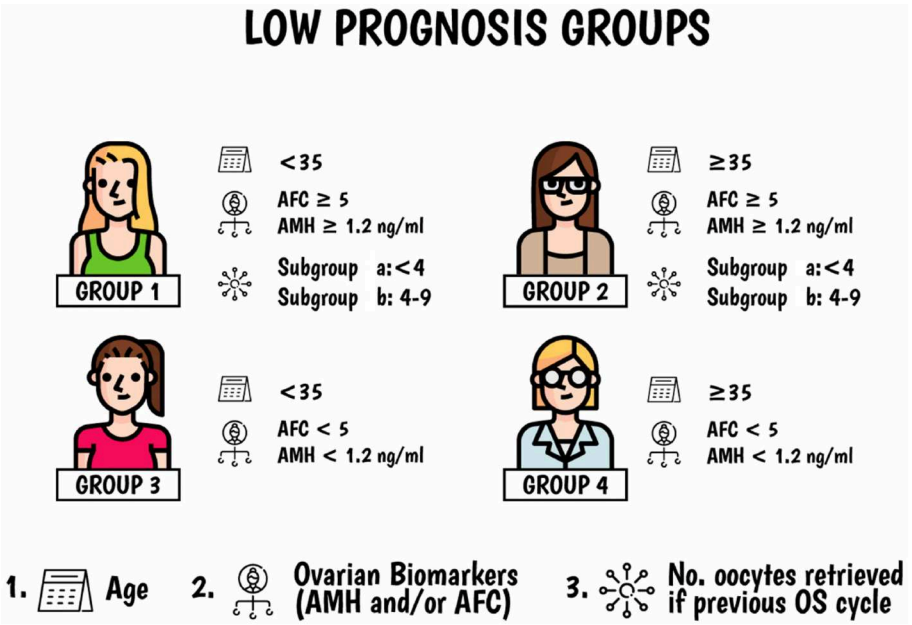

FIGURE 1 | Poseidon criteria of low prognosis patients in ART. Four distinct groups of low prognosis patients can be established based on quantitative and qualitative parameters, namely: 1. The age of the patient and the expected embryo aneuploidy rate; 2. Ovarian biomarkers (antral follicle count [AFC] and/or anti-Müllerian hormone [AMH]), and 3. The ovarian response of the patient in terms of oocyte quantity provided a previous cycle of stimulation was carried out. Art drawing by Chloé Xilinas, EXCEMED, Rome, Italy. Adapted from Esteves et al. (10).

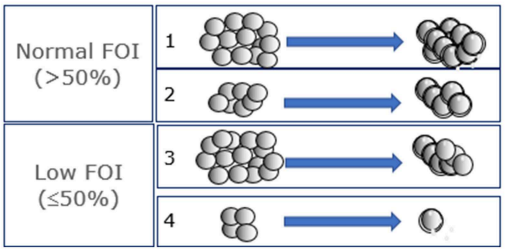

FIGURE 2 | Ovarian resistance using the follicle-to-oocyte index (FOI). Reprint from Alviggi et al. (17).

of FSHR polymorphism on OS. The authors showed that higher FSH consumption is expected in homozygotes for the A allele of the FSHR (rs1394205) polymorphism than in carriers of the G allele. Moreover, FSHR (rs6166) Serine carriers seem to be less responsive to OS treatment, with fewer oocytes retrieved at the end of OS than Asparagine carriers (22). In other words, both Serine carriers of FSHR (rs 6166) and A carriers of FSHR (rs1394205) are at increased risk of exhibiting ovarian resistance to OS, which in turn might lead to a suboptimal response concerning the number of retrieved oocytes at the end of OS.

Ovarian hypo-responsiveness to gonadotropin stimulation remains an undervalued issue in ART both in research and in daily clinical practice. Not surprisingly, the prevalence of this condition is still unclear. However, preliminary data show that ∼10% of women defined as normal responders by demographic characteristics and ovarian reserve testing requires a higher than expected total dosage of gonadotropin to promote adequate follicular development (36). Notably, a recent study indicated that approximately 45% of patients aged 18–40 years who underwent conventional OS using FSH doses of 150–225 IU/day retrieved <10 oocytes at their first stimulation cycle (12, 37). These data may be overestimated as the authors did not specify the ovarian reserve before stimulation. By examining the data from a group of 427 consecutive infertile women who underwent conventional OS in one of the authors' (SCE) clinic, 47% of the treated patients fitted the POSEIDON criteria. Among them, 5 and 35% were within groups 1 and 2 categories, respectively (38). Although larger studies are required, these preliminary data suggest that a remarkable number of women with adequate pre-stimulation ovarian reserve parameters exhibits an unexpected anomalous response to gonadotropin stimulation.

CLINICAL MANAGEMENT OF POSEIDON'S GROUPS 1 AND 2 PATIENTS

As highlighted above, there is a significant number of women classified as normal responders—based on ovarian markers—who show resistance to exogenous gonadotropin stimulation. Unlike those with diminished ovarian reserve in which the

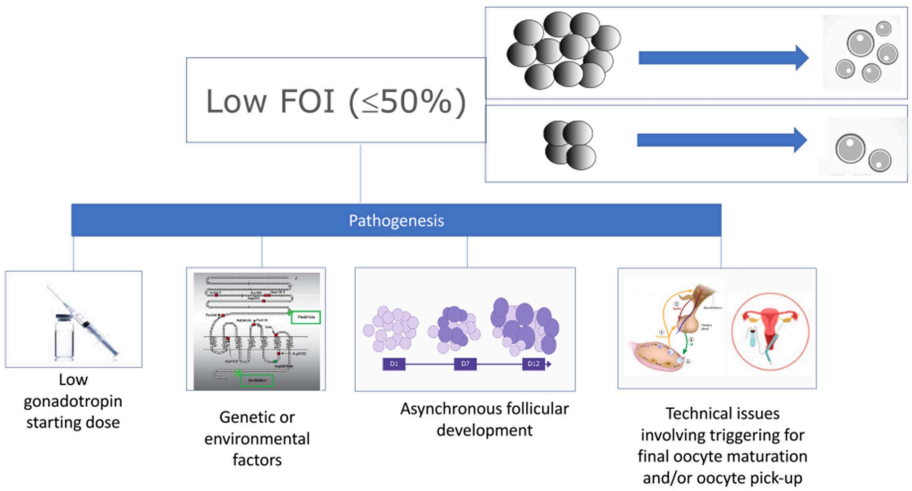

FIGURE 3 | Pathogenesis of low follicle-to-oocyte index (FOI). Reprint from Alviggi et al. (17).

increment of gonadotropin dosage during ovarian stimulation appears to be of limited value (39), POSEIDON's groups 1 and 2 patients seem to benefit from a pharmacological intervention concerning the OS protocol. Given the possibility of the existence of a polygenic trait in POSEIDON's groups 1 and 2 patients, an option would be for clinicians to assess normal ovarian reserve patients concerning the most common polymorphisms in case of an unexpected poor (≤3 oocytes) or suboptimal response (4–9 oocytes) in previous IVF cycle. Ideally, if large randomized controlled trial confirmed the utility of pharmacogenomic approach, genotype screening could be potentially recommended even before ovarian stimulation avoiding inadequate OS attempts.

Patients with specific polygenetic traits would be at high risk of being classified as POSEIDON's groups 1 and 2 after conventional OS using empirical approaches. Thus, such patients could be identified *a priori* and treated accordingly using a pharmacogenomic rather than a trial and error approach. Indeed, pharmacogenomic algorithms have been used to evaluate how genetic differences among individuals might affect drug response, thus ultimately leading to the development of personalized drug therapies to compensate for these differences (30). The individual genomic variation could influence sensitivity to antimicrobials and response to cancer strategies (40).

Furthermore, genetic traits could influence fertility, including ovarian response to gonadotropin stimulation (41, 42), despite no obvious clinical signs or symptoms. These polymorphisms are widespread in population and women with infertile disorders (43). Considering that genotype analysis could be provided at lower cost compared with the past, it is plausible that a genotype mapping of women who showed an unexpected low response during OS could be of use to optimized management strategy in such women. Nevertheless, since the availability of polymorphism panel testing is still limited, another option would be to apply empirical pharmacological interventions to increase the oocyte yield—and eventually pregnancy outcomes—in such patients.

Estimating the Number of Oocytes Needed to Obtain at Least One Euploid Blastocyst for Transfer

In 2016, the POSEIDON group introduced a new marker of success in ART, namely, the ability to retrieve the number of oocytes needed to obtain at least one euploid embryo for transfer (38). This marker represents a logical endpoint to guide clinicians develop an individualized treatment plan for ART patients, including those of POSEIDON's groups 1 and 2. Indeed, availability of a euploid embryo for transfer may change the fate of the low prognosis patient, as ∼50–60% euploid blastocysts implant across all age categories (44). Thus, the higher the number of oocytes retrieved, the higher the probability of obtaining an embryo cohort that may include at least one euploid blastocyst (45, 46). However, retrieval of an optimal number of oocytes may not be feasible in patients of groups 1 and 2 due to reasons discussed in previous sections. The matter is further worsened by female age, which is the most critical predictor of embryo euploidy. In a recent study, using preimplantation genetic testing data of patients undergoing ART, we showed that blastocyst euploidy not only markedly decreases with female aging but also that the magnitude of decrease is progressive with every year of age (46). It would be therefore useful to estimate the individualized minimum number of oocytes needed to achieve at least one euploid blastocyst for transfer. A pretreatment predictive model—the ART Calculator- has been developed to assist clinicians to counsel and plan treatment regarding the number of oocytes required for at least one euploid blastocyst in IVF/ICSI procedures. Briefly, the model was constructed based on the results of the LASSO (Least Absolute Shrinkage and Selection Operator) regression analysis, which was utilized for both variable selection and regularization

to enhance the prediction accuracy and interpretability of the statistical model. It is out of the scope of this paper to discuss the technical aspects of the calculator, but detailed information is provided in other papers within this Frontiers Research Topic. The "ART Calculator" is available online at http://www.groupposeidon.com/ and is fully aligned with the POSEIDON marker of successful outcome.

Individualized Controlled Ovarian Stimulation

Based on limited data, five main strategies might be considered, which can be used alone or combined, namely (i) use of recombinant FSH in preference over urinary gonadotropin preparations, (ii) FSH dose increase, (iii) rec-LH supplementation, (iv) Dehydroepiandrosterone supplementation before OS, and (v) the combination of follicular and luteal phase stimulation in the same ovarian cycle. A flow chart listing the suggested management of Posedoin group 1 and 2 is illustrated in **Figure 4**.

Use of Recombinant FSH

The main problem behind suboptimal response or poor response is that the number of retrieved oocytes might not be consistent with the ovarian reserve. With the aim to retrieve more oocytes

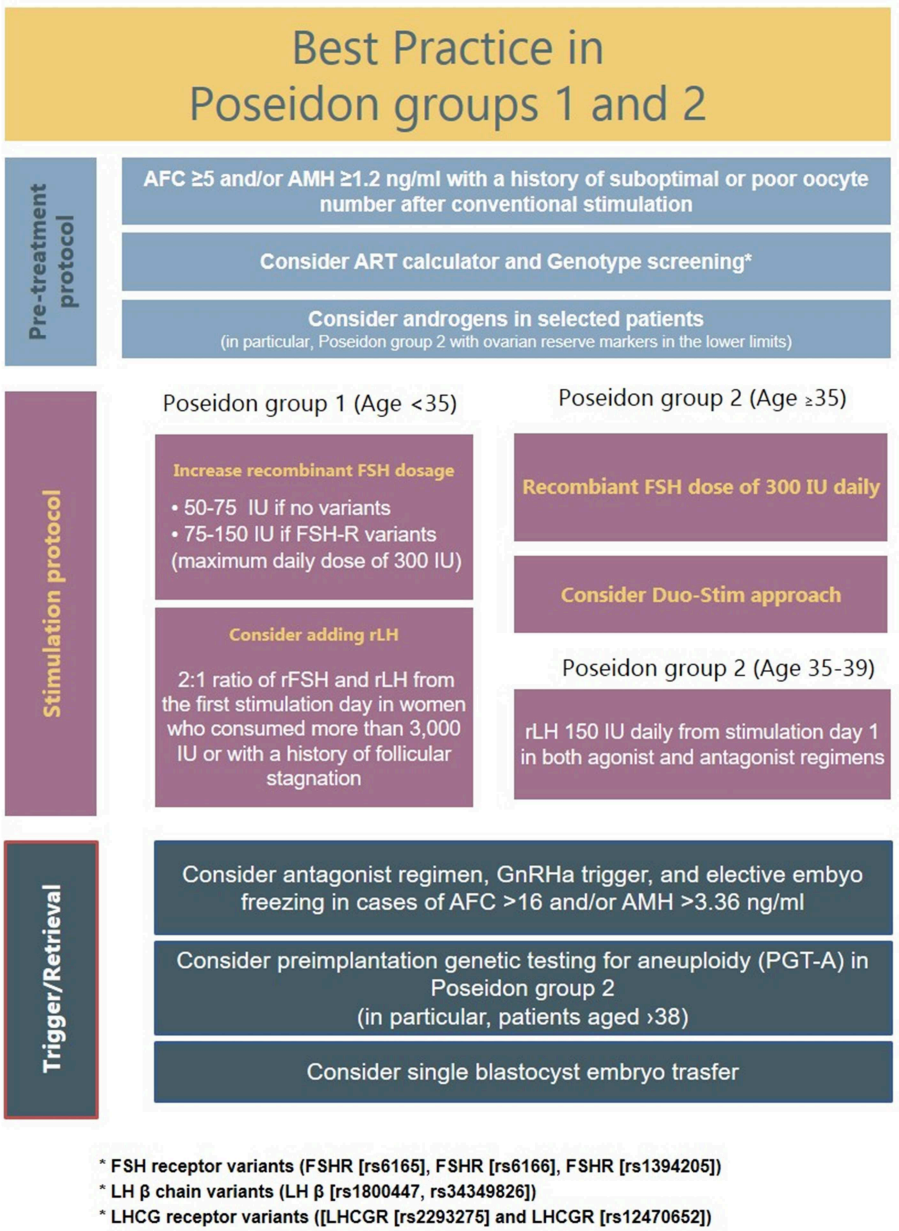

FIGURE 4 | Suggested management of Poseidon groups 1 and 2 patients.

at the beginning of stimulation, a more "potent" gonadotropin formulation should be considered. Several randomized controlled trials and meta-analyses demonstrated that the use of recombinant formulations is associated with significantly higher number of retrieved oocytes than with urinary formulations irrespectively of the pituitary suppression strategy (47–49). These findings seem to relate to the higher bio-potency of recombinant formulations (50). In conclusion, the use of more potent (rFSH) recombinant formulation might be suggested in Poseidon Group 1 and 2.

FSH Dose Increase

Serine carriers of FSHR polymorphism undergoing OS for ART seem to benefit from increased recombinant FSH doses. In this regard, the first attempt to develop a pharmacogenomic approach to OS was conducted by Behre et al. (51). In their study, Ser680/Ser680 carriers were randomly allocated to two subgroups to receive a daily rec-FSH dose of 150 IU or 225 IU. The dose of 225 IU/day was able to restore the estradiol levels at the end of OS in Ser680/Ser680 carriers, which was similar to that of women with the wild-type genotype (51). Along the same lines, Genro et al. showed that when a high FSH dose (300 IU per day) was given, the FORT index was not significantly different in patients undergoing OS for ART, regardless of FSHR rs6166 genotype distribution (52). As for the FSHR rs1394205 polymorphism, we are unaware of any trials examining the effect of increased FSH doses. Furthermore, increasing FSH dosage appears to be a valid strategy in women with a history of suboptimal response *per se*. Specifically, a 2018 retrospective analysis evaluated the effect of FSH dose adjustment in women with a history of suboptimal response (4–9 oocytes retrieved) after conventional OS (37). In this study, 160 women <40 years with normal ovarian reserve undergoing their second ovarian stimulation cycle in a fixed gonadotropin-releasing hormone (GnRH) antagonist protocol with daily recombinant FSH (rFSH) were recruited. A dose increment of rFSH in the subsequent cycle carried out 4 months later on average lead to higher number of oocytes retrieved (9 vs. 6, $p < 0.001$) and good quality embryos (4 vs. 3, $p < 0.001$) than that of previous cycle. A regression analysis showed that an increase of 50 IU of the initial rFSH dose would lead to 1 more oocyte. Although there is evidence that resistance in term of the number of oocytes retrieved and follicle output rate could be associated with specific genotype anomalies, the suboptimal responders were not tested for genetic polymorphisms in this study.

Recombinant Luteinizing Hormone Supplementation

Several trials have examined the clinical utility of adding recombinant Luteinizing Hormone (rLH) in women with ovarian resistance to gonadotropin (53–58). In the larger ones, the ovarian resistance was identified in the form of an "initial slow response" in follicle growth (54, 55). In others, involving a lower number of cases, the hypo-response was retrospectively diagnosed in women who required higher-than-expected doses of gonadotropins considering their age, body mass index, and ovarian reserve (53, 56). Data from the more robust studies indicate that in slow responders, LH supplementation starting from stimulation days 7–10 might be more efficient than increasing the dosage of rFSH to rescue the ongoing cycle.

In detail, Ferraretti et al. study was a single-center randomized trial involving a total of 126 women aged 37 or younger undergoing pituitary suppression with the agonist protocol. The number of oocytes retrieved was significantly higher in hypo-responders treated with the rFSH plus rLH step-up regimen than in those who received a higher dose of rFSH (11.1 vs. 8.2, $p < 0.05$). Along the same lines, higher pregnancy rates per embryo transfer (54 vs. 24.4%, $p < 0.05$), live birth rates (40.7 vs. 22%, $p < 0.005$) and implantation rate (36.8 vs. 14.1%, $p < 0.05$) were observed in women supplemented with rLH than in those who received only an increment of rFSH. Another study was a multicenter RCT involving 229 IVF/ICSI cycles (55). The population, definition of hypo-response, and OS regimen were similar to Ferraretti et al. study. In this trial, the number of oocytes retrieved was significantly higher in patients who received rLH supplementation (9.0 ± 4.3) than in those treated with increased rFSH dosage (6.1 ± 2.6, $P < 0.01$). The use of rLH supplementation was able to restore both rescue implantation (14.2 vs.18.1%, $p > 0.05$) and ongoing pregnancy rates (32.5 vs. 40.2%, $p > 0.05$), which turned out to be resulted similar to that observed in normal responders. Regarding dosage, the use of 150 IU of rLH is apparently better than the use of 75 IU in the long GnRH agonist protocol (59). In a randomized trial, 46 hypo-responders identified by similar criteria as in Ferraretti et al. and De Placido et al. studies (54, 55) were randomized to receive a supplementation with 150IU or 75IU of rLH, respectively. Hypo-responders supplemented with 150 IU/day of rLH had higher number of oocytes retrieved (9.65 ± 2.16 vs. 6.39 ± 1.53, $p < 0.05$) and showed higher percentage of mature oocytes (79 vs. 65.7%, $p < 0.05$) than in those supplemented with 75 IU/day of rLH (59).

More recently, Yilmaz et al. (60) performed a single-center prospective study that corroborated the results of the randomized controlled trials mentioned above. In their study, hypo-responders were identified as in De Placido et al. study. A total of 137 patients were enrolled, 85 of whom had a hypo-response to OS diagnosed on stimulation on day 7 (at least six follicles between 6 and 10 mm; no follicle over 10 mm, and Estradiol levels below 180 pg/mL), and 52 had a normal response (regular follicular growth and Estradiol level >180 pg/mL). In the hypo-response group, 50 women received 75 IU daily of rLH, whereas the rFSH dosage was increased by 75 UI in the remaining 35. Implantation rates were significantly higher in controls (34.7%) and in the rLH supplementation (36.1%) groups than in the increased-dose rFSH group (15%, $P < 0.02$). The pregnancy rates were also higher in the two former groups than in the latter group (64.7 and 57.8%, respectively vs. 32.4%, $P < 0.05$). The findings of the studies mentioned above should be interpreted with caution because the GnRH agonist long protocol was utilized in all studies. Currently, there are no data concerning the use of rLH supplementation to hypo-responders undergoing OS under a GnRH antagonist regimen.

The mechanism by which rLH exerts its beneficial effect in hypo-responders is not fully understood. Although it was advocated that the excessive suppression of endogenous LH after

down-regulation with GnRH analogs may create the need for exogenous LH supplementation, neither Ferraretti et al. nor De Placido et al. found a significant association between serum LH levels during OS and the response to rLH supplementation (54, 55, 61). A more plausible hypothesis would be related to genetically determined characteristics of LH itself or its receptors. Indeed, Alviggi et al. (36, 41) demonstrated that carriers of LH β chain variant had ovarian resistance to exogenous gonadotropin and required a higher dosage of recombinant FSH during OS (36). This variant was initially discovered by Pettersson and Söderholm (62) as an immunologically anomalous form of LH caused by two-point mutations in the β subunit gene, both altering the amino acid sequence (Trp8Arg and Ile15Thr). The LH variant has elevated bioactivity *in vitro* but significantly shorter (5–9 min) half-life in circulation than the wild-type LH (12–22 min) (63). This variant is common worldwide, with carrier frequency varying from 0 to 52% in various ethnic groups. Its incidence in Italy ranges between 12 and 13%. Another polymorphism that might be implicated in impaired ovarian response relates to those altering the Luteinizing hormone/human chorionic gonadotropin receptors (LHCGR) (64, 65). Specifically, a prospective cohort study investigated the effect of multiple gonadotropin polymorphisms on ovarian response in 94 normogonadotropic Caucasian women who underwent OS with a starting dose of 150 IU of recombinant FSH daily. In this study, the presence of allele C on both FSHR-min29 and LHCGR-291 was associated with an increased ratio between the cumulative r-FSH consumption and the total number of oocytes as well as mature oocytes (RR: 5.47, CI 95%: 3.13–7.81, $p < 0.001$) (65).

Lastly, a 2018 systematic review and a further meta-analysis evaluating the role of rLH in ART concluded that adding rLH to the stimulation protocol could be beneficial in two subgroups of patients, namely, (i) women with adequate prestimulation ovarian reserve parameters and an unexpected hyporesponse to rFSH monotherapy, and (ii) those with 36–39 years of age (57, 66). As discussed in the previous sections, many patients classified as POSEIDON's groups 1 and 2 will fit in the former subgroup. It seems, therefore, sound to consider adding rLH to OS. For them, 75–150 IU rLH can be started at the mid-follicular phase in an attempt to rescue the ongoing cycle or at stimulation day 1 in a subsequent cycle.

Dehydroepiandrosterone

Dehydroepiandrosterone (DHEA) supplementation before OS has been proposed to counteract the age-related fertility decline (67–69). A last Cochrane meta-analysis including 12 RCTs concluded that pretreatment with DHEA could significantly improve live birth rate in poor responders and in advanced age women (69). Two RCTs trials was conducted in women fitting Poseidon groups 2, namely those characterized by advanced age and good ovarian reserve (70, 71). In detail, Tartagni et al. conducted a RCT including 109 women between 35-40 years old with good ovarian reserve (i.e., AMH levels above 2 ng/mL). Patients recruited were assigned to DHEA supplementation ($n = 53$, 75 mg/die) or placebo ($n = 56$) eight weeks before OS. Higher live birth rate (22/53 vs. 18/56, $p < 0.05$) and lower miscarriage rate (0/53 vs. 5/56, $p < 0.05$) was observed in women supplemented with DHEA than in the placebo group. Similar findings was observed by Moawad et al. (71) in another RCT in which population study was randomized to receive DHEA (75mg/die) supplementation for 12 weeks before OS. Indeed, higher ongoing pregnancy rate (11/58 vs. 7/47, $p < 0.05$), was observed in women supplemented with DHEA versus no supplemented group. The rational behind the use of androgens could be related to the fact that an impaired theca function and androgens production is observed in advanced age women (72). Notably, it was observed that rFSH administration alone is not able to sustain androgens production in advanced age women (73). Furthermore, these findings corroborate the hypothesis that LH supplementation, which is the main regulator of theca cells, could be of use in advanced age women.

Double Stimulation in the Same Ovarian Cycle

A novel controlled ovarian stimulation approach has been proposed in women of low prognosis as a mean to increase the number of retrieved oocytes and the number of blastocyst available to biopsy for preimplantation genetic test for aneuploidies (PGT-A) in a single ovarian cycle (74). This method, referred to as "DuoStim," combines the conventional follicular phase stimulation (FPS) with luteal phase stimulation (LPS). In both the FPS and LPS, patients undergo co-treatment with a maximal dose of rFSH (300 IU/day) plus rLH (150 IU/day) using a GnRH antagonist regimen (75) (**Figure 4**). The final maturation of oocytes in FPS and LPS was triggered by a subcutaneous bolus of buserelin (dose 0.5 mL) to reduce the time of luteolysis and the second stimulation started five days after the first retrieval. The DuoStim protocol might be considered a putative strategy in patients classified as Poseidon's groups 2 and 4, who are characterized by advanced maternal age. In this regards, a case-control study included 188 poor prognosis patients undergoing DuoStim with PGT-A, fitting at least two of the following conditions: poor ovarian reserve (i.e., AMH < 1.5 ng/mg, AFC ≤ 6), advanced maternal age ($\geq$35 years) and history of few numbers of metaphase II (MII) oocytes ($\leq$5) demonstrated that oocytes/embryos derived from LPS showed similar oocytes competence as FPS-derived ones. Moreover, the authors provided evidence that on average more MII oocytes can be retrieved after LPS than after FPS (75, 76). Therefore, in these patients maximizing the number of oocytes would result in a dramatically higher opportunity to obtain a competent embryo per menstrual cycle in comparison to conventional stimulation. DuoStim was then explored in a large multicentre experience involving 310 women indicated to PGT-A, which confirmed comparable fertilization, blastocyst, euploidy, and pregnancy rates after euploid single-embryo-transfer oocytes/embryos from the FPS and LPS. In turn, the rate of patients obtaining at least one euploid blastocyst significantly increased from 42.3% ($n = 131/310$) after FPS-only to 65.5% ($n = 203/310$) with the contribution of LPS (77). Nevertheless, these results should be interpreted with caution since the treated patients did not explicitly fulfill the Poseidon groups 1–2 criteria. Thus, since

promising, the use of DuoStim in Poseidon Groups 2 patients should be investigated further.

CONCLUSIONS

Infertile patients undergoing ART may respond poorly (<4 oocytes retrieved) or suboptimally (4–9 oocytes retrieved) to gonadotropin stimulation despite the presence of adequate ovarian parameters. According to the new POSEIDON's criteria of low prognosis patients undergoing ART, they are classified as group 1 if younger than 35 years-old or group 2 if ≥35 years-old. Both groups are likely to have lower cumulative live birth rates than normal or high responders, an effect that is modulated by age. The pathophysiology mechanisms explaining this phenomenon are not fully understood but seem to be mainly associated with a polygenic trait involving gonadotropins and their receptors. The primary goal of management in POSEIDON's groups 1 and 2 patients is to maximize oocyte yield as to increase the likelihood of having at least one euploid embryo for transfer. For this, indices such as FORT (follicle output rate) and FOI (follicle-to-oocyte index) should be used to both identify the subset of hypo-responders and to determine if the ovarian reserve was adequately explored during a previous stimulation.

Moreover, testing for the presence of common polymorphisms affecting gonadotropins and/or their receptors might be considered in women belonging to Poseidon groups 1 and 2. Added to this, an individualized estimation of the number of oocytes needed to achieve at least one blastocyst for transfer-for instance, using the ART calculator- can make treatment more focused and cost-effective. Given the overlapping between POSEIDON's groups 1 and 2 categories and hypo-response to OS, several pharmacological interventions may be considered as regards clinical management of such patients. According to the best available literature, there are at least five strategies to be considered, which are not mutually exclusive, namely (i) use of recombinant FSH in preference over urinary gonadotropin preparations, (ii) FSH dosage increase, (iii) use of rLH supplementation, (iv) Androgens supplementation before OS, (v) DuoStim. Nevertheless, no trial explicitly examining the role of interventions to POSEIDON's groups 1 and 2 patients has been carried out yet. The introduction of POSEIDON criteria and practical indices such as FOI, along with polymorphism testing, could help to understand better this specific subgroup of patients undergoing ART. Also, such an approach can be used to design robust clinical trials aiming at finding the optimal clinical management, thus making it an area open for further research.

AUTHOR CONTRIBUTIONS

AC, CA, and SE designed the manuscript and scrutinized the literature. All authors contributed to drafting and critical discussions. All authors contributed to revised and accepted the final manuscript.

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Future Perspectives of POSEIDON Stratification for Clinical Practice and Research

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A total of 50% of patients undergoing IVF treatment has previously been estimated to fulfill the POSEIDON classification criteria; importantly, although the reproductive prognosis differs between patients, POSEIDON patients share the same characteristic of a low ovarian response to exogenous gonadotropin stimulation—independent of age. POSEIDON patients require focused attention as regards ovarian stimulation in order to increase the chances of having at least one euploid blastocyst for transfer—the success criterion for stimulation set forth by the POSEIDON Group. The key to success seems to be individualization in all steps of treatment. In this perspective article we discuss the future impact of the POSEIDON stratification for daily clinical practice as well as for research.

Keywords: ART calculator, Bologna criteria, blastocyst, controlled ovarian stimulation, low ovarian response, pregnancy, POSEIDON criteria

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FUTURE PERSPECTIVES OF POSEIDON STRATIFICATION FOR DAILY CLINICAL PRACTICE

As previously mentioned in this supplement, the incitement to propose the POSEIDON criteria was the high degree of heterogeneity seen in the ESHRE Bologna criteria population (1, 2). With the new POSEIDON stratification significantly more homogenous sub-populations were created, taking age, ovarian reserve, and previous ovarian responses after stimulation with gonadotropins into account. Thus, the overall idea of the POSEIDON stratification was to not only guide clinicians regarding clinical management of the patient, but also to be a counseling tool to help set patient expectations prior to initiation of ovarian stimulation. For this purpose, a number of clinical recommendations in terms of type of GnRH analog, gonadotropin type and dosing were suggested in order to obtain the new marker of success: the number of oocytes needed in each individual

patient to obtain one euploid blastocyst. This quite naturally led to the subsequent development of the ART calculator (3). As seen from **Figures 1**, **2** the main factor distinguishing Groups 1 and 2 from Groups 3 and 4 is the ovarian reserve and a previous response to stimulation with exogenous gonadotropins (1, 2, 5).

Until now clinical management of the low prognosis patient has primarily been based on small studies including heterogenous populations which left clinicians with poor evidence to manage the low prognosis patient—and often a "trial and error" strategy was adopted by individual clinicians. With the POSEIDON stratification the clinician will very quickly get an impression of whether the individual patient fulfills the criteria of being a POSEIDON patient—and if positive (∼50%)—to which of the four groups the patient belongs (6). This places POSEIDON as a daily partner in the clinic; moreover, the POSEIDON patient generally is a patient who needs more clinical consideration and individualization when compared to the other half of patients, constituted by non-POSEIDON patients.

As mentioned previously, patients in POSEIDON groups 1 and 2 underwent one or more stimulations leading to an unexpected impaired ovarian response. Either a low response resulting in <4 oocytes (Groups 1a and 2a) or a suboptimal response, resulting in 4–9 oocytes (Groups 1b and 2b). As seen from **Figure 1** it is suggested that POSEIDON groups 1 and 2 patients undergo their next stimulation with an increase in rFSH dosing, rLH supplementation from day 1 of stimulation as well as GnRH antagonist co-treatment. The main difference between groups 1 and 2, is age and consequently, a difference in oocyte euploidy, and thus, reproductive potential. In general, one could classify the Group 1 patient as a patient with a good ovarian reserve—and due to her age also an expected good oocyte quality (7). In contrast, the Group 2 patient has an age-related increased oocyte aneuploidy although the ovarian reserve is good (7). This means that the number of oocytes needed to obtain success is significantly higher for the aging patient, but with her good ovarian reserve she is likely to reach the estimated number of oocytes needed for one euploid blastocyst (3). This means that future use of the POSEIDON stratification with or without the use of the ART calculator will help clinical decision-making as well as counseling.

Groups 3 and 4 are characterized by a low ovarian reserve which *per se* induces a poor reproductive prognosis. However, age makes a significant difference for success, and it is expected that the younger patient will have a four-times higher probability of a live birth per transfer as compared to the older patient– 20 vs. 5% (8). Again, the POSEIDON stratification will help clinical decision-making and counseling. As shown in **Figure 2**, the suggested handling of the POSEIDON group 3 patient would include either a long GnRHa down-regulation or a "primed" GnRH antagonist co-treatment (synchronization with short term estradiol or progestin treatment or oral contraceptive pill treatment) followed by stimulation with a maximum dose of 300 rFSH. In selected cases with a low oocyte yield and based on the estimate made by the ART calculator, DUO-Stim should be recommended for oocyte or embryo accumulation to shorten time to pregnancy (9–11).

With the increasing delay in child bearing, POSEIDON group 4 patients become more and more prevalent—in some centers constituting 55% of the POSEIDON population (6).The dual negative effect of a reduced ovarian reserve (quantity) as well as an age related increase in aneuploidy (quality) makes this category of patients difficult to handle (7). The POSEIDON recommendation for this patient would include either long GnRHa down-regulation or a "primed" GnRH antagonist co-treatment, followed by stimulation with a maximum dose of 300 rFSH and 150 IU rLH from day one of stimulation. In selected cases with low oocyte yield, DUO-Stim should be recommended for oocyte or embryo accumulation bearing in mind cost-efficiency—especially in women >40 years old (9–11). Although the initial attitude toward oocyte donation could be negative in a large proportion of older Group 4 patients (12), from a scientific point of view the best chance for a live birth would be oocyte donation.

Taken together, we see the POSEIDON stratification as a daily tool in future clinical practice, supporting not only clinical, but also patient decision-making.

FUTURE PERSPECTIVES OF POSEIDON STRATIFICATION FOR RESEARCH

Currently, retrospective analyses of large databases may match patients so as to fit into one of the four POSEIDON groups. However, it is quite unlikely that patients were treated according to the recommendations made by the POSEIDON stratification. Thus, future RCT's are necessary to evaluate the stratification and the recommendations set forth in this supplement. In this aspect, POSEIDON groups 1 and 2 need to be studied separately from groups 3 and 4.

Future Research in Groups 1 and 2

Groups 1 and 2 encompass good reserve patients, some of whom have the presence of FSH-R and LH-R polymorphisms or variant LHβ (13). Previous reports show that an increase in rFSH in patients with an unexpected low response to ovarian stimulation in the first stimulation cycle increases the number of oocytes retrieved which could be the effect of FSH receptor polymorphisms (14, 15). As regards rLH supplementation this has previously been proven to significantly increase clinical pregnancy rates (16–19). Future studies should evaluate the benefit of screening patients prior to their future stimulation for FSH-R and LH-R polymorphisms as well as variant LHβ (20). From the findings, the subsequent stimulation should be tailored accordingly; thus, patients with FSH-R polymorphisms should have an increase in FSH dosing, whereas patients with LH-R polymorphisms and presence of variant LHβ should be treated with rLH from day 1 of stimulation. The primary end point of these studies should be cumulative

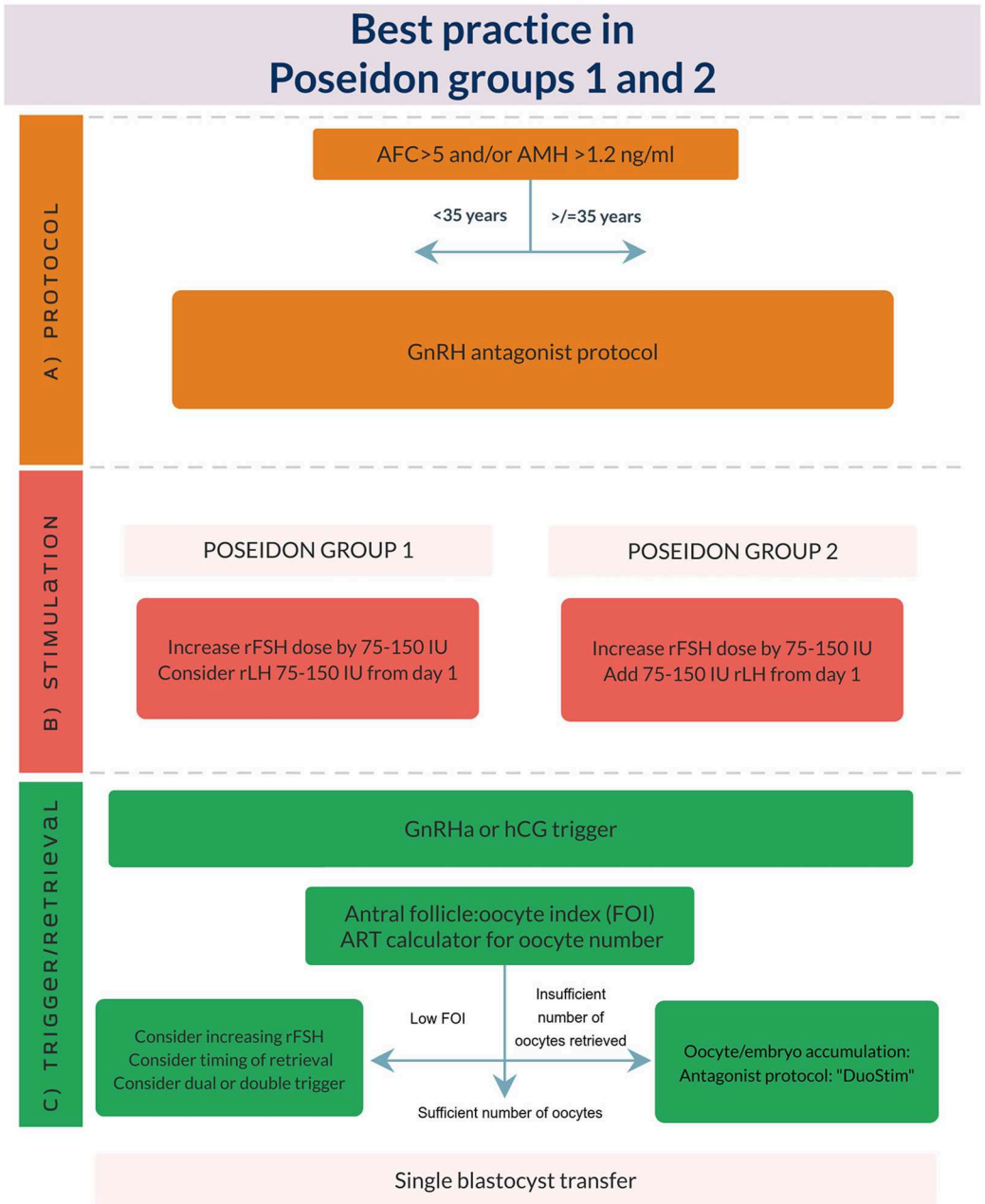

FIGURE 1 | Best practice in POSEIDON groups 1 and 2. **(A)** POSEIDON recommends use of GnRH antagonist co-treatment for all patients in POSEIDON groups 1 or 2. **(B)** Ovarian stimulation strategy: First choice should be an increase in daily rFSH dose by 75–150 IU. In POSEIDON Group 1 patients with follicular stagnation at follicle sizes 1–12 mm, rLH 75–150 IU daily should be added from day 1 of stimulation. In POSEIDON 2 patients rLH 75–150 IU daily should be added to all patients from day 1 of stimulation. **(C)** Ovulation trigger strategy: GnRHa is mandatory in the follicular phase stimulation of the DuoStim protocol. All trigger agents can be used in the luteal phase stimulation. In non DuoStim GnRH antagonist cycles, the choice of trigger between GnRHa and hCG should rely on the embryo transfer strategy (fresh or frozen), patient characteristics (e.g., hypo-hypo) and clinical experience. In cases with a low FOI (4), clinicians should consider pretreatment including short term estrogen or progestin therapy, or OCP for synchronization of the follicles prior to stimulation, adjuvant LH activity during stimulation, or changing trigger strategy to either dual or double trigger. In case of an insufficient number of oocytes retrieved as determined by the ART calculator, the probability of transferring a euploid embryo should be discussed with the patient to counsel whether an immediate transfer or a new stimulation should be suggested (3).

live birth (CLBR), i.e., the live births obtained after one embryo transfer and the subsequent frozen cycles within a 2–3-years period. The suggested secondary endpoint is the achievement of the number of mature oocytes needed to obtain at least one euploid blastocyst as per the ART calculator estimation (3).

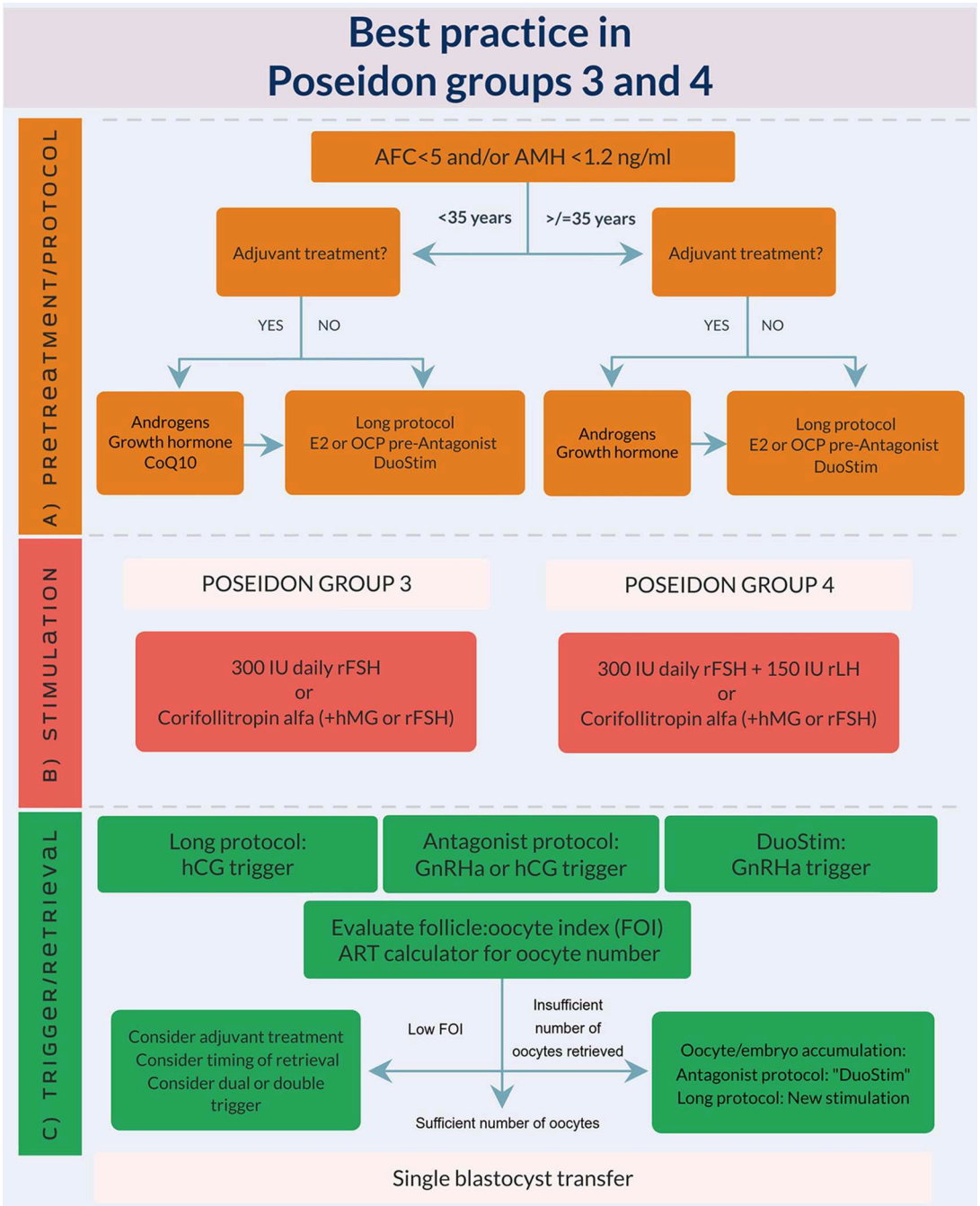

FIGURE 2 | Best practice in POSEIDON groups 3 and 4. **(A)** Pretreatment is rarely the first option in low prognosis patients, but in case of low response to ovarian stimulation, e.g., asynchrony of follicular growth and inadequate ovarian response, pretreatment should be considered. The choice should rely on availability, clinical experience and patient preference. Stimulation protocol might start using GnRH antagonist co-treatment keeping in mind the possibility of converting to DuoStim to achieve the individualized oocyte number (according to the ART calculator). Otherwise a long GnRHa protocol should be considered first choice. **(B)** Ovarian stimulation strategy: First choice in Poseidon group 3 is the GnRH antagonist cycle using either 300 IU daily of rFSH alone or Corifollitropin alfa followed by either rFSH or hMG. In POSEIDON group 4 patients, rLH (75–150 IU daily) should be added from day 1 of stimulation, unless the combination of Corifollitropin alfa and hMG was chosen. GnRH antagonist co-treatment allows the use of Duostim. **(C)** Ovulation trigger strategy: In the long GnRHa down-regulation protocol hCG is mandatory as ovulation trigger, whereas GnRHa is mandatory in the follicular phase stimulation of the DuoStim protocol. All trigger agents can be used for the luteal phase stimulation. In non DuoStim GnRH antagonist cycles, the choice of trigger between GnRHa and hCG should rely on the embryo transfer strategy (fresh or frozen), patient characteristics, and clinical experience. In cases with a low antral follicle to oocyte ratio (FOI) as determined on trigger day (4), clinicians should consider: pretreatment including short term estrogen or progestin therapy, or OCP for synchronization of the follicles prior to stimulation, adjuvant LH activity during stimulation, or changing trigger strategy to either dual or double trigger. In case of an insufficient number of oocytes retrieved as determined by the ART calculator, the probability of transferring a euploid embryo should be discussed with the patient to counsel whether an immediate transfer or a new stimulation should be suggested (3).

Future Research in Groups 3 and 4

The question asked for groups 3 and 4 is—can we increase the number of growing follicles and subsequently the number of competent oocytes? First of all, which GnRH analog regimen is the most optimal for Groups 3 and 4: the long GnRHa down-regulation protocol—or the GnRH antagonist protocol primed with either daily estradiol for 5 days prior to the unset of menses, or 12–14 days of oral contraceptive pills. Moreover, will long term pretreatment with androgens, or short-term pretreatment with growth hormone before and during stimulation have an effect on the number of growing follicles and oocytes? This question needs to be explored in future RCT's. Another pending question is whether DUOstim reduces time to live birth for groups 3 and 4. An RCT comparing DUOStim to a long GnRHa down-regulation protocol or a "primed" GnRH antagonist protocol is necessary to answer this question. Here again, cumulative live birth (CLBR), i.e., the live births obtained after one embryo transfer and the subsequent frozen cycles within a 2–3-years period will be the primary endpoint whereas the POSEIDON marker of success in ART, namely, the number of mature oocytes needed to obtain at least one euploid blastocyst as per the ART calculator estimation will be the secondary endpoint (3).

CONCLUSIONS

The POSEIDON stratification has been well-accepted by reproductive endocrinologists and infertility specialists worldwide, however, this novel classification system needs to be prospectively investigated. It is our hope that during the following years POSEIDON and the ART calculator will be an integral part of daily clinical practice used for decision-making and counseling with the aims of providing the most optimal treatment of the patient and reducing time to live birth.

AUTHOR CONTRIBUTIONS

The style and concept were developed by PH and TH. All authors actively contributed to writing the manuscript and accepted the final version of this manuscript.

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REVIEW
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Management Strategies for POSEIDON Groups 3 and 4

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In the POSEIDON classification, patients belonging to groups 3 and 4 share the same common feature of a poor ovarian reserve which independently of age renders them at high risk of a poor reproductive outcome. Overall, POSEIDON groups 1–4 constitute approximately 47% of patients attending assisted reproductive technology (ART) treatment. With the increasing delay in childbearing, POSEIDON group 4 seems to increase in numbers now in some centers constituting more than 50% of the total POSEIDON population, whereas group 3 patients constitute approximately 10%. Both POSEIDON groups 3 and 4 patients require special attention as regards pre-treatment strategy, ovarian stimulation, adjuvant treatment, and ovulation trigger strategy in order to optimize the probability of having at least one euploid blastocyst for transfer. Although more evidence is needed, recent advances seem to have increased the reproductive outcomes in the poor prognosis patient. The key to success is individualization in all steps of ART treatment. Herein, we review the recent evidence for the management of POSEIDON groups 3 and 4.

Keywords: poor ovarian response, Bologna criteria, POSEIDON criteria, controlled ovarian stimulation, blastocyst, pregnancy, ART calculator

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PREVALENCE OF POSEIDON GROUPS 3 AND 4

The POSEIDON (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number) population constitutes 47% of patients referred to Assisted Reproductive Technology (ART) treatment (1). POSEIDON group 3 constitutes 10% whereas POSEIDON group 4 constitutes 55% (1). In a group of Bologna criteria poor ovarian response (POR) patients, the prevalence of POSEIDON group 3 and 4 patients was recently reported to be 24% (13/54) and 76% (41/54), respectively (2). As these patients have a high risk of ending up with no high quality embryos for transfer (3), they often undergo repeated numbers of ovarian stimulations with a subsequent increase in both physical, emotional and financial cost. In this review, we add to the prior work

considering POSEIDON classification (4–6) by giving recommendations for clinical management and further research in POSEIDON groups 3 and 4.

EVIDENCE FOR MANAGING POSEIDON GROUPS 3 AND 4 PATIENTS

Although studies in POSEIDON groups 3 and 4 patients are emerging (7, 8), there are currently very few prospective studies comparing different treatment strategies. Hence, the present suggestions for clinical management is mainly based on evidence from patients labeled with POR. In this aspect, it is important to distinguish between studies performed before and after the introduction of the Bologna criteria for POR. Prior to the Bologna criteria, studies used multiple definitions of POR, introducing heterogeneity and subsequently a poor clinical value of the reported results, in particular those of meta-analyses (9). In the latest Cochrane review from 2010 in POR management, it was reported that there is no evidence to support one particular intervention (10). However, Cochrane meta-analyses may not be the optimal tool to evaluate treatment strategies while such strategies are still undergoing development and additional fine tuning (11, 12). In this aspect, and while waiting for better evidence, this review may help clinicians plan how to most optimally manage the poor prognosis patient which is an integral part of daily clinical life.

MEASURE OF SUCCESS

According to the POSEIDON concept, the measure of success is to increase the probability of having at least one euploid blastocyst for transfer in the individual patient (6). Recently, a predictive tool so-called "ART Calculator" was launched to estimate the number of oocytes needed to have at least one euploid blastocyst for transfer, available on http://www.members.groupposeidon.com/Calculator/. This calculator provides the estimation mentioned above based on a number of predictors such as female age and type of sperm, which were found to be relevant concerning blastocyst euploidy, see **Figure 1** (1, 13). Thus, using mathematical equations and the age-related probabilities of a blastocyst being euploid per mature oocyte as a function of sperm source, the ART calculator makes two types of predictions automatically, one using pre-treatment information to estimate the minimum number of mature oocytes to achieve at least one euploid blastocyst, and another based on

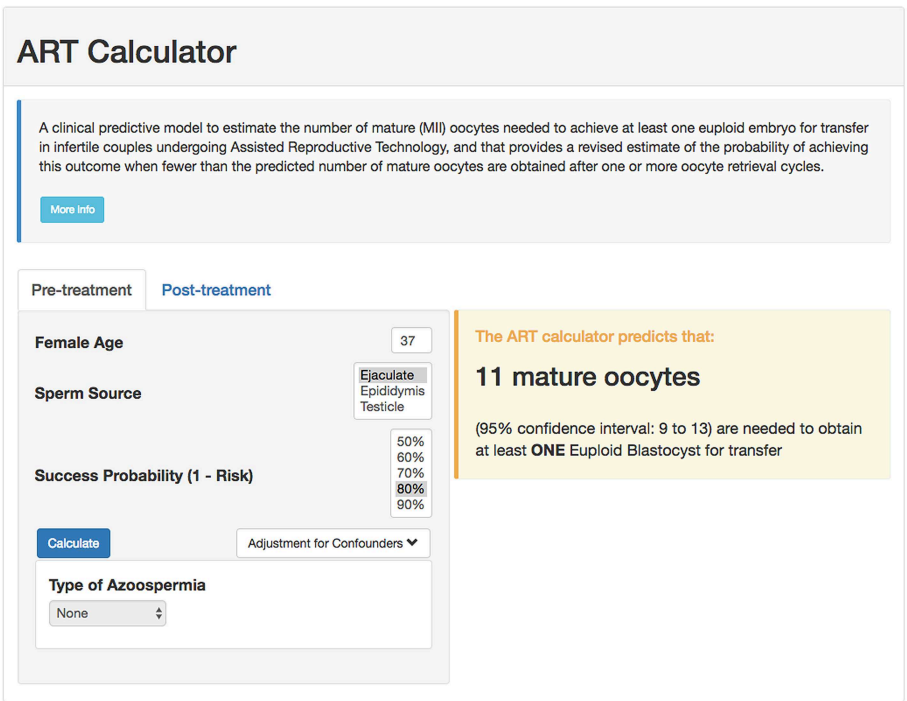

FIGURE 1 | Online calculator to determine the minimum number of mature oocytes required to obtain at least one euploid blastocyst for transfer in infertile patients undergoing IVF/ICSI cycles. The figure shows how the online calculator can be used in an office-based setting. Pre-treatment, clinicians should input the patient age and the sperm source to be used for IVF/ICSI. If the option "Testicle" is marked, then the type of azoospermia should be also defined. The probability of success is set by the user and indicates the chance of having ≥1 euploid blastocyst when the predicted number of mature oocytes is achieved. Its complement is the risk, that is, the chance of having no (zero) euploid blastocysts when the predicted number of oocytes is achieved. Once the button "calculate" is pressed, a text box will pop-up on the right side of the screen, indicating the predicted minimum number of mature oocytes needed for obtaining at least one euploid blastocyst, with its 95% confidence interval (reprinted with permission of the author).

the actual number of mature oocytes collected/accumulated to estimate the chances of having a euploid blastocyst using that oocyte cohort for IVF/ICSI, see **Figure 2**. Apart from guiding the clinician in individualized management, the ART calculator constitutes an ideal tool to counsel patients about their prognosis when embarking on ART treatment, and subsequently, at the time of oocyte retrieval where some patients might be counseled to go directly to a luteal phase stimulation in order to increase the chances of having at least one euploid blastocyst for transfer (13). As an example, the ART calculator estimates that at least 6–9 and 10–15 mature oocytes are needed to obtain one euploid blastocyst for transfer in POSEIDON groups 3 and 4 patients aged 33 and 36 years-old, respectively, assuming a 90% probability of success in the estimations when ejaculated sperm is used for IVF/ICSI. Hence, planning of the most optimal ovarian stimulation regimen is of paramount importance to achieve the highest success rate.

OVARIAN STIMULATION IN POSEIDON GROUPS 3 AND 4

Natural Cycle or Stimulation With Exogenous Gonadotropins

Previously, some authors expressed concern that stimulation *per se* would increase embryonic aneuploidy rates, suggesting that natural cycle IVF might be an option for the POR patient (14, 15). However, abundant evidence does not support this concern neither in young oocyte donors nor in PGS IVF-ET patients (16–19). Moreover, natural cycle IVF results in extremely low live birth rates in the POR patient with a reported live birth rate per cycle of only 2.6% and a cumulative live birth rate of only 7% after six natural IVF cycles in Bologna POR patients (20). Similarly, extremely low live birth rates after natural cycle IVF have been corroborated by others (21). In contrast the largest RCT aligned with the ESHRE Bologna POR criteria reported a live birth rate per cycle of 11% using a combination of a long gonadotropin releasing hormone agonist (GnRHa) down-regulation protocol and daily gonadotropin dosing with 300 IU recombinant FSH and 150 IU recombinant LH (22). Recently, combining follicular and luteal phase stimulation in the same ovarian cycle two trials reported ongoing pregnancy rates above 20% per DuoStim cycle in poor prognosis patients (2, 23). Thus, ovarian stimulation rather than natural cycle should be the preferred first line treatment in the poor prognosis patient with a poor ovarian reserve.

Stimulation Protocol

A meta-analysis in non-Bologna criteria POR patients explored the optimal GnRH analog treatment (24). From this analysis, it was concluded that there was no significant difference in clinical

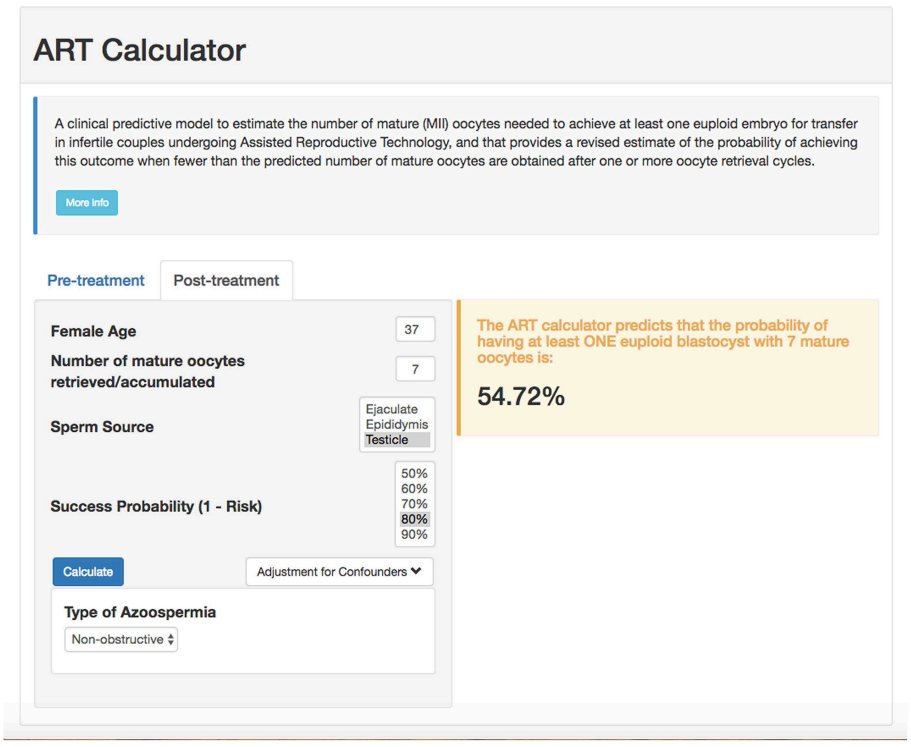

FIGURE 2 | ART online calculator. The figure shows how the online calculator can be used post-treatment, i.e., when fewer than the predicted number of mature oocytes are obtained after one or more oocyte retrieval cycles. Clinicians should input the pre-treatment information and the actual number of mature oocytes collected or accumulated. The probability of success is set by the user; it reflects the chance that the estimation is correct given the number of oocytes input. Once the button "calculate" is pressed, a text box will pop-up on the right side of the screen, indicating the predicted probability of achieving ≥1 euploid blastocyst with the number of mature oocytes available (reprinted with permission of the author).

pregnancy rates comparing the long GnRHa down-regulation protocol to the GnRH antagonist protocol, although the trend favored the long GnRHa down-regulation protocol. Later, a study published in Bologna POR patients reported that the long GnRH agonist protocol, albeit non-significantly, increased the number of mature oocytes by one oocyte as compared to the GnRH antagonist protocol (25). Moreover, the cancellation rate was significantly lower for the long GnRHa protocol. The biological plausibility for this finding may be follicular synchronization obtained after downregulation, which is paramount for the poor ovarian reserve patient as this patient usually has an increased late luteal FSH level, promoting early recruitment of the leading follicle, which in turn will suppress the early growth of the few other follicles residing in the ovary. This inhibitory effect on endogenous FSH—and early recruitment—can also be achieved in GnRH antagonist cycles, using short term daily estradiol 4 mg, or oral contraceptives for 12–16 days as pre-treatment without compromising reproductive outcome as compared to the long GnRHa down-regulation protocol (26, 27). As one more oocyte increases the live birth rate (LBR) by approximately 5% (28, 29), a long GnRH agonist down-regulation protocol or a "primed" GnRH antagonist protocol as mentioned above should be considered first line treatment for the poor prognosis patient. A recent retrospective study in POSEIDON groups 3 and 4 patients reported that a higher live birth rate per initiated cycle can be achieved in group 3 patients by using hMG in GnRHa down-regulation protocol as compared to hMG in GnRH antagonist protocol ($7/54 = 13.0\%$ vs. $78/283 = 27.6\%$, $p = 0.024$) (7). This effect was not noted in POSEIDON group 4 patients. However, a retrospective analysis of 999 poor prognosis patients (defined as AFC < 11 and AMH < 1.1 ng/ml) in the long down-regulation protocol and comparing a rLH + rFSH regimen to hMG showed that rLH + rFSH was superior to hMG regarding the clinical pregnancy rate per started cycle (12.5 vs. 8.1%, $P < 0.02$) (30). Interestingly, this effect was even more pronounced in the patients with AFC<4 (10.2 vs. 1.5%, $P < 0.01$). Another protocol is the so-called mild stimulation protocol (31, 32), but this approach is poorly defined most often involving the GnRH antagonist protocol using low dose gonadotropin stimulation compared to a long GnRH agonist protocol with higher doses of gonadotropin (33). Although recommended in the American clinical guideline for POR (34), mild ovarian stimulation for POSEIDON groups 3 and 4 is an approach not in line with the POSEIDON stratification as discussed extensively in the paragraphs on natural cycle and FSH dosing.

The recent advances in dual stimulation ("DuoStim") represents an interesting solution to accumulate embryos (blastocysts) within a short time span in order to obtain the number of blastocysts needed to increase the probability of having at least one euploid blastocyst for subsequent elective frozen embryo transfer (eFET) (35–38). In a recent publication, Vaiarelli et al. (23) reported that poor prognosis patients (essentially POSEIDON group 4) undergoing a single "DuoStim" cycle resulted in a total of 65.5% (203/310) of patients having at least one euploid blastocyst for transfer (23). Following single euploid blastocyst transfer the ongoing pregnancy rate per transfer was similar comparing blastocysts obtained from follicular phase stimulation and blastocysts obtained from luteal phase stimulation, 39.5% (32/81) and 49.4% (41/83), respectively (23). Although the study excluded patients with no response to DuoStim (43/353), an ongoing pregnancy rate per DuoStim cycle of 20.7% (73/353) in POSEIDON group 4 patients can be considered highly successful in this difficult patient group. Another recent study used the combined advantages of Corifollitropin alfa and Duostim in Bologna criteria patients ($N = 54$), 24% (13/54) and 76% (41/54) were POSEIDON group 3 and 4 patients, respectively. In this study, authors reported an ongoing pregnancy rate per DuoStim cycle of 20.4% (11/54) (2). Hence, evidence suggest that even in poor prognosis patients ongoing pregnancy rates of around 20% can be achieved. However, there are currently no results from prospective randomized trials comparing DuoStim to two conventional stimulation cycles with cumulative live birth rate and time to live birth as end points. Importantly, in DuoStim a freeze-all policy is mandatory, which includes additional manipulations with biological material and costs for the patient or the health care system. Until further, we have to await the results of registered ongoing trials before final conclusions can be made.

Choice of Gonadotropin

A Cochrane meta-analysis covering the normogonadotropic IVF/ICSI population concluded that the type of gonadotropin should be based on availability, convenience and costs (39). Likewise, a large survey involving 314 centers from 73 countries worldwide concluded that the majority of respondents (62.2%) did not believe that there was a difference in efficacy between urinary (u) FSH and rFSH preparations and that the choice of gonadotropin was most often based on the individual preference of the clinician (40). Despite no significant results comparing all types of uFSH with rFSH in normogonadotrophic women (28 trials, 7,339 couples, odds ratio (OR) 0.97, 95% CI 0.87–1.08) (39), a sub analysis observed that hMG was superior to rFSH as regards live birth rate per woman (11 trials, $N = 3{,}197$, OR 0.84, 95% CI 0.72–0.99). However, a recent meta-analysis of 70 prospective studies considering all gonadotropin combinations and all ART outcomes, reported that recombinant FSH alone resulted in greater number of oocytes than hMG or rFSH+rLH (41). The addition of LH activity was useful to reduce the amount of FSH needed and to improve pregnancy outcome, but only if LH activity was provided by rLH rather than hCG. In the context of this review, the question is whether these results can be extrapolated to poor prognosis patients and, admittedly, the results are difficult to interpret. When the effectiveness of the gonadotropin regimen is the focus of the investigation, the primary endpoint should also include the ovarian response, which is a critical measurable parameter of gonadotropin action (42). By contrast, pregnancy is the final result which is influenced by a multitude of factors, including endometrium receptivity, sperm factors, etc. In this regard, high quality evidence overwhelmingly indicates that recombinant FSH is superior to urinary FSH and hMG as a means to increase the oocyte yield (43–47). Since the POSEIDON criteria relies on the individualized oocyte number to increase the likelihood of having at least one euploid blastocyst for transfer, it seems sound

to conclude that recombinant FSH, used alone or combined with recombinant LH, is the natural choice in Poseidon group 4 patients. The use of gonadotropin regimens combining recFSH and LH activity supplementation by recLH in Poseidon group 4 might offer additional clinical benefit, as discussed in the next section, owing to a fine-tuned modulation of the PKA pathway and proliferative/antiapoptotic signals, unlike hCG (42). In conclusion, hMG does not seem to add any clinically significant benefit as regards reproductive outcomes in the GnRH antagonist protocol, and likewise added LH activity in the long GnRHa down-regulation protocol seems to be better covered by r-LH than by hMG.

Another agent for ovarian stimulation is Corifollitropin alfa which has the pharmacokinetic advantage of a rapid increase in the FSH serum level which optimizes early recruitment and increases the number of pre-ovulatory follicles (48). In a RCT including Bologna PORs only, there was no significant difference in live birth rate after fresh embryo transfer, however, significantly more embryos were cryopreserved in the group treated with Corifollitropin alfa followed by hMG as compared to a rFSH only regimen which hypothetically would increase the cumulative live birth rate (49). From a POSEIDON point of view, it is important to achieve more embryos in order to maximize the chance of having one euploid blastocyst for transfer, however, a larger sample size would be needed to reach statistical significance as regards live birth rates (49).

FSH Dosing: Individualization or One Size Fits All?

Recently, the OPTIMIST trial reported that a starting dose of 150 IU FSH (91% used rFSH) provided a similar cumulative LBR after 18 months follow-up as compared to individualized dosing with either 225 or 450 IU FSH in poor prognosis patients ($N = 511$), who were defined as having an antral follicle count of either 8–10 or <8, respectively (50, 51). Subsequently, the study was heavily criticized by many clinical researchers and for a multitude of reasons (52–54). First of all, the definition of poor prognosis was not in line with neither the ESHRE Bologna nor the POSEIDON criteria (52). Secondly, the individualized dosing significantly reduced cycle cancellation and increased the number of good quality embryos for transfer and, finally, the 18-month follow-up period for cumulative live birth rate was criticized for not sufficiently covering supernumerary FET cycles (54). In fact, to show an increase from 20 to 25% in LBR, more than 2,000 patients should have been randomized in order to achieve significant results (53). Hence, the conclusion of the OPTIMIST trial suffered from many shortcomings and, in our opinion, the current best practice in managing poor prognosis patients should be to individualize the ovarian stimulation in order to increase the oocyte yield which is the only key to optimize LBR as seen in large cohort studies (29, 55, 56). In fact, a pivotal study by Sunkara et al. ($N = 400{,}135$ cycles) found that increasing the oocyte yield from 2 to 3 resulted in a 25% relative increase in LBR across all age groups (29). Thus, results from large databases with LBR as outcome have a significantly higher clinical value as compared to small and underpowered studies which came to the conclusion that a higher oocyte number does not lead to a higher number of good quality embryos (57) As regards the maximum daily FSH dose, it was shown that rFSH dosing above 300 IU rFSH daily does not seem to increase the LBR (58). In fact, a large retrospective study ($N = 658{,}519$) reported that daily dosing above 300 IU of FSH (including both uFSH and rFSH) significantly decreased the odds of a live birth (59).

ADJUVANTS TO OVARIAN STIMULATION

Over the years many adjuvants to standard ovarian stimulation have been proposed to increase LBR for the POR patient. In this paragraph we focus on relevant adjuvant therapy where evidence is relatively extensive; thus, excluding e.g., use of platelet enriched plasma, mitochondrial transfer and stem cells treatment where evidence is based primarily on case series.

Androgens

Pretreatment with androgens has been used for the POR patient in several trials. This approach could be considered for Poseidon groups 3 and 4 where, independently of age, the ovarian reserve is reduced and POR is expected. The main biological evidence from the primate model is that androgens induce FSH receptors on granulosa cells (60), which in turn increases the recruitability and growth of pre-antral and antral follicles, through the IGF-1 system (61, 62). In 2012, two independent meta-analyses reported a significant positive effect of transdermal testosterone on the LBR of POR patients (63, 64). However, only a total of 82 patients and 113 patients were included in the intervention arm of the respective meta-analyses, which again included studies performed prior to the Bologna criteria. In another meta-analysis of four RCT's and 2 observational studies including a total of 528 patients, Zhang et al. (65) reported that long-term DHEA treatment, the precursor of testosterone, had a significant positive effect on the LBR of POR patients as compared to controls (RR 1.87, 95% CI, 1.22–2.88) (65). Similarly, the latest Cochrane meta-analysis reported moderate quality evidence supporting that DHEA and testosterone pre-treatment may improve LBR in POR patients (66). Although basic scientific and recent clinical evidence seems to support the use of androgen pre-treatment in POR, a recent commentary argued that the "androgen chapter" needs further study before recommendations can be made (67). Especially, the dosage and the timing of pre-treatment needs to be further elucidated; hence an international clinical research group designed the so-called TTRANSPORT TRIAL for Bologna POR patients (Clinicaltrial.gov identifier NCT02418572), evaluating androgen pre-treatment exceeding 60 days, and using a daily dose of 5.5 mg transdermal testosterone. This study designed to include a large population of Bologna POR patients uses androgen pre-treatment in a daily physiological dose and for an extended time compared to previous trials, taking the time needed for folliculogenesis into account. The results of this trial -when completed- could help clarify the clinical utility of pre-treatment with androgens in poor prognosis patients.

LH Supplementation

The physiological rationale for LH supplementation is primarily based on the "two cell two gonadotropin" concept (68, 69), in which LH supplementation stimulates the conversion of cholesterol into androgens in the theca cell, thus, increasing endogenous intra-ovarian androgen production and follicular growth. On one hand, androgens (i) stimulate FSH receptor expression on granulosa cells (60) (ii) act synergistically with IGF1 for the growth of the follicle (62) and in animal models increase the number of pre-antral and antral follicles (70). On the other hand, LH binding to granulosa cell LH receptors–expressed from the mid-follicular phase onwards- sustains FSH dependent granulosa cell activities, including aromatase induction, release of growth factors and regulates final follicle/oocyte maturation (71, 72). To study the possible clinical effect of rLH supplementation Lehert et al. (73) published a meta-analysis based on 6,443 cycles in normal and poor prognosis patients (non-Bologna criteria) who were supplemented or not with rLH (73). Importantly, in that analysis it was not possible to distinguish between hypo responder and POR patients. While rLH supplementation improved clinical pregnancy rates by 9% (NS) in the overall population, the effect was more pronounced in PORs with a relative risk (RR) of 1.30 (95% CI, 1.01–1.67). Recently, Humaidan et al. (22) published the results of the largest RCT in poor prognosis patients aligned with the ESHRE Bologna criteria and POSEIDON group 4 criteria. In this trial, a total of 939 patients were randomized to either a fixed daily dose of either 300 IU rFSH plus 150 IU r-LH or rFSH 300 IU alone (22). The results indicated no significant differences between groups regarding LBR. However, a *post-hoc* analysis, stratifying patients into mild, moderate or severe POR observed that the moderate and severe PORs benefitted significantly from 150 IU rLH supplementation in terms of a higher LBR and a lower total pregnancy loss (22). Finally, two more recent systematic reviews indicated that rLH supplementation is beneficial in women with hypo-response and in women 36–39 years of age, reinforcing the idea of testing this approach in Poseidon group 4 (74, 75).

Growth Hormone

Growth Hormone (GH) has been explored therapeutically in ART for more than 30 years. The biological rationale for its use is that GH itself has a synergistic effect to that of FSH on follicular development and also through its downstream mediator, Insulin-like Growth Factor 1 (IGF-1), as seen in animal models (76, 77). All models which block or impair the action of GH, result in a delay in puberty, a significant reduction on litter size and a delay in the exhaustion of the follicular pool (78). Subsequent microscopic examination of the ovaries in these animal models shows an increase in primordial and primary follicles and a decrease in the number of growing antral and pre-ovulatory follicles (78–80). Knock-out female mice failed to ovulate either spontaneously or under the influence of gonadotropins, proving the importance of GH and IGF1 in increasing the sensitivity to gonadotropins during the whole process of selection and follicular growth to ovulation (81). Until now, scientific evidence suggests that adjuvant treatment with GH for POR patients in IVF leads to a higher number of oocytes retrieved and a lower gonadotropin consumption (82–84). However, meta-analyses until now failed to show differences in LBR, perhaps as a result of most trials being underpowered and using different definitions for POR. Moreover, there is high interstudy variability regarding the route, timing and dose of GH administration. The general pattern has been to explore GH adjuvant treatment using the same rationale as for androgen supplementation i.e., pre-treatment for some weeks before stimulation to hypothetically increase the number of recruitable follicles. In this line, a recent double-blind, placebo-controlled randomized trial was performed in 10 centers throughout Australia and New Zealand in POR patients, however, and importantly not aligned with the Bologna or the POSEIDON criteria (85). After 4 years that study was stopped after randomization of a total of 130 patients. Unlike other studies, no statistical differences were reported between groups regarding the mean number of oocytes retrieved (5 vs. 4, rate ratio 1.25, 95% CI 0.95–1.66) and the chance of reaching embryo transfer [53/61 [86.9%] vs. 42/51 [82.4%], OR 1.42, 95% CI 0.50–4.00]. However, results from this study should be interpreted with caution as the study was pre maturely stopped and as such was underpowered.

Coenzyme Q10 (CoQ10)

CoQ10 pre-treatment for 60 days prior to ovarian stimulation was very recently investigated in a RCT in POSEIDON group 3 patients ($N = 169$ patients) (8). The hypothesis was that CoQ10 would reduce mitochondrial oxidative stress and thus, improve oocyte competence. The study showed a significant difference in the CoQ10 supplemented group regarding number of oocytes retrieved [4 (mean), IQR 2–5] as compared to controls [2 (mean), IQR 1–2], $p = 0.002$, despite the fact that significantly less FSH was consumed in the CoQ10 supplemented group. In addition, the CoQ10 group had more high-quality day 3 embryos defined as embryos that reached 6 to 8-cell stage with cytoplasmic fragmentation occupying <10% of the embryo surface and had equal size blastomeres. The major limitation, however, was the lack of a placebo group. More studies are definitely needed in the area of pre-treatment with CoQ10, including antioxidants in general and specifically for POSEIDON group 3 and 4 patients. Importantly, CoQ10 and other antioxidants are promising adjuvants keeping in mind that they seem to cause no or very limited adverse reactions and side effects (8).

OVULATION TRIGGER STRATEGY

In a recent review (86), the subject of individualized ovulation triggering (OT) was covered in detail. For the present review we extract the important message that achieving the maximum number of mature oocytes can be improved not only by the use of an individualized COS protocol, but also by individualizing the OT strategy. The key for success when using an OT agent is to reach an optimal LH activity level after trigger, resulting in the retrieval of more than 75% mature oocytes and without increasing the risk of OHSS development (87). A previous cycle with a low follicle:oocyte ratio (FOI) could reflect lack of an appropriate follicular response to trigger which could be associated to ovarian aging, poor ovarian reserve or even to

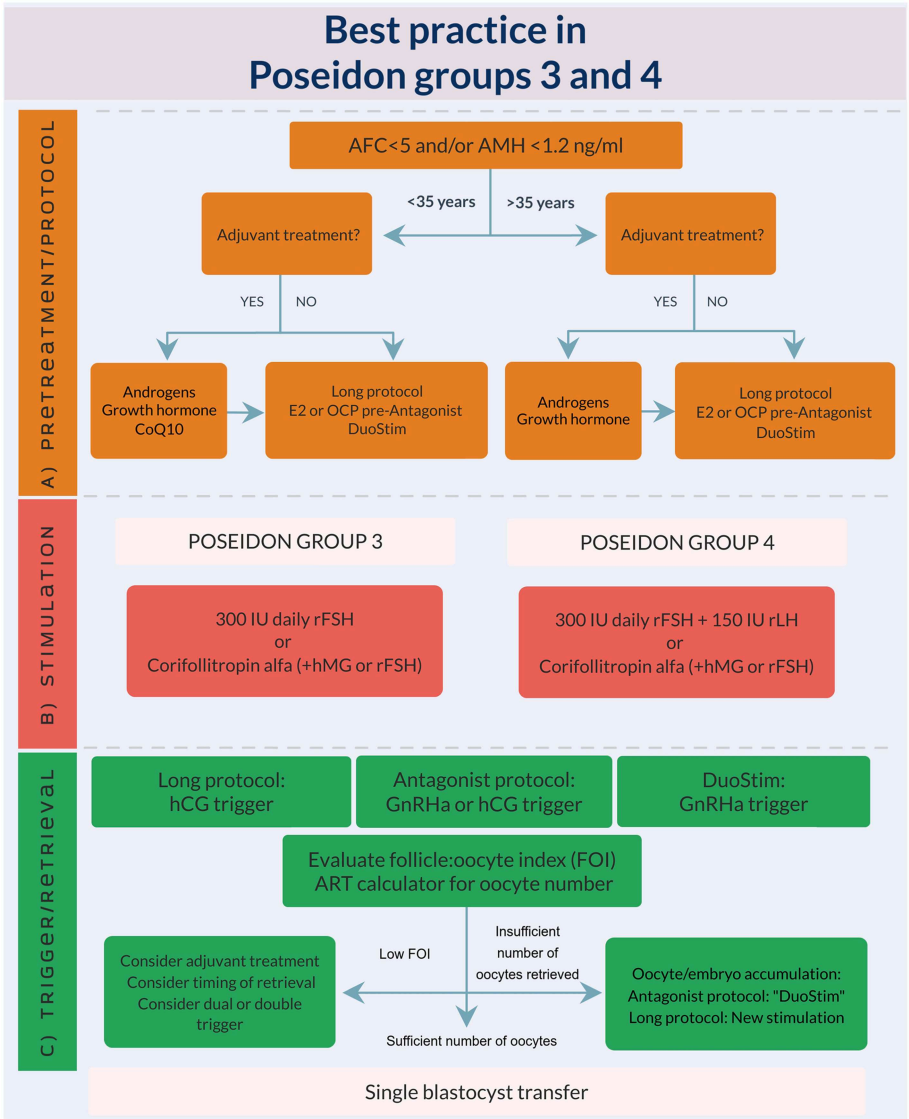

FIGURE 3 | Best practice in POSEIDON groups 3 and 4. **(A)** Pre-treatment is rarely the first option in poor prognosis patients, but in case of unsuccessful ovarian stimulation, i.e., inadequate ovarian response, pre-treatment should be considered. The choice should rely on availability, clinical experience and patient preference. Stimulation protocol might start using GnRH antagonist co-treatment keeping in mind the possibility of converting to DuoStim to achieve the individualized oocyte number (according to the ART calculator). Otherwise a long GnRHa protocol should be considered first choice. **(B)** Ovarian stimulation strategy: First choice in Poseidon group 3 is the GnRH antagonist cycle with either 300 IU daily of rFSH alone or Corifollitropin alfa followed by either rFSH or hMG. In POSEIDON group 4 patients, rLH (75–150 IU daily) should be added from day one of stimulation unless the combination of Corifollitropin alfa and hMG was chosen. The GnRH antagonist cycle allows use of Duostim, unlike the long-agonist GnRH analog. **(C)** Ovulation trigger strategy: In the long GnRHa down-regulation protocol hCG is mandatory as ovulation trigger, whereas GnRHa is mandatory in the follicular phase stimulation of the DuoStim protocol. All trigger agents can be used in the luteal phase stimulation. In non-DuoStim GnRH antagonist cycles, the choice of trigger between GnRHa and hCG should rely on the embryo transfer strategy (fresh or frozen), patient characteristics (e.g., hypo-hypo) and clinical experience. In cases with a low FOI as determined on trigger day, clinicians should consider pre-treatment including short term estrogen therapy or OCP for synchronization of the follicles prior to stimulation, adjuvant LH activity during stimulation, or changing trigger strategy to either dual or double trigger. In case of an insufficient number of oocytes retrieved as determined by the ART calculator, the probability of transferring a euploid embryo should be discussed with the patient to counsel whether an immediate transfer or a new stimulation should be suggested.

mutations of the LH receptor (5, 86, 88). However, a low FOI can be largely improved by carefully considering the OT strategy.

Human Chorionic Gonadotropin

Human chorionic gonadotropin (hCG) has been used as a surrogate to LH for more than 30 years. Both gonadotropins stimulate the LH receptor due to molecular similarities (89); nevertheless hCG is characterized by having a longer half-life compared to LH (90) and this fact conditions the physiology of the corpora lutea and luteal phase hormonal profile. Using hCG as OT agent ensures an action at the level of the follicle regardless of the pituitary status and hCG trigger with a standard luteal

phase support has been shown to yield comparable reproductive outcomes as compared to GnRHa trigger and a modified luteal phase support policy (11).

GnRHa

GnRHa is a synthetic peptide that interacts with the GnRH receptor releasing LH and to a lesser extent, FSH after activation. In a GnRH antagonist cotreated-cycle, a bolus of GnRHa displaces the GnRH antagonist from the receptor which induces a flare of LH and FSH and subsequently, oocyte maturation and ovulation (91). The amount of LH (and FSH) secreted after GnRHa trigger is significantly reduced in comparison with the natural cycle (92) which leads to implantation failure and early pregnancy loss after fresh embryo transfer, when using a standard LPS, only (93). However, good quality oocytes and embryos were obtained after GnRHa as well as after hCG triggering (94). Moreover, significantly more MII oocytes and embryos were obtained after GnRHa trigger as compared to hCG trigger in a recent retrospective analysis in cancer patients undergoing COS and cryopreservation (95). This finding was supported by a recent systematic review and meta-analysis, in which two RCTs showed a significant increase in the number of good quality embryos after GnRHa trigger as compared to hCG trigger, MD 0.94, 95% CI 0.01, 1.87 (11).

Combination of OT Agents

OT strategies such as "dual trigger" and "double trigger" have been explored mainly in patients with a low FOI in a previous cycle or a low proportion of mature oocytes and these strategies have been suggested to improve IVF outcomes, to some extent overcoming impairment in follicular function, oocyte meiotic maturation and cumulus expansion (86). Dual trigger is defined as the combined use of GnRHa and a low-dose of hCG, administered simultaneously (96). In contrast, Double trigger is defined as the administration of GnRHa and hCG for OT at 40 and 34 h, respectively, prior to oocyte retrieval (97). Both strategies combine the advantages of GnRHa and HCG: the direct intrafollicular LH activity mediated by hCG, the simultaneous induction of an endogenous FSH surge mediated by GnRHa, and the support of the early luteal phase LH activity mediated by hCG (98). Double trigger adds the aspect of prolonging the interval between OT and the oocyte retrieval which has been described as a strategy to increase the maturity rate of retrieved oocytes. The physiological rationale being that some patients may need a longer exposure time to the OT agent to allow cumulus expansion and detachment of the oocyte (99). However, the evidence for the use of double trigger in patients with low oocyte/follicle yield, low M-2 rate or poor responders is very limited, reported by 2 groups, only, both from Israel (87, 97, 98, 100); thus, awaiting confirmation by further large scaled RCTs. Importantly, the number of cycles included in these series was 1, 12, 8, and 33, only.

HOW TO TAILOR THE MOST OPTIMAL ART TREATMENT ENCOMPASSING THE DIFFERENT TOOLS MENTIONED TO ACHIEVE AT LEAST ONE EUPLOID BLASTOCYST FOR TRANSFER

Based on the abovementioned evidence, we developed an expert opinion algorithm on how to manage POSEIDON group 3 and 4 patients, see **Figure 3**. As explained earlier, the suggestions for management is based on "very poor evidence" in terms of GRADE (Grading of Recommendations Assessment, Development and Evaluation). Thus, more research is needed and the suggested recommendations should preferably be used in future RCT's or at least clinicians should have retrospective database capture of their results. Despite the poor evidence until now, we believe our suggestions represent current best practice.

CONCLUSIONS

Poor prognosis patients challenge IVF clinicians every day. Herein, we extracted and discussed best practice for these patients. Although more research is needed to make firm clinical recommendations, it is interesting that the treatment concepts discussed herein resulted in ongoing pregnancy rates above 20% per cycle (Duostim) for POSEIDON groups 3 and 4. Future trials investigating pre-treatment strategy, ovarian stimulation strategy and ovulation trigger strategy are warranted and should be based on a more detailed patient stratification such as suggested by the POSEIDON Group.

AUTHOR CONTRIBUTIONS

The style and concept were developed by TH and PH. TH produced **Figure 3** using visme.co with critical revisions from all authors. All authors contributed to writing the manuscript, contributed with critical review and discussions regarding the final version of this review, and accepted the submission of this manuscript for publication.

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GENERAL COMMENTARY
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Commentary: Management Strategies for POSEIDON Groups 3 and 4

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A Commentary on

Management Strategies for POSEIDON Groups 3 and 4
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The discussion about poor ovarian response (POR) is very reminiscent of the current global climate debate. From all sides, we hear that something should be done immediately. There are also a variety of suggestions, mostly considering only a partial aspect. But nowhere are they summarized, and it is not proven that only one of these measures used in a singular manner really improves the situation at all in a sustainable way. And we also experience these problems with "poor responder" patients of ovarian stimulation therapy. That is why we have to be grateful to Haahr et al. that they have summarized in their publication "Management Strategies for POSEIDON Groups 3 and 4" (1) the current state in various aspects.

It starts with the definition of a so called POR. It has been shown that many studies with randomized controlled trials used heterogeneous definitions (2) and so were not applicable to perform, for example, a meta-analysis. It was with the so-called Bologna criteria (3) that a first step was done to standardize this group of patients in a better way facilitating better studies. However, women with POR comprise several different subgroups, and Bologna criteria for POR do not eliminate clinical heterogeneity within the POR population. Especially, the influence of age on prognosis in *in vitro* fertilization (IVF) seems undervalued. So, these criteria are not able to discriminate patients with reduced ovarian reserve from patients having low/suboptimal response to gonadotrophins due to inherent ovarian resistance (e.g., genetic polymorphisms) (4), and they do not formulate recommendations for clinical decision making. More and more we have to realize that postponement of childbearing and maternal age at first pregnancy are on the rise. So, we see a considerable increase in age-related infertility and the demand for assisted reproductive technologies (ART) treatment (5). In POR patients, oocyte number (meaning ovarian reserve) and oocyte quality (age) need to be distinguished. This aspect was realized with the introduction of the so-called POSEIDON classification in which Oocyte Quality and Quantity for Identification and Stratification of the "Low Prognosis" were combined. One consequence of this can be seen in the fact that in the four POSEIDON classification groups, also "hypo-responders" were included as a distinct category of "low prognosis" patients (6). And an intermediate marker of success was introduced: the number of oocytes needed to obtain at least one euploid blastocyst for transfer in each patient. For this purpose, also a so-called "ART-calculator" to determine the minimum number of mature oocytes required according to the patient's individual situation was developed and is available on the POSEIDON homepage.

It is clear, and this represents our increasing dilemma: The older the patient, the more mature oocytes are needed but the less oocytes are retrieved. Therefore, properly personalized stimulation becomes even more important, especially in women of POSEIDON group IV (older and poor ovarian reserve prestimulating parameters). The authors showed very well that the sometimes

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proposed "natural cycle" and/or the "mild stimulation" do not provide an advantage, regarding neither aneuploidy, nor number of oocyte retrieved, nor (cumulative) pregnancy rates [(c)PR].

The most important questions for ovarian stimulation are: Which GnRH analog regimen and which gonadotrophin? Concerning the first question, the authors were able to show, based on recent literature, that the use of the long GnRH agonist (GnRHa) downregulation protocol that was frowned upon in the last years in the IVF community offers several advantages regarding early recruitment, synchronization, and cancelation rate. Intriguingly, in very POR patients (AFC < 4), the combination of long GnRHa protocol with recombinant FSH and LH [r(FSH + LH)] resulted in significantly higher PR per started cycle. Another alternative in which exclusively a GnRH antagonist regimen (GnRHant) can be applied is the "DuoStim." With this kind of oocyte accumulation, previously recommended in single and consecutive cycles (7), one of five patients in POSEIDON IV showed an ongoing pregnancy.

A central concern of the POSEIDON management strategy is to achieve the necessary number of mature oocytes to increase the likelihood of transferring at least one euploid embryo. In this regard, the authors show very well that rFSH is superior to urinary gonadotrophins. This is also confirmed in a study with real world data of nearly 5,000 women with low ovarian reserve parameters (low AMH, elevated FSH): Whenever rFSH was used, more oocytes could be obtained (8). In those women, older than 35 years, the highest number of oocytes could be achieved with the combination of r(FSH + LH), and significantly less FSH was needed.

The authors look also if adjuvants, as a pretreatment or during stimulation, can improve the outcome in women in POSEIDON IV. Even if the **Androgen** chapter is not exhaustively written yet, especially in terms of dose and duration, it seems that pretreatment with androgens leads to better live birth rate (LBR) in women with POR. This must also be said in relation to pretreatment with antioxidants, for example, **CoQ10**. With regard to **growth hormone**, there is currently no convincing indication of its effectiveness in POR. **LH supplementation** appears to improve oocyte quality in moderate and severe POR patients, as it was also recently reported in women with repeated implantation failure (9). In POR patients, a lower pregnancy loss and so higher LBR were seen. Also, "LH priming" before rFSH stimulation in POR patients (defined by cycle cancellation or <4 oocytes collected in a previous cycle) can ameliorate the situation. If in the previous cycles, only a PR of 7% and no live birth were achieved after the LH priming, an LBR/pat. of 29% could be reported (10).

This seems to be a more physiological way to increase follicular androgen concentration because it is doubtful if exogenous administration will increase intrafollicular concentrations (11).

Also the trigger strategy for final oocyte maturation and oocyte retrieval preparation (hCG, GnRHa in GnRHant cycles, or combination of both) may influence the outcome in POR patients.

Although some recommendations are based only on "very poor evidence," it is nevertheless the authors' merit not only to point out possible therapies for improving the situation in POSEIDON IV patients but also to show which urgent studies are now required to treat such patients with POR—whose numbers are constantly increasing—to be able to treat them better and more successfully in the future.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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Conflict of Interest: KB is a current board member of POSEIDON (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number). KB got honoraria fess for giving lectures from Merck KgaA, Bayer AG and Scientific Endometriosis Research Foundation.

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GENERAL COMMENTARY
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Commentary: Management Strategies for POSEIDON Groups 3 and 4

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Keywords: clinical management guidance, poor responder, individual stimulation, chromosomal problem, live birth rate

A Commentary on

Management Strategies for POSEIDON Groups 3 and 4
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As elaborated in the excellent paper by Haahr et al. (1) the POSEIDON group 3 (POR patients below the age of 35 years) represent a much easier to treat entity than their older counterparts. In general their chance of producing aneuploid embryos is considerably lower than in women of older age. According to Franasiak et al. (2) the aneuploidy rate identified on the basis of 221 trophectoderm biopsies is 31.3% at the age of 34 years, increasing steadily to over 80% at age 43 and onwards. Therefore, the likelihood of transferring a euploid embryo is high in POSEIDON group 3 patients, even in cases where only two embryos develop from fertilized oocytes. This nevertheless underlines the importance of maximizing the number of good quality mature oocytes by choosing the best individual stimulation approach possible (3). Because there may be considerable high individual variation in the rate of oocyte aneuploidy and resulting embryos even in young patients as has been shown by Minasi et al. (4) it may be worth to clarify the situation of chromosomal problems in the oocytes at an early stage of treatment—i.e., during the first treatment cycle. In countries where embryo biopsy is legally not permitted (like Germany) this can be achieved by performing biopsies on the two polar bodies from normally fertilized oocytes. This will cover only the maternal contribution to chromosomal mal-distribution which nevertheless represents the vast majority of these problems. The high concordance rate of polar body results and the chromosomal constitution of the corresponding oocytes has been well-documented (5). If the results for an individual patient show normal-for-age aneuploidy rates subsequent therapies can focus on optimization of oocyte yield while PGT-A may be added as an adjunct technology for cases identified to have higher rates to spare the patient unnecessary transfers or spontaneous abortions. This may facilitate even POR patients of younger age to shorten the time-to-pregnancy or rather time-to-Live-Birth.

The paper by Haahr et al. (1) is presently the best available guidance for the clinician faced with patients presenting with reduced ovarian reserve to individually tailor the approach to therapy to

offer the maximum chance for pregnancy and birth. The additional detailed presentation of information on adjuvant therapies opens the path for further clinical research about their relevance in improving the perspective for all POR patients. Especially for women meeting the POSEIDON group 3 criteria this is the perfect assistance to enable the achievement of live birth rates above 20% by taking the best possible path from the very beginning of the treatment.

AUTHOR CONTRIBUTIONS

VB prepared the manuscript. RF reviewed the final version.

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OPINION
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Management Strategies for POSEIDON's Group 1

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Keywords: poor responders, POSEIDON criteria, suboptimal responders, FSH receptor, LH, FSH polymorphisms, IVF

Poor ovarian responders represent one of the most difficult group of patients in every day clinical fertility practice. Still, a major limitation of the available published research is the striking diversity in the definitions used to define poor ovarian response, which could hamper the validity of the results (1, 2).

Despite the recent attempt by the European Society of Human Reproduction and Embryology (ESHRE) to apply a uniform definition for women who respond poorly to ovarian stimulation, the so called "Bologna" criteria (3), it seems that clinicians are still reluctant to use them in clinical studies (4), mainly due to the inability of these criteria to distinguish alterations in oocyte quantity vs. oocyte quality, grouping together women with different biologic characteristics and therefore altered clinical prognosis.

Recently, the POSEIDON group proposed a more detailed stratification of low responders, taking into account essential baseline characteristics of infertile women, which could have a significant impact on their reproductive outcome (5). In this context, patient classification is not only based on the number of oocytes retrieved, but also on various other features that may affect treatment success and should be carefully taken into consideration in the era of tailored-approach treatment, such as age and ovarian "sensitivity" to exogenous gonadotropins.

In this regard, four different patients' categories have been identified through the POSEIDON criteria, taking into account patients' age, ovarian reserve markers and response to stimulation in order to define patients' actual prognosis.

POSEIDON Group 1 apparently includes the best prognosis patients, compared to other POSEIDON groups, referring to young infertile women (<35 years old), with adequate ovarian reserve markers (AFC $\geq$ 5; AMH $\geq$ 1.2 ng/ml), and unexpected poor (<3 oocytes retrieved) or suboptimal (4–9 oocytes retrieved) response following conventional ovarian stimulation (5).

Management of women belonging to the POSEIDON group 1 requires a distinct diagnostic and therapeutic approach in relation to patients' characteristics, which should be specifically tailored to their young age and the adequate ovarian reserve of these women (6).

Age is undeniably the strongest determinant of treatment success in women seeking fertility advice (7). The age-related decline in fertility, owing to a significant decrease in both oocyte quantity (as reflected by lower oocyte yield) and quality (as reflected by higher aneuploidy and spontaneous abortion rates), is directly associated with the very low LBR observed in older women (8). Therefore, although prognosis is very bad in old poor responders, irrespective of the treatment modality used (9, 10), substantial benefit could be anticipated in younger women if an adequate number of oocytes is harvested. If we further consider that suboptimal response to stimulation significantly impairs cumulative live birth rates (11–13) and that women with unexpected poor/suboptimal responders may have better prognosis compared to patients with predicted low response (14–16), it could be stated that POSEIDON group 1 patients may represent the most interesting group, on which clinical research should focus in the future.

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Several pathophysiological explanations have been proposed in order to clarify the nature of unexpected poor/suboptimal response. Ovarian sensitivity in relation to gonadotropin treatment has been the dominating theory, with evidence deriving from the investigation of the genetic variations of gonadotropins and their receptors (17). In particular, FSHR polymorphisms (e.g., Ser680Asn and Thr307Ala) have been associated with reduced sensitivity to gonadotropins (18) and may be the most reasonable explanation for the inadequate response following ovarian stimulation (19). This, in addition to the established need for higher gonadotropins in these patients (18), despite their normal ovarian reserve markers (20), suggests that genetic variation in the FSHR is a marker of ovarian sensitivity, irrespective of ovarian reserve.

On the other hand, a common variant of the β subunit of luteinizing hormone (LH) (v-LH) has been shown to affect FSH sensitivity and the ovarian response to FSH in normogonadotrophic women. Previous studies demonstrated that patients with this genetic variant of LH may experience an unexpected suboptimal response to stimulation and actually require higher cumulative-dose of gonadotropins (21, 22); thus, it may be imperative to consider the potential presence of such a genetic variant among several patients belonging to POSEIDON group 1.

Furthermore, less studied polymorphisms including FSH/LHCGR genes and their combinations may also be relevant, although evidence is sparse (23, 24).

Clear treatment guidelines have not been established for POSEIDON group 1 patients; still, this needs to be tailored in accordance to the underlying pathophysiological mechanism responsible for the impaired response to stimulation (6).

Utilization of higher gonadotropin doses of more "potent" recombinant formulations may be the solution in a significant percentage of these women, especially in the ones with polymorphisms identified in the FSHR gene. Taking into account that the Ser680Asn polymorphism of the FSHR gene may negatively influence the ovarian response to FSH stimulation and women with the genotype Ser/Ser appear to be more resistant to FSH action, a pharmacogenetic study demonstrated that treatment with higher FSH starting dose (225IU) in women homozygous for Ser680 (SS) resulted in similar serum estradiol (E2) levels with women who are homozygous for Asn680 (AA)/heterozygous (AS) treated with lower FSH doses (150IU) and significantly higher E2 levels compared to SS women treated with low 150IU dose (25). Moreover, a recent retrospective study evaluated the effect of FSH dose adjustment in women with a history of suboptimal response (4–9 oocytes retrieved) and demonstrated that an increase in the starting dose of FSH was significantly associated with a higher oocyte yield in the following IVF cycle (26).

On the other hand, administration of r-LH supplementation could be another option in these women, especially in cases of genetic variations of LH gene. Given the accumulating evidence from clinical research demonstrating that recombinant LH (rLH) could potentially increase the number of oocytes retrieved and result in higher pregnancy rates in women with non-pathological ovarian reserve tests and previous unexpected poor (27) or inadequate response (28), the use of rLH in these women is fully justified, and future research is essential to confirm these initial findings.

The utilization of novel promising approaches such a dual stimulation should not be overlooked and may be of benefit for POSEIDON group 1 patients. The rationale of this strategy is that poor prognosis women may undergo both follicular and luteal phase ovarian stimulation in the same menstrual cycle, in an attempt to maximize the number of oocytes retrieved and in turn increase the chance to obtain a genetically normal embryo in a short time interval (29). However, more evidence is needed for the applicability of luteal phase stimulation in poor responders, before implementation in clinical practice.

The synchronization of the follicular cohort through luteal phase estradiol/oral contraceptive pills (OCP) pre-treatment could be an option in young patients with unexpected poor or suboptimal response; albeit evidence extrapolated from studies in poor responders is controversial (2, 30).

Finally, adjuvant treatments with growth hormone (GH) or testosterone have been of great interest as an option to improve the outcome in women with a poor ovarian response and certainly merit evaluation in POSEIDON group 1. However, it should be stated that even if previous meta-analyses support the use of these regimens in poor responders (31, 32), results need to be interpreted with great caution due to limited evidence and small sample size of the relevant RCTs (33).

In conclusion, young women with normal ovarian reserve markers with a previous unexpected poor or suboptimal response seem to form a distinct group of infertile patients with different clinical prognosis compared to poor responders according to the "Bologna" criteria. Genetic polymorphisms of gonadotropins and their receptors may be a plausible explanation for the poor/suboptimal response following conventional ovarian stimulation; albeit more evidence is needed (NCT03007043, available at: clinicaltrials.gov). The management of these patients may imply the increase in the starting dose of recombinant FSH and/or supplementation with rLH or even double ovarian stimulation in an attempt to increase the number of oocytes retrieved and therefore the final reproductive outcome. The use of GH/testosterone and priming protocols including estradiol/OCPs represent other promising options. Nonetheless, further studies are warranted in order to validate these therapeutic approaches.

AUTHOR CONTRIBUTIONS

NP and PD contributed equally to the design and the writing of the manuscript.

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The POSEIDON Criteria and Its Measure of Success Through the Eyes of Clinicians and Embryologists

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This article represents a viewpoint on the POSEIDON criteria by a group of clinicians and embryologists. Its primary objective is to contextualize the Poseidon criteria and their metric of success for the relevant Frontiers Research Topic "POSEIDON's Stratification of Low Prognosis Patients in ART: The WHY, the WHAT, and the HOW". "Low prognosis" relates with reduced oocyte number, which can be associated with low or sometimes a normal ovarian reserve and is aggravated by advanced female age. These aspects will ultimately affect the number of embryos generated and consequently, the cumulative live birth rate. The novel system relies on female age, ovarian reserve markers, ovarian sensitivity to exogenous gonadotropin, and the number of oocytes retrieved, which will both identify the patients with low prognosis and stratify such patients into one of four groups of women with "expected" or "unexpected" impaired ovarian response to exogenous gonadotropin stimulation. Furthermore, the POSEIDON group introduced a new measure of clinical success in ART, namely, the ability to retrieve the number of oocytes needed to obtain at least one euploid blastocyst for transfer in each patient. Using the POSEIDON criteria, the clinician can firstly identify and classify patients who have low prognosis in ART, and secondly, aim at designing an individualized treatment plan to maximize the chances of achieving the POSEIDON measure of success in each of the four low prognosis groups. The novel POSEIDON classification system is anticipated to improve counseling and management of low prognosis patients undergoing ART, with an expected positive effect on reproductive success and a reduction in the time to live birth.

Keywords: POSEIDON criteria, ovarian stimulation, low prognosis, poor ovarian response, oocyte, blastocyst, assisted reproductive technology, ART calculator

CURRENT SCENARIO

The proportion of patients of advanced female age and low ovarian reserve seeking fertility treatment is increasing worldwide. It is well-known that pregnancy rates are lower in these women than in younger counterparts. However, it is also important to realize that repetition of assisted reproductive technology (ART) treatments using a "trial and error" approach does not seem to help these patients, since the gap between older and younger patients, as regards cumulative pregnancy rates, increases after multiple IVF cycles (1).

In the era of personalized medicine, success in ART goes far beyond pregnancy. It should be redefined considering other quality dimensions, without overlooking the patient perspective (2–4). We believe that provision of proper evaluation, counseling about the chances of success, and development of an effective and safe time-limited treatment plan taking into full consideration the patients' values and preferences should be the cornerstones of healthcare delivered to infertile couples undergoing ART.

As far as evaluation is concerned, ovarian reserve biomarkers, like anti-Müllerian hormone (AMH) and antral follicle count (AFC), are now widely used to predict ovarian response to gonadotropin stimulation. Despite their clinical utility in this regard, the value of ovarian reserve biomarkers to predict reproductive success in ART is suboptimal (5–7). Furthermore, ovarian reserve markers cannot identify the hypo-responder patient, a concept firstly introduced by the Evian Annual Reproduction (EVAR) Workshop Group in 2008. These women, who differ from Bologna criteria poor responders in terms of age and ovarian reserve, have a stagnant response to exogenous FSH during ovarian stimulation and might end up having an unexpected poor or a suboptimal number of retrieved oocytes after conventional ovarian stimulation (8, 9).

By contrast, what became clear over the last years is a strong positive association between oocyte number and live birth rates (10–13). Nevertheless, the oocyte number should be combined with female age since the likelihood of achieving a live birth among patients with similar oocyte yield ultimately depends on the age of the patient (10). It means that the number of oocytes needed to maximize live birth should be individualized considering the age of the patient, and more importantly, patient-oriented strategies should be used to achieve the estimated individualized oocyte number.

THE POSEIDON CRITERIA OF "LOW PROGNOSIS" PATIENTS UNDERGOING ART

The issues mentioned above constitute the cornerstones of the novel POSEIDON (**P**atient-**O**riented **S**trategies **E**ncompassing **I**ndividualize**D** **O**ocyte **N**umber) criteria for "low prognosis" patients undergoing ART (14–17) (www.groupposeidon.com). The POSEIDON criteria propose a shift from the terminology of poor ovarian response (POR) to the concept of low prognosis. The low prognosis patient is classified into four groups according to the results of ovarian reserve markers (AMH, AFC, or both), female age, and the number of oocytes retrieved in previous cycles of conventional ovarian stimulation (OS)—in cases where this information is available (**Figure 1**). Patients fitting the POSEIDON criteria have low prognosis in ART owing to a decreased number of oocytes, which will limit the number of embryos produced. This condition might be aggravated further by advanced female age, thus negatively impacting the availability of genetically normal embryos for transfer, ultimately affecting the cumulative live birth rates (CLBR) per started cycle (13, 18).

Hence, the "low prognosis" concept fundamentally relates to cumulative live birth delivery rate, which is defined by the International Committee for Monitoring Assisted Reproductive Technologies (ICMART) (19) as, "*the number of deliveries with at least one live birth resulting from one initiated or aspirated ART cycle, including all cycles in which fresh and/or frozen embryos are transferred, until one delivery with a live birth occurs or until all embryos are used, whichever occurs first, expressed per 100 cycles (initiated or aspirated).*"

According to the POSEIDON criteria, the patients are classified as groups 1 and 3 if younger than 35 years old, and as groups 2 and 4 if older than 35 years of age (14–16). Female age is a critical element in the POSEIDON classification because age is crucially related to embryo ploidy and more importantly live birth outcome. In a study by the POSEIDON group involving infertile patients subjected to IVF-ICSI and pre-implantation genetic testing for aneuploidy (PGT-A) by next-generation sequencing analysis (NGS), the blastocyst euploidy probabilities were calculated as a function of female age (20). The probabilities mentioned above sharply declined after the age of 34 and were overall lower than 50% in women aged 35 years of age and over (**Figure 2**). This biological phenomenon, in combination with the already reduced ovarian reserve in patients with advanced female age, might increase the risk of having no euploid embryos for transfer (20). In the above study, the percent decline in blastocyst euploid probability increased progressively with advancing female age. The geometric mean of the yearly variation was 13.6%. However, it increased progressively year on year. At age 30 it was 2.0%, whereas, at ages 35, 39, and 44, the relative loss in the blastocyst euploidy probabilities were 6.7, 13.6, and 24.5%, respectively (**Figure 2**). These figures indicate that the older the patient, the higher the number of oocytes and embryos needed to increase the chances of having at least one euploid blastocyst within the cohort of embryos (20).

Collectively, patients fitting POSEIDON's groups 1 and 3 are young and, therefore, the risk of embryo aneuploidy is relatively low. By contrast, groups 2 and 4 include older patients with an increased risk of embryo aneuploidy (**Figure 1**). As a result, irrespective of the group, the number of embryos generated would be likely low, thus affecting the CLBR per started cycle. Importantly, despite having an overall low prognosis, the CLBR per started cycle will differ according to the classification group as it is affected by female age and oocyte number.

CLINICAL VALIDATION DATA

Leijdekkers et al., in 2019, used the data from the OPTIMIST prospective study to assess the CLBR in low-prognosis patients stratified according to the POSEIDON criteria (22). The authors showed that the prognosis concerning CLBR differed among

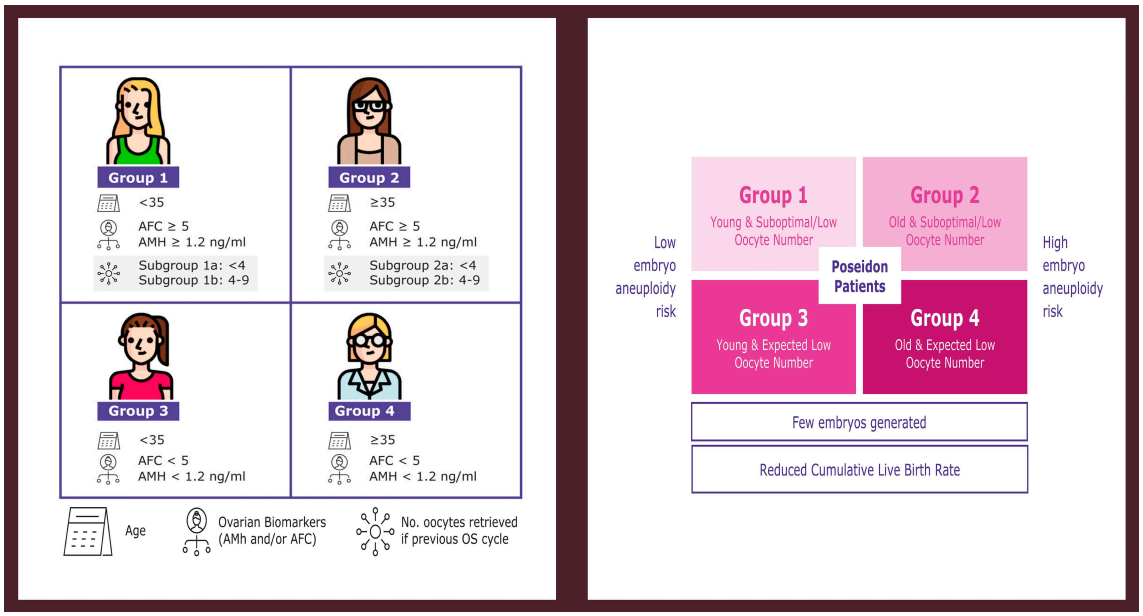

FIGURE 1 | POSEIDON criteria of low prognosis patients in ART. The novel system relies on female age, ovarian reserve markers, ovarian sensitivity to exogenous gonadotropin, and the number of oocytes retrieved, which will both identify the patients with low prognosis and stratify such patients into one of four groups of women with "expected" or "unexpected" impaired ovarian response to exogenous gonadotropin stimulation. According to these criteria, four distinct groups of low prognosis patients can be established **(left)**. Owing to low oocyte numbers and less embryos produced, POSEIDON patients have lower cumulative live birth rates per started cycle than non-POSEIDON counterparts. However, the prognosis is differentially affected by oocyte quantity and female age, as the latter relates to the risk of embryo aneuploidy **(right)**. Art drawing by Chloé Xilinas. Modified from Esteves et al. (16). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY).

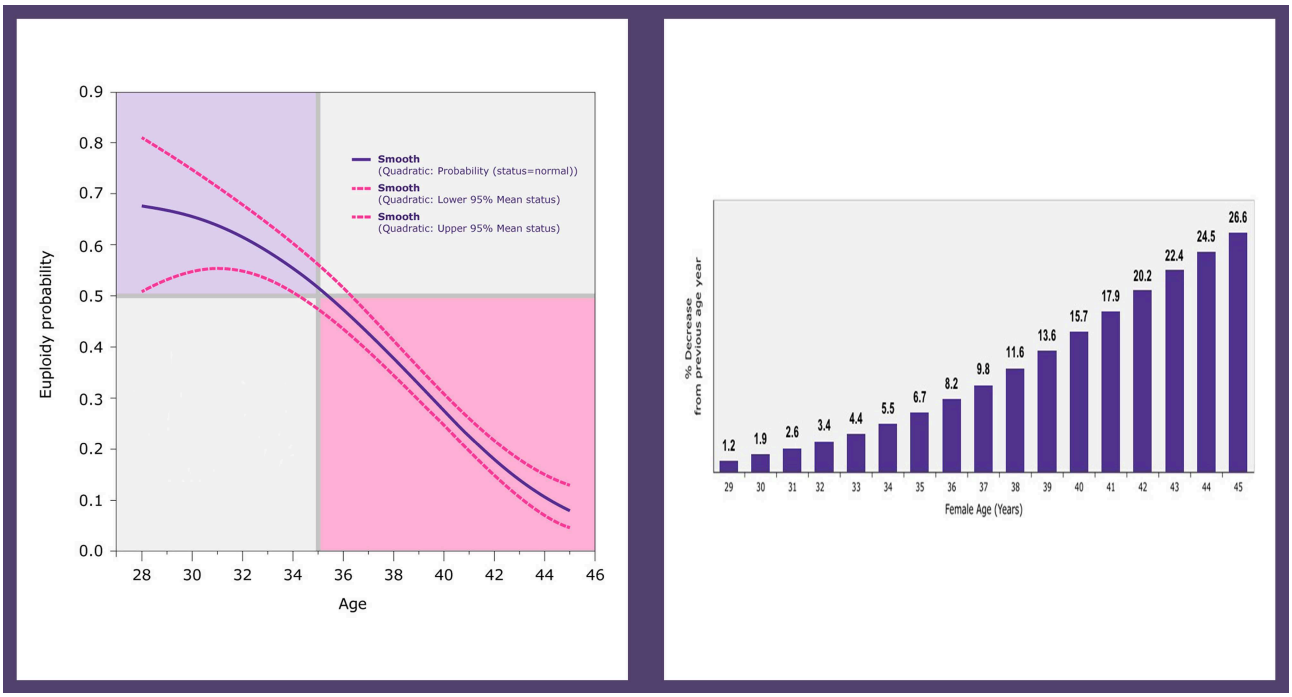

FIGURE 2 | Logistic regression analysis of 1,220 trophectoderm biopsies from 436 patients undergoing ICSI and PGT-A by NGS. The plot depicts the fitted probabilities (with 95% confidence intervals) of blastocyst euploidy as a function of female age **(left)**. The graph shows the percent decrease in the probability of a blastocyst being euploid, which increases progressively with every year of female age **(right)**. Reprinted with permission of Edizioni Minerva Medica from Esteves et al. (21).

the low-prognosis groups, with maternal age (and hence oocyte quality) being the dominant determinant of CLBR rather than the quantitative ovarian reserve. Thus, the authors concluded that given the fact that the differences in CLBR among POSEIDON groups are primarily due to the effect of maternal age on oocyte quality, the new criteria have limited value for clinical management, although it might be used for patient counseling.

Having scrutinized the authors' data, we found that in addition to confirming what clinicians already know about the primary role of maternal age on the likelihood of achieving a live birth in ART, the study of Leijdekkers et al. also confirm that CLBR is affected not only by age but definitely by the number of oocytes retrieved (22). In their study, the authors showed that the CLBR in low-prognosis patients was ∼56% over 18 months follow-up. Notably, the CLBR was surprisingly high in all POSEIDON groups, reaching ∼68 and 39% in Poseidon groups 1b and 4, respectively, as compared to 72 and 58% in younger and older non-POSEIDON patients. However, such figures were achieved after an average of two fresh transfer cycles per woman, which is not in line with the CLBR definition by the ICMART (19). It is important to realize that the concept of low-prognosis introduced by the POSEIDON group concerns CLBR per started cycle, as defined by the ICMART (16). By contrast, the per-period estimation might inflate CLBRs owing to the high dropout rate after the first failed IVF treatment (23).

Indeed, when Leijdekkers et al. (22) evaluated CLBRs per cycle, there was a remarkable difference between POSEIDON patients (21, 43, 10, 25, 29, and 17% in groups 1a, 1b, 2a, 2b, 3, and 4, respectively) and non-POSEIDON counterparts (52%). Moreover, their data show that CLBR per cycle was twice as high in patients with a suboptimal response to stimulation (4–9 oocytes) compared to those with a low response to stimulation (<4 oocytes) both in women <35 years-old (group 1b: 43%; group 1a: 21%) and ≥35 years-old (group 2b: 25%; group 2a: 10%). Not surprisingly, the CLBRs per cycle in young (29%) and old (17%) expected poor responders (POSEIDON groups 3 and 4, respectively) were similar to those of POSEIDON groups 1a and 2a. These figures have clinical importance because CLBR per started cycle might be increased in low prognosis women by increasing the number of retrieved oocytes, which may be achieved mainly in POSEIDON patients who have adequate ovarian reserve markers, that is, groups 1 and 2 (8, 17, 18, 24, 25).

Thus, in addition to serve as a counseling tool, we suggest that the POSEIDON criteria should be used to guide clinical management with a specific focus on optimizing the follicle:oocyte ratio (FOI) to achieve higher reproductive outcomes. In patients with an unexpected poor/suboptimal oocyte number due to a low FOI (e.g., groups 1 and 2), it has been suggested that individualization of ovarian stimulation might increase the number of oocytes retrieved (17, 18). However, patients with an expected low oocyte number could also benefit from individualized regimens, in which pharmacological interventions should be combined with oocyte/embryo accumulation (26, 27).

THE RATIONALE OF INDIVIDUALIZING THE OOCYTE NUMBER

Big data indicates that there is a positive association between the number of oocytes and CLBR per started cycle, with higher oocyte thresholds for better outcomes (13, 18). Although this information gives the clinician some guidance, high oocyte numbers might be hard to achieve in POSEIDON patients. Thus, the POSEIDON group introduced a new metric of success in ART, namely, *the ability to retrieve the number of oocytes needed to obtain at least one euploid blastocyst for transfer in each patient* (14, 15). The POSEIDON marker of success seems to be a logical endpoint for clinicians providing care to women undergoing ART because the transfer of a euploid embryo provides –at any given age– implantation rates in the range of 50–60% overall (28). Importantly, this endpoint does not imply that PGT-A should be routinely performed during ART.

We acknowledge that live birth rate (LBR) is the primary endpoint for couples undergoing ART (29). Nonetheless, LBR has been reported in only a small proportion of studies and depends on a multitude of controlled and uncontrolled factors, thus making it challenging to use LBR for making individualized predictions about the number of oocytes needed to achieve the desired outcome. In particular, LBRs in low responders and advanced age women are influenced by the age-dependent miscarriage rate observed in these subgroups. For instance, the miscarriage rate in women over 40 years was estimated to be ∼30% (30). Not surprisingly, a dramatic drop-out before delivery is observed during trials. Moreover, LBR is prone to biases not related to ART. For instance, intrauterine fetal death after 12 weeks of gestation occurs in about 5% of ongoing pregnancies, whose risk further increases in women of advanced age (31).

Hence, other endpoints, such as the one proposed by the POSEIDON group, might be considered as we feel it is essential to acknowledge the continuum of reproductive outcomes like implantation rates, pregnancy rates, clinical pregnancy rates, ongoing pregnancy rates, and LBR. Naturally, infertility is a couple's problem, and a single intermediate metric (such as the one introduced by the POSEIDON group) is limited to predict treatment outcome. Thus, we are not suggesting that LBR should be replaced by the new metric but do believe it adds independent information that may allow for better treatment planning. The clinician can objectively estimate the individualized oocyte number to achieve at least one euploid embryo for transfer by either looking at the embryonic data of her/his particular clinic or using predictive models.

THE ART CALCULATOR

Recently, a new predictive tool, called the "ART Calculator," was developed to estimate the minimum number of metaphase II (MII) oocytes required to have at least one euploid blastocyst for transfer in patients undergoing ART (21). To achieve this goal, firstly, there was a search for relevant predictors. The observational unit and the response variable were respectively

(i) the woman, and (ii) the pair (*m*, *n*), where *n* is the number of retrieved metaphase II oocytes and m the corresponding number of euploid blastocysts. A penalized regression model, with the negative binomial for the distribution of euploid blastocysts and the log link function, was used for the selection of predictors. The negative binomial was chosen from first principles and from the heuristic fact that this distribution fitted the data very closely. The selection of predictors was carried out by the Lasso method, a procedure that allows for the fitting of correlated and high-dimensional data. Among 26 predictors tested from ∼350 infertile couples undergoing IVF/ICSI and PGT-A, female age, and type of sperm used for IVF/ICSI were found to be the relevant predictors concerning blastocyst euploidy.

The final predictive model provides the age-related probabilities of a blastocyst being euploid per metaphase II (MII) oocyte as a function of sperm type (ejaculated, epididymal, or testicular sperm, and adjusted for the type of azoospermia, that is, obstructive or non-obstructive azoospermia). The data indicated that the estimated probability of an MII oocyte turn into a euploid blastocyst decreases progressively with female age, an effect that is negatively modulated by the use of testicular sperm from men with non-obstructive azoospermia (NOA) (21). The above results are consistent with previous reports. Indeed, with aging, oocyte chromosomal abnormalities and cytoplasmic dysfunctions increase, whereas the number of primordial follicles progressively decline (20, 32–35). Moreover, the use of testicular sperm from men with NOA was shown to be a negative predictor for obtaining a euploid blastocyst per oocyte pickup, most probably related to the fact that the blastocyst rate per fertilized oocyte is significantly reduced (21, 36).

Using the probabilities mentioned above and mathematical equations, the ART calculator provides individualized estimations about the minimum number of MII oocytes required to obtain at least one euploid blastocyst, with 95% confidence interval [CI]. Specifically, the ART calculator makes two types of predictions automatically, one using pretreatment information to estimate the minimum number of MII oocytes to achieve at least one euploid blastocyst, and another based on the actual number of mature oocytes collected/accumulated to estimate the chances of having a euploid blastocyst using that oocyte cohort for IVF/ICSI (http://www.members.groupposeidon.com/Calculator/).

As an example, a hypothetical couple undergoing IVF/ICSI whose female partner is 36 years old and the male partner has moderate oligoasthenoteratozoospermia—thus ejaculated sperm will be used for sperm injections—needs at least nine metaphase II oocytes (confidence interval: 7–10) to obtain at least one euploid blastocyst for transfer, considering a 80% probability of success (set by the user) (source: http://www.members.groupposeidon.com/Calculator/). Let us now consider that the patient under discussion belongs to POSEIDON's group 2. Using the POSEIDON criteria and the ART calculator, the treating physician can plan the ovarian stimulation strategy with the mindset of optimizing the FOI to achieve the predicted number of metaphase II oocytes or higher (17, 25). If the target oocyte number is achieved, the exemplary couple's chance of having at least one euploid blastocyst for transfer in the resulting embryo cohort will be 80% (or 20% risk of failure). It is well-known that single euploid blastocyst transfer gives ∼50–60% implantation rates (30). Thus, given the risk of spontaneous miscarriage and intrauterine fetal death after 12 weeks of gestation of about 10%, the ultimate live birth rate for the hypothetical couple will be about 40% (28). These figures are remarkably higher than the LBR of ∼30% reported for such couples without the "POSEIDON's approach" (37). On the other hand, if the exemplary couple belonged to Poseidon's group 4 and the number of retrieved metaphase II oocytes were four after the above exercise, the revised estimates would indicate a ∼51% probability of having at least one euploid blastocyst with that oocyte number (source: http://www.members.groupposeidon.com/Calculator/). In this scenario, the health care provider and the affected patients could decide the best way to move forward, which might include, for instance, going ahead with fertilization, embryo culture and transfer (with or without PGT-A), or exploring oocyte/embryo accumulation (27).

Detailed information about the calculator development is available in a dedicated article within this Frontiers Research Topic (21). Although other female factors, such as obesity, ethnicity, previous pregnancy, infertility etiology, and ovarian reserve markers are important for ovarian stimulation success, they were not deemed informative for the ART calculator predictive model, which used blastocyst euploidy per MII oocyte as the response. However, it is worth mentioning that there was no attempt to determine fundamental associations between the predictors and the number of euploid blastocysts. Along these lines, "power" is not a relevant concept in predictive modeling nor are sequential temporal associations concerning the ability of an MII oocyte turn into a euploid blastocyst. The primary objective of the ART calculator study was the development of a prediction formula for the number of euploid blastocysts. The resulting model was subjected to validation by the holdout sample method. The quality of the predictive model was assessed by the ROC curve, calculated on the holdout sample. The predictive ability of the model assessed by the area under the ROC curve was ∼72%, thus suggesting that unknown factors intrinsically related to the biological variability of oocytes and embryos might also influence their ploidy status (21).

From both clinical and embryological perspectives, the ART calculator provides objective information, which might help patients prepare themselves both emotionally and financially for the treatment journey. Moreover, the ART calculator provides clinicians an estimation of the minimum number of mature oocytes required for at least one euploid blastocyst in IVF/ICSI procedures, which improves the planning of the specific treatment. Nonetheless, clinicians should not deny treatment to infertile women if the predicted number of oocytes needed to achieve at euploid blastocyst is too high or the probabilities of achieving this goal—based on the actual number of oocytes retrieved—is too low. The embryos are statistically independent concerning the ploidy status, which primarily depends on

maternal age (31). Thus, the euploid embryo could be anywhere within the patient embryo cohorts.

PATIENT-ORIENTED STRATEGIES TO ACHIEVE THE INDIVIDUALIZED OOCYTE NUMBER

Using the POSEIDON criteria, the clinician can, first of all, identify and classify patients who are likely to have reduced success in ART, and secondly, develop a treatment plan to achieve the individualized oocyte number related to the optimal probability of generating at least one euploid blastocyst for transfer in each POSEIDON's patient category.

In practical terms, the individualized oocyte number can be achieved using patient-oriented strategies. For instance, the type of GnRH analog, type of gonadotropin, the starting dose, and the regimen may be tailored according to POSEIDON stratification (8, 38–41). Importantly, patient-oriented gonadotropin dosing aimed at retrieving more oocytes does not seem to affect the embryo ploidy status. In an ongoing multicenter study by the POSEIDON group, we observed that the age-controlled probability of a blastocyst being euploid is not affected by the size of embryo cohort (unpublished data), thus confirming previous observations of a lack of detrimental effect on embryo ploidy in patients who had more oocytes retrieved (20, 32). Our observations also indicate that the use of minimal or mild stimulation –as compared to conventional stimulation– has no apparent positive effect on embryo genetic competence. What matters most concerning embryo ploidy is female age and not the intensity of ovarian stimulation (42–45).

In reality, low gonadotropin dosing or suboptimal gonadotropin regimen might result in hypo-response and the retrieval of fewer than expected oocytes (8, 16, 18, 24, 40). This phenomenon can be better appreciated in POSEIDON groups 1 and 2, who despite adequate pre-stimulation ovarian parameters end up having a poor or suboptimal oocyte yield, possibly due to inappropriate gonadotropin dosing/regimen and/or the presence of genetic polymorphisms affecting the gonadotropins and their receptors (9, 17, 25, 46). Therefore, a thorough evaluation of the patient is critical to help the clinician identify the low prognosis patient and plan a treatment tailored to the patient's specific needs. It has been suggested that individualization of ovarian stimulation might increase the number of oocytes retrieved among patients with an unexpected poor/suboptimal oocyte number (POSEIDON's groups 1 and 2), in particular, those with a low FOI (8, 9, 24). Naturally, the use of the right gonadotropin starting dose and the possibility to adapt the dose and the regimen during the cycle is essential to optimize oocytes yield while securing patient safety (18, 47–50).

Notwithstanding, even using the best protocol, the individualized oocyte number might be difficult to achieve with a single ovarian stimulation. This observation is particularly relevant for patients in POSEIDON's groups 3 and 4, who all have a reduced ovarian reserve. In such cases, treatment should be planned with the mindset that the number of oocytes needed to achieve at least one euploid blastocyst is lower in young (group 3) than in older (group 4) patients (21). Individualized regimens, possibly combining pharmacological interventions and oocyte/embryo accumulation, could also benefit these patients as a means of shortening the time frame to reach the target oocyte number (26, 27, 41, 48–54).

FUTURE DIRECTIONS

The critical data necessary to support the clinical uptake of the POSEIDON criteria would involve the confirmation that (i) patients fitting the four groups have low prognosis as compared to non-POSEIDON patients concerning the CLBR per started cycle, and (ii) patient-oriented strategies with the mindset to achieve the POSEIDON's measure of success increase the continuum of reproductive outcomes, including the time to live birth. The patient population characteristics, discovery set, and the independent validation steps for building and confirming the associative success of the POSEIDON classification are ongoing, and the first results have been recently published (22, 26, 55, 56). While awaiting the results of randomized trials to clarify the role of interventions in this vast and important group of ART patients, we would suggest that individualization of the ovarian stimulation is superior to a "one size fits all" policy in POSEIDON patients.

CONCLUSIONS

The novel POSEIDON classification of the low prognosis patient in ART combined with the use of patient-oriented strategies to achieve the individualized oocyte number—as predicted by the ART calculator—should be considered by clinicians to reduce the time to live birth. This new system may help improve patient counseling and management, with an expected positive effect on IVF success and time to live birth. We invite readers to learn more about the POSEIDON initiative and the ART Calculator at both www.groupposeidon.com and this Frontiers Research Topic https://www.frontiersin.org/research-topics/6849/poseidons-stratification-of-low-prognosis-patients-in-art-the-why-the-what-and-the-how. The POSEIDON group is an open access initiative; thus, we encourage our colleagues to join us as POSEIDON members (please find out more at http://www.groupposeidon.com/member-benefits/).

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

SE and PH contributed to the conception and designed the manuscript. SE wrote the first draft of the manuscript. CA, PH, RF, CYA, AC, KB, SS, NP, DG, MG, HY, IÖ, MR, LV, MB, LR, AV, DC, and FU wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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Validation of ART Calculator for Predicting the Number of Metaphase II Oocytes Required for Obtaining at Least One Euploid Blastocyst for Transfer in Couples Undergoing *in vitro* Fertilization/Intracytoplasmic Sperm Injection

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This multicenter study evaluated the reliability of the recently published ART calculator for predicting the minimum number of metaphase II (MII) oocytes (MIImin) to obtain at least one euploid blastocyst in patients undergoing *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI). We used clinical and embryonic retrospective data of 1,464 consecutive infertile couples who underwent IVF/ICSI with the intention to have preimplantation genetic testing for aneuploidy. The validation procedure followed a stepwise approach. Firstly, we assessed the distribution of euploid blastocysts per patient and found that it followed a negative binomial distribution. Secondly, we used generalized linear models and applied the Lasso procedure–including MII oocytes to adjust the data–to select the factors predicting the response variable "euploid blastocyst." Third, a logistic regression model–fit to the binomial response euploid (yes/no) for each MII oocyte–was built using the relevant factors. The observational unit was the "woman" whereas the response was the pair (m, n), where n is the number of retrieved MII oocytes and m the corresponding number of euploid blastocysts. The model was internally validated by randomly splitting the data into training and validation sets. The R-squares (∼0.25) and the area under the ROC curve (∼0.70) did not differ between the training and validation datasets. Fourth, mathematical equations and the calculated probabilities generated by the validation model were used to determine the MIImin required for obtaining at least one euploid blastocyst according to different success probabilities. Lastly, we compared the fittings generated by the validation model and the ART calculator and assessed the predictive value of the latter using the validation dataset. The fittings were sufficiently close for both the estimated probabilities of blastocyst euploid per MII oocyte ($r = 0.91$) and MIImin ($r = 0.88$).

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The ART calculator positive predictive values, i.e., the frequency of patients with at least one euploid blastocyst among those who achieved the estimated MIImin, were 84.8%, 87.5%, and 90.0% for 70%, 80%, and 90% predicted probabilities of success, respectively. The ART calculator effectively predicts the MIImin needed to achieve at least one euploid blastocyst in individual patients undergoing IVF/ICSI. The prediction tool might be used for counseling and planning IVF/ICSI treatments.

Keywords: assisted reproductive technology, ART calculator, intracytoplasmic sperm injection, preimplantation genetic testing for aneuploidy, decision support models, POSEIDON criteria, validation study

INTRODUCTION

In modern society, the age of the population seeking assisted reproductive technology (ART) is increasing steadily as both women and men tend to postpone childbearing. It is well-known that the female age is the central factor for pregnancy success, with higher ages associated with poorer outcomes (1). However, the frequency of couples with coexistent male infertility has also increased (2, 3). Recent studies have demonstrated that both female age and the etiology and severity of male infertility independently affect reproductive outcomes even under ART settings (4–6).

ART success has been commonly reported as the delivery of a live birth resulting from one initiated or aspirated ART cycle (7). The most comprehensive studies indicate that there is a positive association between the number of retrieved oocytes and live birth rates (LBR), in particular, cumulative LBR, with higher oocyte thresholds for better outcomes (8–10). Although the LBR is the preferable endpoint for couples, it depends on a multitude of controlled and uncontrolled factors, thus making it challenging to use this metric for individualized predictions about the number of oocytes needed to achieve the desired outcome. In 2016, the POSEIDON (**P**atient-**O**riented **S**trategies **E**ncompassing **I**ndividualize**D** **O**ocyte **N**umber) collaborative group introduced a new metric of success, namely, the ability to obtain the number of oocytes needed to achieve at least one euploid blastocyst for transfer (11–13). Besides the critical role of oocyte numbers on ART success, the transfer of euploid embryos markedly reduces the female age-related decrease in implantation rates (14–16), thus suggesting that the POSEIDON's marker might be a useful endpoint for clinicians providing care to infertility patients.

Recently, a clinical predictive model named "ART Calculator" was developed to estimate the number of metaphase II (MII) oocytes needed to achieve at least one euploid embryo for transfer in each patient undergoing ART (17). The model was built based on clinical and embryonic data of over 300 infertile couples who underwent *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) and trophectoderm biopsy for preimplantation genetic testing for aneuploidy (PGT-A). The fitted model selected female age, sperm source –adjusted by type of azoospermia whenever appropriate–, and MII oocytes as predictors. A final logistic regression analysis model was developed based on the above predictors to estimate the probability of an MII oocyte become a euploid blastocyst as a function of female age and sperm source. Lastly, an online calculator was created–based on mathematical equations and the probabilities mentioned above–to predict the minimum number of MII oocytes (MIImin) required to obtain at least one euploid blastocyst for specified probabilities of success.

We propose that using pretreatment factors to predict the MIImin could be useful in shared decision-making concerning ART treatments. Herein, we investigated the reliability of the ART calculator using real-world data from couples undergoing ART.

MATERIALS AND METHODS

After ethics committee approval, we formed a multicenter collaborative group to enroll consecutive infertile couples who underwent IVF-ICSI treatment intending to have trophectoderm biopsy for PGT-A from July 2017 to August 2018. The ethics committees of *Instituto Investiga*, Campinas, Brazil (CAAE 64291417.0.0000.5599), Hacettepe University, Ankara, Turkey (KA-180069), and Clinica Valle Giulia, Rome, Italy have approved the study.

Study Population and Patients' Eligibility Criteria

The patients were retrospectively selected using pre-defined inclusion/exclusion criteria from three institutions: Anatolia IVF and Women's Health Center, Ankara, Turkey (Anatolia), G.E.N.E.R.A. center for Reproductive Medicine, Rome, Italy (GENERA), and ANDROFERT, Andrology and Human Reproduction Clinic, Campinas, Brazil (Androfert).

All patients were subjected to IVF/ICSI with the intention to have PGT-A, a test to analyze the DNA of blastocysts for determining genetic abnormalities (aneuploidies). PGT-A was indicated due to advanced maternal age, recurrent miscarriage, repeated implantation failure, severe male factor, and due to patients' concerns about their embryonic ploidy status.

Eligible patients were consecutive infertile couples undergoing their first IVF/ICSI cycle irrespective of the protocol used for ovarian stimulation. We only included patients who reached at least the oocyte pick-up stage, regardless of whether or not a blastocyst was available for biopsy. Moreover, patients were only included if all retrieved MII oocytes were inseminated for own use and the resulting viable blastocysts biopsied. Patients who had PGT for balanced translocations or single-gene diseases were

excluded. We also excluded patients treated with donor oocytes, those whose cycles involved injection with both ejaculated and surgically retrieved sperm, and those who used both fresh and frozen-thawed gametes (e.g., fresh and frozen-thawed sperm or fresh and vitrified-warmed oocytes) simultaneously. Cycles in which PGT-A was carried out on vitrified-warmed blastocysts were also excluded.

The participating centers used a unique case report form (CRF) for data collection. Each included couple contributed data concerning only one IVF/ICSI cycle. A total of twenty-three variables were included (**Supplementary Table 1**). Demographic data included age, body mass index (BMI), infertility duration, infertility factor, antral follicle count (AFC), anti-Müllerian hormone (AMH) levels, and semen parameters. Treatment data comprised the type of ovarian stimulation, gonadotropin regimen, total gonadotropin dose, sperm source for ICSI, and gamete status (fresh or frozen-thawed). Lastly, treatment outcomes included the number of oocytes retrieved, number of MII oocytes retrieved, number of two-pronuclei (2PN) zygotes, number of blastocysts, and number of euploid blastocysts. Codes replaced the records linking patients' identification. Each center's dataset was sent to a third-party statistical company for compilation and analysis.

Treatment Characteristics

The included couples were evaluated and treated according to each institution's policies, as previously described (18–21). In brief, the ovarian reserve was determined by either AFC or AMH levels, or both, using standardized protocols (22, 23). The AMH values were obtained with the aid of the modified Beckman Coulter generation II assay (23), whereas the AFC was evaluated on the early follicular phase using a two-dimension ultrasound scan (22). Semen analyses were carried out according to the 2010 World Health Organization manual for the examination of human semen (24, 25). The type of azoospermia, when applicable, was determined by the treating physician using a combination of clinical and laboratory data.

The process of ART included ovarian stimulation, oocyte retrieval, fertilization, blastocyst culture, blastocyst biopsy, PGT-A, and subsequent vitrified-warmed embryo transfer. The choice of the ovarian stimulation regimen and gonadotropin dosage was based on the clinician's assessment of ovarian reserve, female age, and history of previous response to ovarian stimulation (18, 19, 26). One of the three protocols was used for ovarian stimulation, namely, (i) long GnRH agonist protocol (Lucrin; Abbott), (ii) GnRH antagonist protocol [Cetrotide (Merck) or Orgalutran (MSD)], and (iii) minimal stimulation protocol. Recombinant FSH [Gonal-F (Merck) or Puregon (MSD)] monotherapy, recombinant FSH combined with recombinant LH [2:1 ratio, Pergoveris (Merck)], recombinant FSH (Gonal-F, Merck) combined with either hMG (Menopur, Ferring) or recombinant LH (Luveris, Merck), or highly purified hMG monotherapy (Menopur; Ferring) were used for ovarian stimulation with initial daily doses varying from 150 to 450 IU. After 5 days of stimulation, the ovarian response was monitored with the use of transvaginal ultrasonography and serum estradiol measurements to adjust daily gonadotropin dosing. Both fixed and flexible GnRH antagonist protocols were used. The antagonist was started on the fifth or sixth day of ovarian stimulation or when the leading follicle achieved 12 mm mean diameter in the fixed and flexible regimens, respectively. The minimal stimulation protocol consisted of either clomiphene citrate or letrozole, followed by a low dose of injectable gonadotropin.

Trigger of final oocyte maturation was achieved by a single subcutaneous injection of (i) recombinant hCG (250 mcg; Ovitrelle, Merck), (ii) urinary hCG (10,000 IU; Gonasi, IBSA), or (iii) GnRH agonist [0.2 mg triptorelin (Decapeptyl; Ferring) or 50 IU buserelin (Suprefact, Sanofi-Aventis)] according to each Center's policies. Oocyte retrieval was carried out under intravenous anesthesia with the use of transvaginal ultrasound-guided puncture of follicles 35–37 h after triggering final oocyte maturation.

In vitro Fertilization Procedures

After 2–4 h of incubation, cumulus-oocyte complexes were denuded by exposure to 80 IU/mL hyaluronidase solution diluted 10-fold with buffered media, and also mechanically by denuding plastic pipettes. Sperm preparation was carried out as previously described (6, 27–29). Insemination of oocytes through ICSI was carried out immediately after denudation (28, 30). Each inseminated oocyte was then placed in a microdroplet of culture medium, covered by pre-equilibrated mineral oil in a micro-well, and loaded into the incubator. Fertilization was checked 16–18 h post-insemination and defined as the presence of two pronuclei (2PN) and two polar bodies. The zygotes were kept in culture to reach the blastocyst stage. Embryo culture was carried out at 37°C under ∼6.0% CO_2 and 5% O_2 with either a sequential [Quinn's Advantage cleavage-blastocyst media (Origio), G-family media (Vitrolife), and Sidney IVF (Cook)] or a continuous medium [CSCM (Irvine Scientific)], either using a time-lapse (Embryoscope, Vitrolife) or standard incubators (Minc, Cook). Embryo quality was scored according to the criteria described by Gardner (29–31).

Trophectoderm Biopsy and Preimplantation Genetic Testing

Trophectoderm biopsy was performed on expanding, expanded, and hatched blastocysts (days 5 or 6) (17, 20, 32). In general, zona opening was not performed at the cleavage stage. All biopsies were conducted on a heated stage in a dish prepared with microdroplets of buffered medium overlaid with pre-equilibrated mineral oil. A diode laser was used to assist an opening of 10–15 μm in the zona pellucida (20, 33, 34). Five to ten trophectoderm cells were then aspirated into the trophectoderm biopsy pipette followed by laser-assisted removal of the target cells from the body of the embryo. Biopsied embryos were vitrified.

At Anatolia and Androfert, trophectoderm biopsies were sent to a reference genetic laboratory for the analysis (Genlab, Ankara, and Chromosome, São Paulo, respectively). Samples were processed for whole-genome amplification (WGA) and next-generation sequencing (NGS). In the former, biopsied trophectoderm samples were transferred to 1xPBS solution in

PCR tubes, stored at −20°C until 24 samples were collected, and then shipped to the central laboratory. Whole-genome amplification was performed using the Sureplex amplification kit (Illumina, San Diego, CA, USA) (35). After WGA, amplification was checked in gel electrophoresis, and DNA concentration was measured using the dsDNA high-sensitivity assay kit (Qubit®; Life Technologies, Waltham, MA, USA). After that, the VeriSeq PGS kit (Illumina, San Diego, CA, USA) was used for NGS library preparation following the manufacturer's protocol for fragmentation, tagmentation, indexing, and purification steps. After normalization, samples were pooled, denatured, and sequenced using Miseq (Illumina, SanDiego, CA, USA). The generated data were analyzed using BlueFuse Multi Software (Illumina, SanDiego, CA, USA). In the latter, the biopsied fragments were immersed into 0.2 mL PCR tubes in a total volume of 2.5 μL of Tris-EDTA buffer pH 8.0 (ThermoFisher Scientific Baltics, Vilnius, Lithuania), frozen at −20 Celsius degrees, and then shipped for analysis. Specimens were subjected to cell lysis, WGA, and construction of libraries using the Ion Reproseq kit (ThermoFisher Scientific, Germany). The DNA quantity was estimated using StepOne (ThermoFisher Scientific, Germany) following the manufacturer's protocol, and NGS was performed using the Ion Torrent PGM™ platform (ThermoFisher Scientific, Germany). Euploidy data analysis was carried out on the Ion Reporter software version 5.2 calibrated at medium sensitivity, using Low-Coverage Whole-Genome workflow (20). At GENERA, the chromosomal analysis was performed through a quantitative real-time polymerase chain reaction (qPCR) as previously described (36). In brief, multiplex amplification of 96 loci (four for each chromosome) was carried out, and a method of relative quantification (37) was applied to predict the copy number status of each chromosome. This comprehensive chromosome testing approach passes through a targeted DNA pre-amplification protocol that does not identify segmental and mosaic aneuploidies (34).

Copy numbers were measured quantitatively, and embryos were classified according to the PGDIS criteria for reporting embryo results (38). In NGS, embryos with <20% of abnormal cells were classified as euploids, whereas embryos with >80% of abnormal cells were deemed aneuploids. Mosaic embryos were those with abnormal cells ranging from 20 and 80%. In qPCR, euploidy was reported when normal chromosomal segregation was detected in each of the 24 chromosomes.

STATISTICAL ANALYSIS

Validation Procedure

The validation procedure followed a stepwise approach as depicted in **Figure 1**.

Firstly, we analyzed the distribution of the number of euploid blastocysts per patient to model the logistic regression analysis. Secondly, we applied a generalized linear model using the adaptive Lasso (Least Absolute Shrinkage and Selection Operator) method, including MII oocytes as a factor to adjust the data, for the selection of predictors concerning the response variable "number of euploid blastocysts" (39, 40). The stopping rule on the Lasso procedure was based on the adjusted Akaike Information Criteria (AIC). We included MII oocytes rather than the total number of retrieved oocytes as the former are the gametes with the capacity to support embryo development to the blastocyst stage and beyond (41, 42). Thus, we avoided the confounding factors that could potentially influence the MII rate. Once the predictors were selected, a logistic regression model–fit to the binomial response euploid (yes/no)–was built. The response was the pair m, N [number of euploid blastocysts (m), number of MII oocytes (N)] for each woman. This logistic model generates the probability (p) as the output, where "p" is the probability that an MII oocyte would turn into a euploid blastocyst, given the relevant predictors. Since participating centers might have different success rates and used distinct genetic analysis platforms, we also included "center" as a predictor to quantify any variation among centers.

The predictive ability of the final model was evaluated by the holdout sampling method. This method randomly split the data into training and validation sets. The training dataset size was 75% of the total, and the validation dataset was 25% of the total. The computation was carried out on the training dataset and its results applied to the validation dataset. Since the validation dataset was not used for the estimations, it can be deemed "new" or "future" data. If the quality of the fits–assessed by the area under the receiver operating characteristics (ROC) curve (AUC)–are comparable between the training and validation datasets, the final model would be apt to be used elsewhere. Then, the probabilities generated by the model were used to determine the MIImin for different success probabilities using the formula $\text{MIImin} \geq \frac{\log(1-\pi)}{\log(1-p)}$, as previously described (17). The probability of success was denoted by π, and its complement, $1-\pi$, was the risk, i.e., the probability of having no euploid blastocyst despite achieving the estimated MIImin.

Lastly, we compared the fittings generated by the final (validation) model and the ART calculator (17) (https://members.groupposeidon.com/Calculator/) and assessed the predictive value of the latter using the validation dataset. These parameters were the primary validation tests. Graphs and a descriptive correlation measure were used to compare the outputs generated by the validation model and the ART calculator concerning the calculated probabilities "p" and the MIImin. The predictive value of the ART calculator was assessed by computing the frequency of patients with at least one euploid blastocyst among those who achieved the MIImin as predicted by the ART calculator. It is expected that the frequency of cases reaching the MIImin would be at least equal to the probability of success denoted by π. The movie shows how the ART calculator was used to provide the MIImin (see **Supplementary Video**).

Sample Size Calculation

The sample size was determined based on the accuracy of the prediction model to estimate the probability "p" that an MII oocyte would turn into a euploid blastocyst (43, 44). For this, we used the ROC curve and set the AUC value as 0.75 and the confidence interval (CI) as 0.07. We estimated *a priori* a 20% loss in the valid cases. Using these assumptions, a dropout inflated sample size of 900 subjects

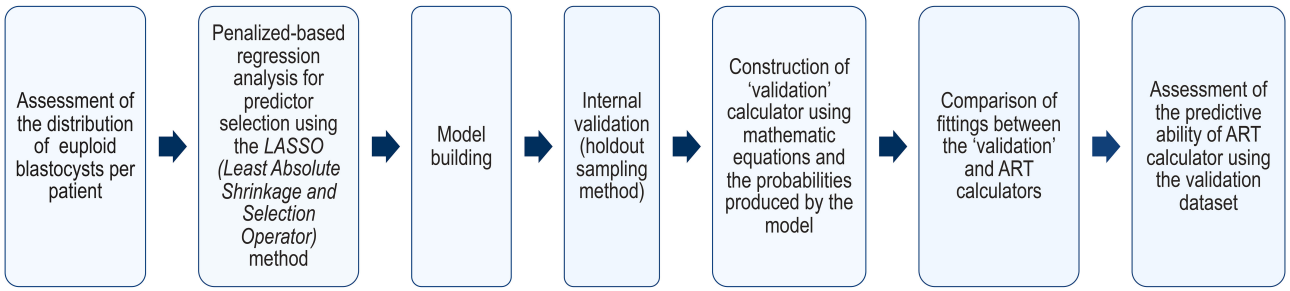


FIGURE 1 | Validation process roadmap.

produces a two-sided 95% CI with a width of 0.07 when the AUC is 0.75.

Missing Data

Data with missing predictor values were excluded *a fortiori* by the regression calculations. Data imputation was not used. Concerning ovarian reserve tests, we included cases in which either the AFC or the AMH value was available.

Sensitivity Analysis

Since critical embryonic outcomes might impact the estimated probabilities of MII oocytes turning into euploid blastocysts, we assessed whether 2PN fertilization rates and blastulation rates differed among study centers. The 2PN fertilization rate was the number of fertilized oocytes on day 1 (presence of 2PN and two polar bodies assessed at 17 ± 1 h post-ICSI), as a function of all MII oocytes injected. The blastulation rate was the proportion of blastocysts observed on days 5 and 6 post-insemination as a function of the number of 2PN zygotes. The Tukey-Kramer HSD (honestly significant difference) test was used to perform multiple comparisons of means for the variables 2PN fertilization rates and blastulation rates. The test is an exact alpha-level test if the sample sizes are the same, and conservative if the sample sizes are different.

Computations were performed using JMP® PRO 13 (SAS Institute, Cary, North Carolina, US) and PASS 15.0.4 software (NCSS, Kaysville, Utah, USA).

RESULTS

Patient Characteristics

A total of 1,464 patients were included, all of which had a complete IVF/ICSI record for 19 predictors (**Figure 2** and **Supplementary Table 2**). **Table 1** shows the distribution of the characteristics of the couples and their IVF/ICSI cycle. The mean female age of our selected cohort was 39.4 years (95% CI: 33.0–44.0 years) with a mean number of MII oocytes retrieved per patient of 6.7 (95% CI: 1.0–16.0). The mean number of blastocysts available for biopsy and PGT-A per patient was 2.1 (95% CI: 0.0–6.0). A total of 9,779 MII oocytes were injected, resulting in 3,108 blastocysts that were subjected to PGT-A. Overall, the percentage of euploid embryos in our cohort was 42.0%. The mean number of euploid blastocysts per patient was 0.9 (95% CI: 0.0–4.0).

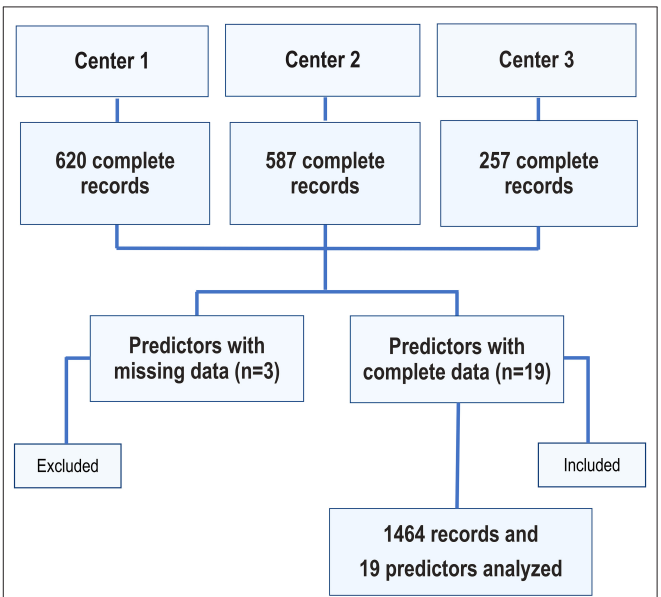


FIGURE 2 | Flowchart depicting the number of included couples and analyzed predictors.

The number of euploid blastocysts per woman followed a negative binomial (Gamma-Poisson) distribution (**Supplementary Figure 1**). The patient demographics and cycle characteristics by the study's Center are provided in **Supplementary Tables 3–5**. A total of 620, 587, and 257 patient records were available for analysis by Anatolia, GENERA, and Androfert, respectively. Among the included patients, 19 (1.3%) and 355 (24.2%) had no retrieved metaphase II oocytes and blastocysts available for PGT-A, respectively.

Development of Validation Model

For the selection of predictors, the stopping rule on the Lasso procedure was based on the adjusted Akaike Information Criteria. The model is a generalized linear model, and the response is the number of euploid blastocysts. The negative binomial distribution was applied to the fit. Accordingly, the link function is the logarithm. For the overdispersion, we chose the identity as the link function. Among the 19 eligible pretreatment

TABLE 1 | Demographics and treatment characteristics of included couples.

Characteristics	*N*	Median	95% CI
Female age (years)	1,464	39.4	33.0–44.0
Male age (years)	1,464	42.0	33.0–52.0
BMI, female (kg/m^2)	1,464	24.8	19.0–34.0
BMI, male (kg/m^2)	333	26.9	21.6–33.1
Infertility factor, N (%)			
Male factor	252 (17.2)	–	–
Unexplained	544 (37.2)	–	–
Endometriosis	58 (3.9)	–	–
Endocrine/Anovulatory	106 (7.2)	–	–
Anatomic/Tubal	55 (3.7)	–	–
>1 type	449 (30.8)	–	–
Baseline FSH (UI/mL)	408	8.6	4.6–14.8
Ovarian reserve marker			
AFC (n)	1,464	9.3	2–22
AMH (ng/mL)	1,287	2.0	0.2–6.8
Semen parameters			
Sperm count (M/mL)	1,464	38.9	0.0–100.0
Total motility (%)	1,395	52.0	20.0–75.0
Sperm morphology (%)	797	3.8	1.0–8.0
DFI (%)	179	21.5	7.0–50.0
Azoospermia, *N* (%)	69 (4.7)		
Non-obstructive; *N* (%)	35	–	–
Obstructive; *N* (%)	34	–	–
Poor ovarian reserve, *N* (%)	482 (32.9)	–	–
Male factor associated (%)	458 (31.3)	–	
Type of ovarian stimulation			
Conventional ovarian stimulation; *N* (%):	1,366 (93.3)	–	–
Minimal stimulation, *N* (%)	98 (6.7)	–	–
Type of gonadotropin; *N* (%)			
rFSH monotherapy		513 (35.1)	–
rFSH + rLH		296 (20.2)	–
rFSH + hMG		538 (36.7)	–
hMG alone		97 (6.6)	–
None		20 (1.4)	–
Total gonadotropin dose (IU)	1,464	3,060.4	850.0–4,950.0
Sperm source for ICSI; *N* (%)			
Ejaculate	1,358 (92.7)	–	–
Epididymis	17 (1.3)	–	–
Testicle	89 (6.0)	–	–
Ejaculated sperm; *N* (%)			
Homologous; normal	761 (56.1)	–	–
Homologous; abnormal	587 (43.2)	–	–
Heterologous	10 (0.7)	–	–
Gamete status for ICSI; N (%)			
Fresh, sperm [S] + oocyte [O]	1,388 (94.8)	–	–
Cryopreserved [S + O]	0 (0.0)	–	–
Combined, fresh [S] + vitrified-warmed [O]	7 (0.5)	–	–
Combined, frozen-thawed [S] + fresh [O]	69 (4.7)	–	–
Oocyte and embryo parameters	1,464		
No. Oocytes retrieved		8.8	1.0–22.0
No. Mature (MII) oocytes		6.7	1.0–16.0
No. Fertilized oocytes (2PN)		4.8	0.0–12.0
No. Blastocysts		2.1	0.0–6.0
No. Euploid blastocysts		0.9	0.0–4.0

BMI, body mass index; AFC, antral follicle count; AMH, anti-Müllerian hormone; DFI, Sperm DNA fragmentation index; FSH, follicle stimulating hormone; POR, poor ovarian reserve defined according to the POSEIDON criteria, namely, antral follicle count (AFC) <5 and/or AMH hormone <1.2 ng/mL; 2PN, two pronuclei zygote; MII, metaphase II.

predictors (see **Supplementary Table 2**), the model selected only female age (**Supplementary Table 6**).

In the validation dataset, however, the number of cases involving azoospermia was small, in particular, when assessing the dataset of Anatolia and GENERA. Given the importance of sperm source in the ART calculator (17), which was highly dependent on the female age, we included sperm source in the final model. Furthermore, owing to the different methods for assessing blastocyst euploid between GENERA (qPCR) and Anatolia/Androfert (NGS), we also included the "study center" in the final model.

Table 2 shows the final fitted predictive model of the binomial response euploid (yes/no) for each MII oocyte using female age, sperm source, and study center, all of which were found to be statistically relevant predictors. In particular, sperm source only applied to the comparisons between ejaculated sperm and testicular sperm from men with NOA. Moreover, the effect of the "study center" was exclusively noted when the Italian center was compared to the two other centers. **Supplementary Table 7** shows the previously published ART calculator predictive model for comparison purposes only. In the latter, only the female age and sperm source were relevant predictions. Notably, the original ART calculator model was developed using a single-center dataset, thus making the "study center" irrelevant for model comparison.

Figure 3 shows the predicted probabilities of an MII oocyte turning into a euploid blastocyst, which decreased progressively as a function of the female age. Overall, the probabilities were negatively modulated by the use of testicular sperm from men with NOA across age. The effect of sperm source was highly dependent on the female age.

Figure 4 shows the relative influence of the "study center" on the calculated probabilities. The figure shows the probabilities according to female age and sperm source. The fittings revealed that the probability of an MII oocyte turning into a euploid blastocyst was overall impacted by the study center. Notably, the fittings were very close between the Turkish and Brazilian centers. Both centers used NGS for blastocyst chromosomal analysis, which coincides with the platform utilized to construct the ART calculator model. By contrast, the probabilities of an MII oocyte turning into a euploid blastocyst were higher in the Italian center

TABLE 2 | Final validation model for prediction of the probability (p) of euploid blastocyst per mature (MII) oocyte.

$Y = -2.728414 - 0.138868$ [I(spermSource=Ejaculate) – I(spermSource=Testicular_NOA)]
-0.13032 [I(spermSource=Testicular_NOA) – I(spermSource=NOA)]
$+0.4928267$[I(spermSource = Ejaculate) – I(spermSource=Testicular NOA)]
$+0.0807783$[I(Center = Anatolia) – I(Center = Androfert)]
$+0.3765617$[I(Center = GENERA) – I(Center = Anatolia)]
Where the indicator function I(x) = 1 if x is TRUE and 0 otherwise. The probability p is $p = \left(\frac{1}{1+e^{-Y}}\right)$

Term	Estimate	Std error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Intercept (a)	−2.728414	0.183	220.281	<0.0001	−3.088	−2.368
spermSource[Ejaculate-Testicular_NOA]:(ageFemale-39.414)	−0.138868	0.007	306.601	<0.0001	−0.154	−0.123
spermSource[Testicular_NOA-NOA]:(ageFemale-39.414)	−0.13032	0.027	22.471	<0.0001	−0.184	−0.076
spermSource[Ejaculate-Testicular NOA]	0.4928267	0.179	7.570	0.006	0.141	0.843
Center [Anatolia-Androfert]	0.0807783	0.105	0.591	0.441	−0.125	0.286
Center [GENERA-Anatolia]	0.3765617	0.068	30.010	<0.0001	0.241	0.511

Response: euploid blastocyst per MII oocytes	–LogLikelihood: 1527.242
Distribution: negative binomial	Number of Parameters: 6
Estimation method: adaptive Lasso	BIC: 3,098.065
Validation method: AICc	AICc: 3,066.544
Probability model link: Logit	Generalized RSquare: 0.258
Number of rows: 1,464	Area under the curve: 0.700

The full equation is written at the top of the table. Each particular characteristic is displayed with an associated P-value (Prob >ChiSquare) giving the indication of how much weight each variable will contribute to the probability of blastocyst euploidy per metaphase II oocytes.

than the Turkish and Brazilian centers. The former analyzed the blastocysts through qPCR comprehensive genetic screening.

Sensitivity Analysis

Analysis of embryonic outcomes that might have influenced the estimated probabilities of an MII oocyte to turn into a euploid blastocyst demonstrated that the means concerning 2PN fertilization rates and blastulation rates were not significantly different among study centers (**Supplementary Data Sheet**).

Assessing Ability to Predict Blastocyst Euploidy Probability

The internal validation by the holdout sampling method revealed that both the R-squares (∼0.26) were very close between the validation and training datasets. Moreover, both the AUC (∼0.70) and the ROC curves were also practically identical (**Figure 5**).

Comparison of Fittings

Figure 6 shows the comparison of predicted blastocyst euploidy probabilities per MII oocyte between the validation model and ART calculator. The curves depict the probabilities according to the female age and sperm source. Both age and type of sperm used for ICSI were influential; younger women and the use of ejaculated sperm for ICSI were associated with a higher chance of having a euploid blastocyst per MII oocyte. The fittings generated by the ART calculator and validation model were similar. The median absolute difference in the predicted probabilities between both models was 0.02 (95% CI 0.00–0.05) (**Supplementary Figure 2**).

Supplementary Figure 3 shows the relative influence of the "study center" for assessing blastocyst euploidy on the calculated probabilities. The fittings of both the Turkish and Brazilian centers were very close to that of the ART calculator, in particular among women of 35 years and older; this subset of patients comprised 94% of the validation dataset (**Supplementary Figure 4**). Furthermore, the fittings of the Italian center and the ART calculator showed similar shapes. Still, the former yielded slightly higher blastocyst euploidy probability per MII oocyte across age than that of the ART calculator. The mean absolute differences on the predicted probabilities between the ART calculator and validation model by country were 0.011 and 0.015 in the Turkish and Brazilian centers [95% interquartile ranges 0.015 (Androfert) and 0.008 (Anatolia)], respectively, whereas it was 0.047 in the Italian center [95% interquartile range 0.005 (GENERA); **Supplementary Figure 5**].

Figure 7 shows the correlation concerning the predicted probabilities of blastocyst euploid per MII oocyte between the validation model and ART calculator; the probabilities were highly correlated ($r = 0.91$). **Figure 8** shows the correlation between the MIImin estimated by the ART calculator and the validation model. The formula $MIImin \geq \frac{\log(1-\pi)}{\log(1-p)}$ was used to compute the minimum number of MII oocytes required to obtain at least one euploid blastocyst. The figure shows the correlations according to three user-defined probabilities of success (π) concerning the estimations, namely, 70% (**Figure 8A**), 80% (**Figure 8B**), and 90% (**Figure 8C**). In all scenarios, the MIImin estimated by the ART calculator was highly correlated with the MIImin estimated by the validation model ($r \sim 0.88$).

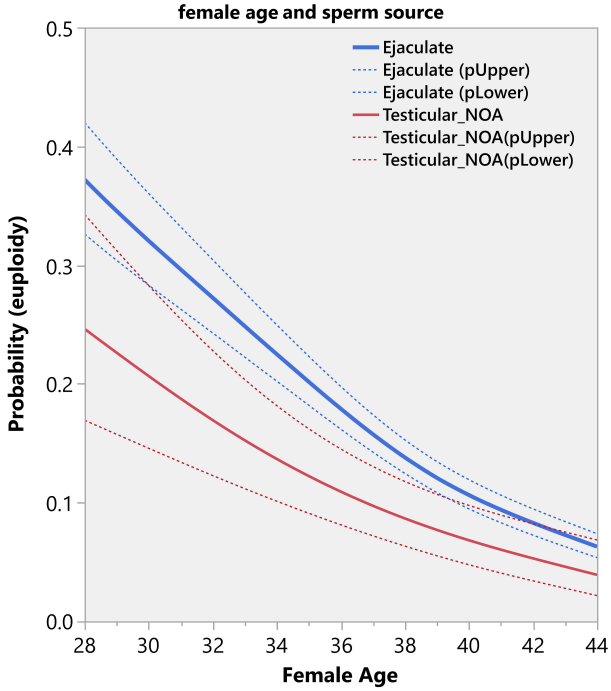


FIGURE 3 | Blastocyst euploidy probability per mature (MII) oocyte. The plots show the probability of an MII oocyte turn into a euploid blastocyst as a function of female age and sperm source. The estimated probabilities (solid curves) and their 95% CI (dotted curves) are presented according to sperm source for IVF/ICSI, namely, ejaculated sperm (blue) and testicular sperm extracted from patients with non-obstructive azoospermia (NOA) (red). The relations are non-linear and characterized by a differential modulatory effect of sperm source across age (see text).

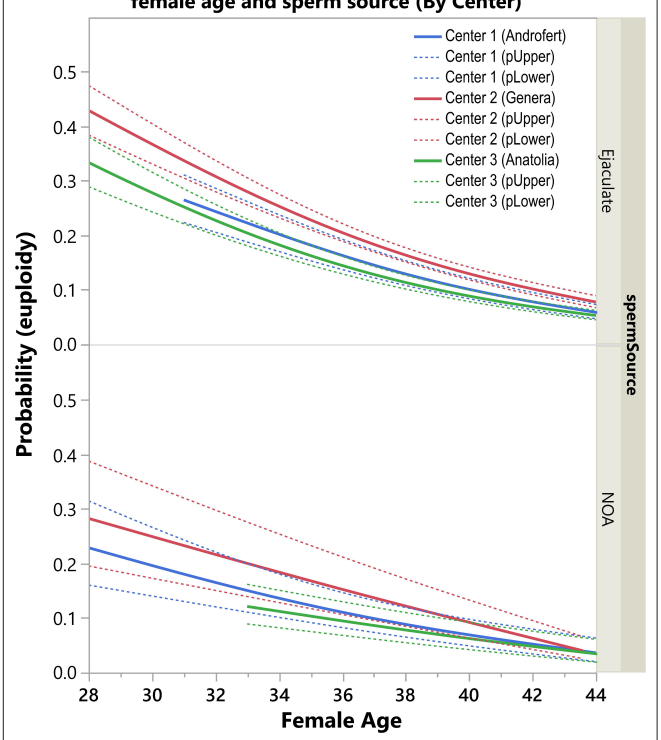


FIGURE 4 | Center effect (genetic analysis method) on blastocyst euploidy probability per mature (MII) oocyte. The plots show the probability of an MII oocyte turn into a euploid blastocyst as a function of female age and sperm source by Center. The estimated probabilities (solid curves) and their 95% confidence interval (dotted curves) are presented according to sperm source for IVF/ICSI, namely, ejaculated sperm (top) and testicular sperm extracted from patients with non-obstructive azoospermia (NOA) (bottom). Centers 1 and 3 utilized next generation sequencing (NGS) for the analysis of trophectoderm biopsies whereas Center 2 used quantitative real-time polymerase chain reaction (qPCR). The relations are non-linear and characterized by a differential modulatory effect of sperm source and genetic analysis method across age (see text).

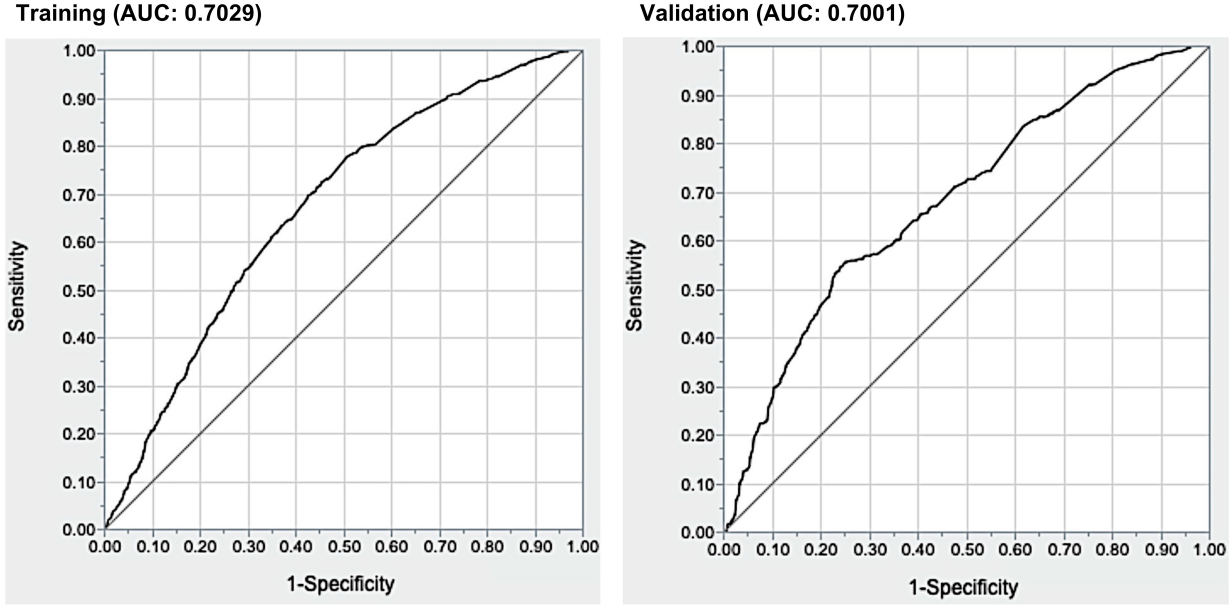
ART Calculator Predictive Ability

The validation dataset comprised of 1,464 patients was used to assess the ART calculator performance. The frequencies of patients with at least one euploid blastocyst among those who achieved the predicted MIImin by the ART calculator (positive predictive value) were 84.8, 87.5, and 90.0% for 70, 80, and 90% probabilities of success (π), respectively (**Table 3**).

DISCUSSION

We have validated a point-of-care clinical tool, named "ART calculator," to assist clinicians in predicting the minimum number of MII oocytes required to achieve at least one euploid blastocyst for transfer in infertile couples undergoing IVF/ICSI through the use of a database obtained from a retrospective analysis of three institutions. The validation procedure followed the same steps applied during the development of the ART calculator (17), but it included an external cohort 5-fold bigger than that used in the latter. The model was reliable and adequately predicted the MIImin for different user-defined probabilities of success. The similarities between the predictive ability of the validation and ART calculator models indicate that the estimations should hold for future data. While the ART calculator performed better when NGS was the method for blastocyst chromosome screening, it also correlated well with qPCR data.

The clinicians counseling infertile couples who are embarking on ART may now have an additional tool to provide individualized recommendations regarding the MIImin required to achieve at least one euploid blastocyst for transfer. Personalized tools to objectively assess the probability of success in ART are urgently needed because patients do not fully understand the association between the availability of oocytes and embryos and pregnancy failure. Thus, proper counseling regarding the chances of success in ART needs improvement. The availability of at least one euploid embryo for transfer has a major impact for the patient undergoing ART, as ~50–60% of euploid blastocysts implant across all age categories (15, 16). The ART calculator may help to discuss



Response	euploidBlastocysts
Distribution	Binomial
Estimation Method	Adaptive Lasso
Validation Method	Validation Column
Probability Model Link	Logit

Measure	Training	Validation
Number of rows	1098	366
Sum of Frequencies	1098	366
-LogLikelihood	1146.5199	382.21863
Number of Parameters	5	5
BIC	2327.917	793.82595
AICc	2303.0963	774.60821
Generalized RSquare	0.2521806	0.2722234

FIGURE 5 | Internal validation. The final model was validated by the holdout method (75% of the data in the training dataset, 25% on the validation data set). The areas under the receiver operating characteristics curves (AUC) curves (∼0.70) and the generalized RSquare results (∼0.26) were similar, thus indicating that the results should hold true for future data.

these issues by providing an objective assessment of the number of oocytes needed to optimize the chances of implantation, with potential clinical utility for guidance concerning the development of a workable therapeutic plan to reduce the time to live birth.

Interpretation

The ART calculator focuses primarily on pretreatment predictors, in particular, female age and type of sperm used for IVF/ICSI, to assist with the informed decision-making process. In this study, we confirmed the role of the female age by assessing a large validation dataset of three ART centers from three countries. Importantly, our dataset included consecutive infertile couples undergoing IVF/ICSI with the intention of having PGT-A. It means patients were included irrespective of having a blastocyst available for biopsy, likewise in the ART calculator original study (17). This feature of the study's design was essential to accurately estimate the number of MII oocytes required to achieve at least one euploid blastocyst because many patients undergoing ART do not have either MII oocytes retrieved or embryos available for PGT-A.

Firstly, we analyzed the distribution of the number of euploid blastocysts per couple and found that it followed a negative binomial (Gamma-Poisson) distribution. This distribution was the same attained in the ART calculator original dataset, thus confirming previous observations (17). Then, we assumed the negative binomial model for the number of euploid blastocysts, and applied a penalized method, named the Lasso, for variable selection (39, 40). The negative binomial was chosen from the first principles and from the heuristic fact that this distribution fitted the data very closely. The method, which allows for the fitting of correlated and high-dimensional data (39, 40, 45), removed redundant variables and selected female age as the only relevant predictor.

Subsequently, we built a generalized regression model–fit to the binomial response euploid (yes/no) for each MII oocyte–using predictors deemed relevant. The response was the pair m, N [number of euploid blastocysts (m), number of MII

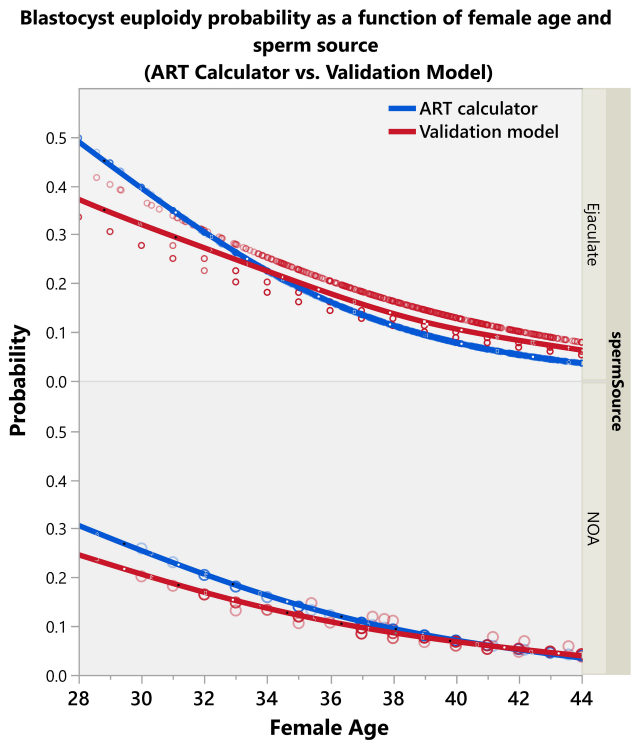


FIGURE 6 | Plots showing the predicted blastocyst euploidy probabilities (per MII oocyte) by the validation model and ART calculator.

oocytes (N)] for each woman. In addition to the female age, we included "sperm source" in the final fitted model for two reasons. Firstly, it was deemed necessary in the ART calculator development study. Secondly, the number of cases involving non-ejaculated sperm was small in the validation dataset, which might have resulted in the removal of this predictor by the LASSO method.

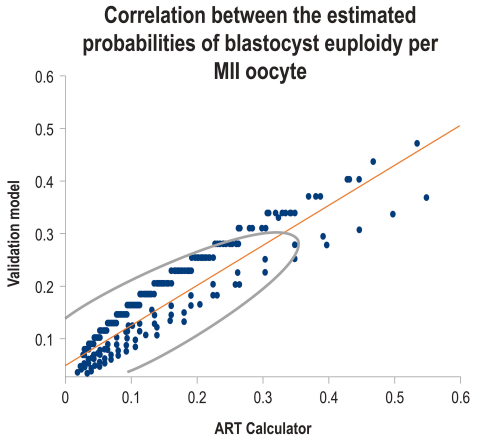
Moreover, we included the technique of blastocyst euploidy assessment as they differed between the study centers. The Italian center utilized qPCR, whereas the Turkish and Brazilian centers applied NGS. Unlike NGS, qPCR does not highlight embryos with a PGT-A result falling in the mosaic range (46).

Indeed, the validation model confirmed that the effect of sperm source was highly dependent on the female age, thus confirming the results of the ART calculator study (17). Our data indicate that the estimated probability of an MII oocyte turn into a euploid blastocyst decreases progressively with female age, an effect that is negatively modulated by the use of testicular sperm from men with NOA, like that observed in the ART calculator development study. While the impact of testicular sperm was meaningful in younger women, it was practically offset in women of 40 years and over, thus indicating that the effect of advanced female age on embryo quality is so dramatic that it cannot be changed significantly by any other factor. Of note, these results must be interpreted with caution given the limited number of men with azoospermia and women younger than 35 years in our dataset. The blastocyst euploidy probabilities–as shown in **Figure 3**–are more meaningful for the female age range between 35 and 44 years and ICSI cases involving the use of ejaculated sperm, which comprised over 95% of our dataset.

Nevertheless, our data are consistent with previous reports, which showed that the use of testicular sperm from men with NOA adversely affects the likelihood of obtaining a euploid blastocyst per oocyte pickup. This effect is caused primarily by the lower fertilization rate and blastocyst development rate with the use of testicular sperm than ejaculated sperm (6, 17, 47). Thus, the sperm source has to be discussed in certain situations, although the most critical factor in predicting the number of mature oocytes for at least one euploid blastocyst is the female age. With aging, oocyte chromosomal abnormalities and cytoplasmic dysfunctions are increased, whereas the number of primordial follicles progressively declines (20, 48–50). Consequently, both embryo quantity and quality are reduced, thus explaining the reasons why IVF success is lower in older women than in younger counterparts (51).

The validation model revealed that the probability of blastocyst euploidy per MII oocyte was affected by the center in which IVF/ICSI was carried out. Since participating centers might have different success rates, we assessed whether critical embryonic outcomes impacted the blastocyst euploidy probability. We found that there were no differences in 2PN fertilization and blastulation rates among centers (**Supplementary Data Sheet**). These findings suggest that the genetic analysis method was the likely reason explaining the differential blastocyst euploidy probability per MII oocyte between the Italian and Turkish/Brazilian centers. As previously mentioned, the genetic analysis platform used to construct the ART calculator was the same as the one used by the Turkish and Brazilian centers. As expected, the mean absolute difference in the predicted probabilities between the ART calculator model and the validation model using the Turkish and Brazilian centers combined was very low (1%). By contrast, the probabilities of an MII oocyte turn into a euploid blastocyst were higher in the Italian center than the Turkish and Brazilian centers. The former analyzed the blastocysts through quantitative real-time polymerase chain reaction (qPCR) comprehensive genetic screening. The higher blastocyst euploidy probabilities per MII oocytes in GENERA relates to the fact that allegedly mosaic embryos are not reported. At GENERA, the decision of not reporting mosaic embryos relies on the current limitations of diagnosing chromosomal mosaicism from a single trophectoderm biopsy rather than to the molecular technique (52–55). Although the effect of "study center"–and its inherent differences concerning the type of utilized genetic analysis–was statistically significant, its clinical impact seems to be less relevant. Indeed, the mean absolute difference in the predicted probabilities generated by the ART calculator model and validation model was still low (4%) when only the Italian center was considered.

The next steps of our validation study were essentially mathematical. We assessed the prediction ability of the validation model by the holdout sampling method, which randomly splits the data in two, known as training and validation datasets.



Summary statistics

	Value	Lower 95%	Upper 95%
Correlation	0.907144	0.897567	0.915865
Covariance	0.004859		
Count	1464		

Variable	Mean	SD
ART calculator (euploidy)	0.103477	0.079794
Validation model (euploidy)	0.124836	0.067124
RSquare		0.822911
Root mean square error		0.028257
Mean of response		0.124836
Observations (or Sum Wgts)		1464

FIGURE 7 | Scatterplot showing the correlation between the ART calculator and validation model concerning the predicted probabilities of blastocyst euploid per MII oocyte. The density ellipse contains 95% of the points.

TABLE 3 | ART calculator predictive value.

			At least one euploid blastocyst				
			Yes (*N*)	**%**	**No (*N*)**	**%**	**Total (*N*)**
ART Calculator	Probability of success (π)						
	70%	MIImin (=yes)	317	**84.8%**	57	15.2%	374
		MIImin (=no)	334	30.6%	756	69.4%	1090
	80%	MIImin (=yes)	217	**87.5%**	31	12.5%	248
		MIImin (=no)	434	35.7%	782	64.3%	1216
	90%	MIImin (=yes)	135	**90.0%**	15	10.0%	150
		MIImin (=no)	516	39.3%	798	60.7%	1314

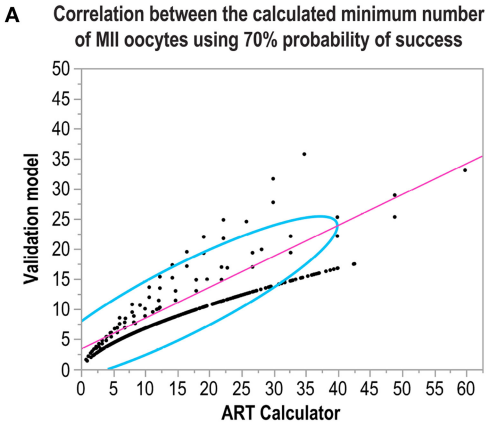
The validation dataset (N = 1,464 patients) was used to compute the frequencies of patients with at least one euploid blastocyst among those who achieved the predicted minimum number of metaphase II oocytes (MIImin) according to the ART calculator (positive predictive value; PPV) for three probabilities of success. The PPV are highlighted in bold.

The quality of the predictive model—assessed by comparing the ROC curves between the training and validation (holdout) datasets—was similar to that of the ART Calculator (0.70 vs. 0.72, respectively), thus suggesting that both models can be used elsewhere. For predictive models, calibration using an external dataset might increase performance owing to the homogeneity of the studied population (56). However, in our study, the calibration of the ART calculator using the external (validation) dataset did not improve its performance. In both models, the infertile couple was the observational unit and the pair (m, n), the response (where "n" is the number of metaphase II oocytes and "m" the corresponding number of euploid blastocysts). A heterogeneous (mixed) Poisson model might have produced the negative binomial distribution for the number of euploid blastocysts. The heterogeneity is expected given the distinct women ages. Thus, given the observations above and the complex nature of the process in which an MII oocyte might end up into a euploid blastocyst, the original ART calculator model with a ∼72% predictive ability should be the one to be used clinically.

Importantly, the objective of the validation model—as well as the ART calculator—was to develop a prediction formula for estimating the minimum number of MII oocytes needed to achieve at least one euploid blastocyst. There was no attempt to determine fundamental associations between the predictors and blastocyst euploidy (57). Thus, other known and unknown predictors might also influence blastocyst euploidy, but the inclusion of additional predictors from the existing dataset did not materially affect the estimates.

After the internal validation discussed above, the same model was run with the full dataset, that is, comprising the training and validation datasets, to predict the probability "p" of blastocyst euploidy per MII oocyte. The model itself was logistic, and the derived coefficients defined the linear expression "y" to obtain "p". The values of "p" were highly correlated between the validation model and the ART calculator ($r > 0.9$). The final endpoint was the MIImin oocytes required to obtain at least one euploid blastocyst. This endpoint was estimated using the value of "p" and the probability of success (i.e., the probability of having at least one euploid blastocyst if the predicted number of MII oocytes is achieved). Again, the MIImin generated by the validation model and ART calculator were highly correlated overall ($r \sim 0.9$).

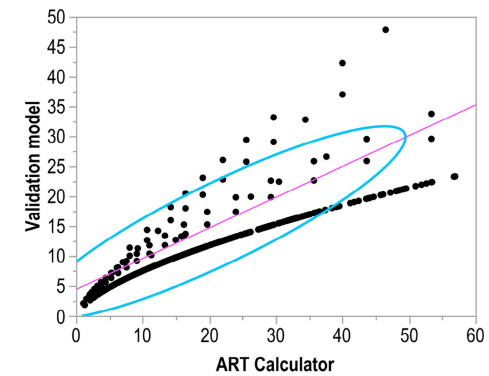
Lastly, we assessed the ART calculator's usefulness by computing its positive predictive ability. It was expected that the



Summary statistics

	Value	Lower 95%	Upper 95%
Correlation	0.886044	0.874354	0.896707
Covariance	47.87672		
Count	1464		

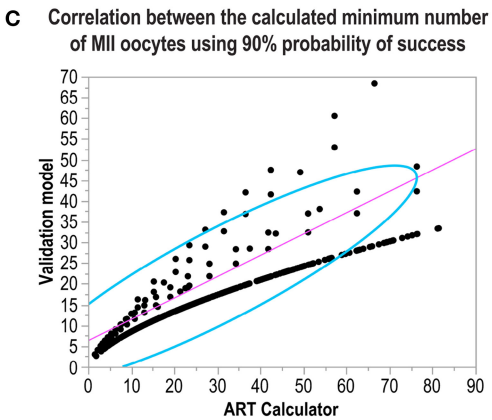
Variable	Mean	SD
RSquare		0.785074
Root mean square error		2.59684
Mean of response		11.66796
Observations (or Sum Wgts)		1464



Summary statistics

	Value	Lower 95%	Upper 95%
Correlation	0.886044	0.874354	0.896707
Covariance	85.55384		
Count	1464		

Variable	Mean	SD
RSquare		0.785074
Root mean square error		3.471385
Mean of response		15.5974
Observations (or Sum Wgts)		1464



Summary statistics

	Value	Lower 95%	Upper 95%
Correlation	0.885941	0.874321	0.896545
Covariance	174.7869		
Count	1464		

Variable	Mean	SD
RSquare		0.784891
Root mean square error		4.97117
Mean of response		22.38999
Observations (or Sum Wgts)		1464

FIGURE 8 | Scatterplots showing the correlation between the ART calculator and validation model concerning the predicted minimum number of MII oocytes required for achieving at least one euploid blastocyst with user-defined 70% **(A)**, 80% **(B)**, and 90% **(C)** probabilities of success. The density ellipse contains 95% of the points.

frequency of couples that achieved the MIImin—as predicted by the ART calculator—and had at least one euploid blastocyst would be at least equal to the user-defined probability of success. Indeed, the positive predictive values were equal or slightly higher than the correspondent user-defined probabilities of success, thus confirming the clinical utility of the predictive tool, which is available online at https://members.groupposeidon.com/Calculator/.

Clinical Importance

In practical terms, the estimations provided by the ART calculator should be analyzed according to the probability of success, denoted by "π" (e.g., 70%, 80%, 90%), set by the user. Based on the ART calculator, an exemplary patient of 35 years-old embarking on IVF/ICSI, whose male partner is non-azoospermic, requires a total of five (95% CI: 5–6), seven (95% CI: 6–9), and ten (95% CI: 9–13) MII oocytes to obtain at least one euploid

blastocyst for 70, 80, and 90% probabilities of success. It means that among couples achieving those figures, the risk, denoted by $1 - \pi$, of having no euploid blastocyst despite achieving the predicted MIImin will be, respectively, 30, 20, and 10%. Since each euploid blastocyst has an implantation potential of ∼50–60% irrespective of the age group (15, 16, 32), then if all other factors are adequate, the cumulative pregnancy rates among patients who achieve the MIImin as per the calculator estimation should be 50–60% or higher.

The model is primarily intended to be a counseling tool for shaping patients' expectations and preparing them both emotionally and financially for the treatment journey. From a clinical and embryological perspective, the ART calculator outputs might also be used to help clinicians design individualized patient-oriented treatment strategies aiming at obtaining the number of MII oocytes needed for achieving at least one euploid blastocyst for transfer in IVF/ICSI procedures. The provision of such an objective estimation could help the clinician with regards to treatment planning. The individualized oocyte number may be achieved using patient-oriented strategies. For instance, the type of GnRH analog, type of gonadotropin, the starting dose, and the regimen could be tailored accordingly (58–63). A comprehensive review of the patient-oriented strategies encompassing individualized oocyte number can be found in a series of articles compiled in a dedicated Frontiers in Endocrinology research topic (https://www.frontiersin.org/research-topics/6849/poseidons-stratification-of-low-prognosis-patients-in-art-the-why-the-what-and-the-how).

Strengths and Limitations

To our knowledge, this is the first study to validate the ART calculator using an external patient cohort. The results are clinically significant for all stakeholders, including patients, healthcare providers, and policymakers. The primary use of the model is to serve as a counseling tool for infertile couples embarking on ART, who would like to gather information about their chances of success. However, the predictions can be used in conjunction with clinical knowledge for treatment planning as well as to develop patient awareness campaigns focusing on fertility preservation and the impact of female age on fertility. We studied the most important predictors for ART success using ∼1,500 couples subjected to IVF/ICSI and PGT-A in Italy, Turkey, and Brazil. Additionally, we used robust methods for developing the validation model and comparing its fittings with the ART calculator. Furthermore, we computed the ART calculator predictive value and confirmed its clinical utility.

Limitations of our study include the inherent bias of a retrospective analysis and the fact that we were not able to assess all potentially relevant predictors. Baseline levels of FSH, sperm DNA fragmentation index, sperm morphology, and male BMI were excluded due to the inconsistent reporting by participating centers. Infertility duration, ethnicity, dietary patterns, smoking habits, alcohol consumption, history of previous pregnancy, and past PGT-A results were not taken into account as these predictors were not available in the dataset. Although these predictors may have an impact on ART success, their role on blastocyst euploidy remains to be elucidated. By contrast, the most important predictors for blastocyst euploidy according to the existing evidence were assessed, including female age, male factor, and ovarian reserve markers. While other validation studies exist for predictive models concerning live birth after a single or multiple IVF/ICSI cycles (64–66), no validation study like ours exists to predict the minimum number of mature oocytes needed to achieve at least one euploid blastocyst for transfer.

We acknowledge the variability in embryonic outcomes among centers and the intrinsic characteristics of different platforms used for comprehensive chromosomal screening, which could play a role in the accuracy of the calculator. Hence, we recommend caution when applying the ART calculator in other settings, as the coefficients of the fitted model might vary between centers.

Future Research

Since our validation model was developed using retrospective data from ART centers, we will retest the model using a large prospective training cohort to provide even more accurate data in the future. Moreover, assessment of the ART calculator predictive value concerning (i) the oocyte genetic status by polar body analysis, and (ii) live birth rates are under consideration. We are currently sourcing suitable databases for conducting these studies.

CONCLUSIONS

This study has validated a novel calculator to predict the minimum number of metaphase II oocytes required to achieve at least one euploid blastocyst in the general population of infertile patients undergoing IVF/ICSI. The ART calculator may be used as a point-of-care clinical toll for counseling and treatment planning in IVF/ICSI treatments.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the articles/**Supplementary Material**.

ETHICS STATEMENT

The ethics committees of Instituto Investiga, Campinas, Brazil (CAAE 64291417.0.0000.5599), Hacettepe University, Ankara, Turkey (KA-180069), and Clinica Valle Giulia, Rome, Italy have approved the study.

AUTHOR CONTRIBUTIONS

SE designed and coordinated the study, wrote the article, affirms that the manuscript is an honest, accurate, and transparent account of the study reported and that no important aspects of the study have been omitted. JC designed the study, carried out the statistical analyses, and helped with data interpretation. FB, İÖ, MP, AV, DC, and LR coordinated data collection. HY, FU, and CA helped with coordination and data interpretation. All

authors contributed intellectually to the writing or revising the manuscript and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2019.00917/full#supplementary-material

Supplementary Table 1 | Patient and treatment variables in dataset.

Supplementary Table 2 | Distribution of missing values and analyzed predictors.

Supplementary Table 3 | Demographics and treatment characteristics of included couples by Anatolia Center (Turkey).

Supplementary Table 4 | Demographics and treatment characteristics of included couples by GENERA (Italy).

Supplementary Table 5 | Demographics and treatment characteristics of included couples by ANDROFERT Center (Brazil).

Supplementary Table 6 | Adaptive Lasso regression analysis for variable selection.

Supplementary Table 7 | ART calculator model for prediction of the probability (p) of euploid blastocyst per mature (MII) oocyte.

Supplementary Table 8 | Raw data.

Supplementary Figure 1 | Negative binomial distribution. Distribution of the number of euploid blastocysts. The summary statistics and quartiles show the descriptive data concerning the distribution of number of euploid blastocysts among 1,464 infertile couples undergoing IVF/ICSI and PGT-A. The distribution of euploid blastocysts per patient followed a negative binomial. This is evidenced by the univariate statistical analysis, where a gamma-Poisson distribution fits very well to the sample distribution. The fitted density function is shown in red in the histogram. The graph depicts the probability plot whose linearity evidences the appropriateness of the negative binomial.

Supplementary Figure 2 | Differences on the predicted probabilities by the ART calculator and validation model.

Supplementary Figure 3 | Comparison of fittings by ART calculator and validation model. The plots show the blastocyst euploidy predicted probabilities (per MII oocyte) by the ART calculator and validation model according to study center (Brazil = Androfert; Turkey = Anatolia; Italy = GENERA). The blue and red lines indicate the probability curves of the ART calculator and the validation model, respectively.

Supplementary Figure 4 | Distribution of female age on dataset.

Supplementary Figure 5 | Distribution of differences concerning the predicted probabilities (difP) according to the ART calculator and validation model by study center. The graphs depict the group of patients with 35 years and older, which correspond to over 90% or the validation dataset (see also **Supplementary Figure 4**).

Supplementary Video | The movie shows how the ART calculator can be used in an office-based setting. Pretreatment, clinicians should input the patient age and the sperm source to be used for IVF/ICSI. If the option "Testicle" is marked, then the type of azoospermia should be also defined. The probability of success is set by the user and indicates the chance of having ≥1 euploid blastocyst when the predicted number of mature oocytes is achieved. Its complement is the risk, that is, the chance of having no (zero) euploid blastocysts when the predicted number of oocytes is achieved. Once the button "calculate" is pressed, a text box will pop-up on the right side of the screen, indicating the predicted minimum number of mature oocytes needed for obtaining at least one euploid blastocyst, with its 95% confidence interval. Posttreatment, i.e., when fewer than the predicted number of mature oocytes are obtained after one or more oocyte retrieval cycles. Clinicians should input the pretreatment information and the actual number of mature oocytes collected or accumulated. The probability of success is set by the user; it reflects the chance that the estimation is correct given the number of oocytes input. Once the button "calculate" is pressed, a text box will pop-up on the right side of the screen, indicating the predicted probability of achieving ≥1 euploid blastocyst with the number of mature oocytes available.

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Management Strategies for POSEIDON Group 2

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Although individualization of ovarian stimulation aims at maximal efficacy and safety in assisted reproductive treatments, in its current form it is far from ideal in achieving the desired success in women with a low prognosis. This could be due a failure to identify such women who are likely to have a low prognosis with currently used prognostic characteristics. Introduction of the patient-oriented strategies encompassing individualized oocyte number (POSEIDON) concept reinforces recognizing such low prognosis groups and stratifying in accordance with important prognostic factors. The POSEIDON concept provides a practical approach to the management of these women and is a useful tool for both counseling and clinical management. In this commentary, we focus on likely management strategies for POSEIDON group 2 criteria.

Keywords: POSEIDON classification, POSEIDON group 2, poor responder, suboptimal responder, IVF (ICSI)

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INTRODUCTION

Success following assisted reproductive treatments (ART) has improved significantly since the early years of *in vitro* fertilization (IVF) treatment. The notable contribution to this success is the introduction of ovarian stimulation into ART in the early 1980s (1, 2). Soon after the introduction of ovarian stimulation, it became apparent that women varied in their response to stimulation. To this end a low responder was first described as being associated with low serum oestradiol levels and requiring higher gonadotrophin stimulation doses (3). Since then, there had been varied descriptions and terminologies such as poor ovarian response (POR), low response, inadequate response, suboptimal response with numerous definitions, several criteria and different thresholds.

A review in 2000 enlisted around 28 criteria used for the definition of POR (4) and a latter review nearly 10 years later reinforced the issue of lacking uniform criteria for defining POR with 41 definitions being used in 47 RCTs that had since been published on the topic (5). Discrepancies in the definition lead to clinical heterogeneity among studies on POR leading to inconsistent and inconclusive findings (6). This lead to researchers and clinicians calling for a unified definition of POR leading to the publication of the ESHRE consensus, Bologna criteria definition of POR (7).

However, there has been skepticism whether the ESHRE consensus, the Bologna criteria for defining POR is fit for purpose and whether the new consensus definition mitigated clinical heterogeneity. The ESHRE consensus definition of POR considers proven poor responders based on previous cycle performances and predicted poor responders as one category, does not consider suboptimal response, does not factor in female age and the oocyte competence in terms of embryos aneuploidy rate (8). Additionally, it comprises of several subpopulations with varied baseline characteristics (9). Furthermore, the Bologna criteria encompasses a very poor prognosis group that is associated with very low live birth rates (10, 11) raising the interrogation if any interventions could enhance clinical outcomes for these women with very poor prognosis (12, 13).

CONCEPT OF INDIVIDUALIZED OVARIAN STIMULATION

The main objective of individualization of ovarian stimulation (OS) is to offer women the best treatment tailored to her own unique characteristics, thus maximizing the chances of pregnancy and eliminating the iatrogenic and avoidable risks resulting from ovarian stimulation (14). It currently entails categorizing women based on their predicted response in order to individualize OS regimens. Women can be identified as having an expected poor response, normal response or a high response based on ovarian reserve tests (ORTs). Among the various ORTs including basal FSH, basal oestradiol, inhibin B, antral follicle count (AFC), and anti-mullerian hormone (AMH), AFC, and AMH have the highest accuracy for the prediction of either a poor or a high response following ovarian stimulation (15–17). However, whether the categorization into the three broad categories of poor, normal and high response is sufficient to categorize all women in an ART programme has been questioned with evidence-based suggestions to refine the categorization by recognizing the suboptimal responder (18). Suboptimal response is the group between poor response with ≤3 oocytes and normal response with 10–15 oocytes. These women with 4–9 oocytes have a better prognosis over poor responders but have a lower prognosis compared to normal responders. Given that poor responders have a very low prognosis with most interventions being futile, it would be justifiable to focus research and interventions toward other low prognosis groups such as the suboptimal responder. This further lead to the notion of "patient oriented strategies encompassing individualized oocyte number"—POSEIDON concept. The recent publication in Frontiers in Endocrinilogy by Conforti and colleagues hughlights the need for intervention studies to test the POSEIDON concept, particularly for groups 1 and 2 where benefit is more likely in the context of a good ovarian reserve (19). This paper discusses interventions in the context of POSEIDON group 2 women that would merit further research.

POSEIDON CONCEPT: THE WHY, THE WHAT AND THE HOW

A systematic review and meta-analysis on predictive factors in IVF evaluated nine common predictors and found the following factors of female age, duration of infertility, basal follicle stimulating hormone (FSH) levels, the number of retrieved oocytes, and embryo quality to be associated with the chances of pregnancy (20). Older female age, longer duration of infertility, higher basal FSH levels were negative predictors whereas higher number of oocytes and good embryo quality were positive predictors. There has been consistent evidence of a strong association between number of oocytes retrieved and live birth reinforcing that the number of oocytes is an important prognostic variable for IVF success (21–24). It is therefore paramount that the OS regimens optimize number of oocytes retrieved to maximize success.

Younger women have a favorable prognosis in achieving a live birth compared to older women. An important reason for this is the increase in aneuploid embryos and consequent decrease in euploid embryos with increasing female age. Whereas, female age influences the embryo euploidy rate, euploidy rate remains stable in relation to the embryo cohort sizes, thereby resulting in more euploid embryos with higher number of embryos (25). It therefore becomes apparent that female age, ovarian reserve, ovarian response to stimulation and number of oocytes retrieved are overriding factors determining the success of ART. Ovarian stimulation regimen should therefore aim to enhance ovarian response to stimulation, particularly for the low prognosis group of patients extending to the suboptimal responder.

The POSEIDON stratification is aimed at clinical management by considering the most important prognostic factors and stratifying women accordingly. Group 1: women aged <35 years with adequate ovarian reserve (AFC ≥ 5, AMH ≥ 1.2 ng/ ml) with an unexpected poor response (<4 oocytes) or a suboptimal response (4–9 oocytes); Group 2: women aged ≥ 35 years with adequate ovarian reserve (AFC ≥ 5, AMH ≥ 1.2 ng/ ml) with an unexpected poor response (<4 oocytes) or a suboptimal response (4–9 oocytes); Group 3: women aged < 35 years with poor ovarian reserve (AFC < 5, AMH < 1.2 ng/ ml); Group 4: women aged ≥ 35 years with poor ovarian reserve (AFC < 5, AMH < 1.2 ng/ ml) (26).

MANGAMENT OF POSEIDON GROUP 2 WOMEN

The aim of defining the POSEIDON groups is to individualize therapeutic approaches by fine tuning OS in terms of the right pituitary suppression regimen, the ideal gonadotrophin selection, along with dosage and optimize ovarian response and number of oocytes to obtain a euploid embryo with the highest implantation potential for transfer. POSEIDON classification reinforces the avoidance of iatrogenic suboptimal response underpinning likely genetic variants such as FSH receptor polymorphism (27), variant luteinising hormone–β (V LH–β) (28) that might benefit from gonadotrophins with different pharmacokinetic profiles and yielding a higher number and competent oocytes for a given dosage (29, 30). The broad stratification based on a female age cutoff, taking cognizance of the declining prognosis in women beyond age of 35 years, is likely to be helpful in clinical decision making for the vast majority of women undergoing IVF below age of 40 years (31).

The long gonadotrophin releasing hormone (GnRH) agonist regimen is associated with a significantly higher oocyte yield over the short GnRH agonist regimen, and as such should be the preferred downregulation regimen with the use of GnRH agonists (32). Given the concurring evidence of comparable efficacy with the use of the long GnRH agonist and GnRH antagonist regimens for both general population and poor responder women undergoing IVF (33), either regimens could be recommended for POSEIDON group 2 women by extrapolating current evidence. *As the aim is to improve egg numbers, these women may benefit from a higher gonadotrophin dose over the*

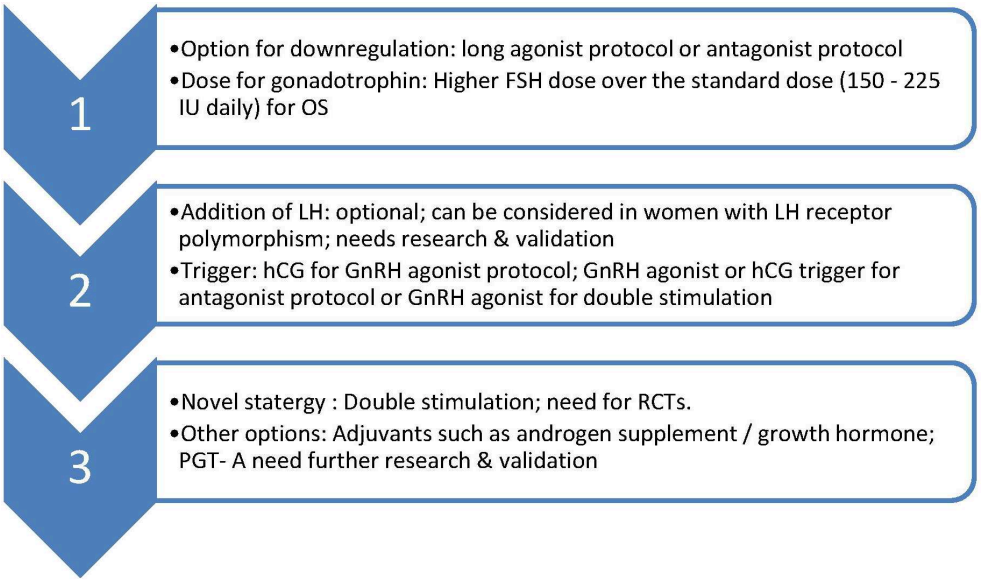

FIGURE 1 | Suggested management of POSEIDON group 2. OS, ovarian stimulation; LH, luteinising hormone; hCG, human chorionic gonadotrophin; GnRH, Gonadotrophin releasing hormone; PGT-A, pre implantation genetic testing-aneuploidy; RCT, randomized controlled trial.

standard 150 IU – 225 IU daily for OS (34). There is the discussion that women in this group have a specific genotype profile accounting for the variability in ovarian response to stimulation that is unexpected based on routine ovarian reserve testing. *There has been evidence on the relevance of genetic variants of gonadotropins and their receptors in ovarian stimulation and benefits of increasing FSH dose or adding recombinant LH for women with a hypo response to recombinant FSH* (35) *depending upon presence of FSH or LH receptor polymorphisms, respectively* (36). *Whether FSH or LH receptor polymorphism screening should be offered to all women with adequate ovarian reserve prior to their first IVF treatment depends on prevalence of such polymorphism in this select IVF population and its impact. Further, whether these women are likely to benefit from gonadotrophins with different pharmacokinetic profile such as additional LH activity to FSH needs evaluation with further research into this area.*

Dual stimulation is a novel strategy in which double stimulation ("DuoStim") is attempted in the same menstrual cycle (36). Earlier, it was proposed that only one wave of follicular recruitment takes place in an ovarian cycle. It has hence been shown that two and three cohorts of antral follicles are recruited during a menstrual cycle (37–39). Double stimulation has been proposed as one of the treatment statergy for management of POSEIDON group 2 which consists of women ≥35 years with an unexpected POR or suboptimal response. Since aneuploidy rates are higher in this group compared to women <35 years, higher oocyte yield is needed to achieve a single euploid embryo. Double stimulation strategies can help in maximizing oocyte yield in a single ovarian cycle. An earlier study has compared the oocyte yield and euploid blastocyst rates following FPS and LPS (40). The study reported no significant difference in retrieved cumulus oocyte complex (5.1 ± 3.4 vs. 5.7 ± 3.3) or euploid blastocyst rates (46.9 vs. 44.8%). A recent case control included 188 women with poor prognosis who under double stimulation (41). The authors reported fewer oocytes collection (3.6 ± 2.1 vs. 4.3 ± 2.8; $P < 0.01$) and euploid blastocysts (0.5 ± 0.8 vs. 0.7 ± 1.0; $P = 0.02$) after FPS compared to LPS. A systematic review which included eight studies and 338 women, reported no compromised in quality or quantity of oocytes retrieved following LPS compared to FPS (42). A "freeze all strategy" is mandatory for double stimulation. It is suggested that double stimulation may reduce the cycle drop out rates in these women with poor or suboptimal response and shorten time to pregnancy (42). Currently, there is very limited data is available on obstetrical and neonatal outcomes following double stimulation.

Double stimulation protocol needs validation in POSEIDON group 2 population along with cost-effectiveness and safety data. *Overall, such group of women in POSEIDON group 2 might benefit recognition and whether they could benefit from novel strategies or inteventions such as adjuvant androgen therapies, addition of growth hormone, preimplantation genetic testing for aneuploidy (PGT-A) warrants further research.* Management options for POSEIDON group 2 women is summarized in **Figure 1**.

AUTHOR CONTRIBUTIONS

SS drafted the manuscript with contribution from GR and MK. All authors approved final version.

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Low Prognosis by the POSEIDON Criteria in Women Undergoing Assisted Reproductive Technology: A Multicenter and Multinational Prevalence Study of Over 13,000 Patients

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Objective: To estimate the prevalence of low-prognosis patients according to the POSEIDON criteria using real-world data.

Design: Multicenter population-based cohort study.

Settings: Fertility clinics in Brazil, Turkey, and Vietnam.

Patients: Infertile women undergoing assisted reproductive technology using standard ovarian stimulation with exogenous gonadotropins.

Interventions: None.

Main outcome measures: Per-period prevalence rates of POSEIDON patients (overall, stratified by POSEIDON groups and by study center) and the effect of covariates on the probability that a patient be classified as "POSEIDON".

Results: A total of 13,146 patients were included. POSEIDON patients represented 43.0% (95% confidence interval [CI] 42.0–43.7) of the studied population, and the prevalence rates varied across study centers (range: 38.6–55.7%). The overall prevalence rates by POSEIDON groups were 44.2% (group 1; 95% CI 42.6–45.9), 36.1% (group 2; 95% CI 34.6–37.7), 5.2% (group 3; 95% CI 4.5–6.0), and 14.4% (group 4; 95% CI: 13.3–15.6). In general, POSEIDON patients were older, had a higher body mass index (BMI), lower ovarian reserve markers, and a higher frequency of female factor as the primary treatment indication than non-POSEIDON patients. The former required larger doses of gonadotropin for ovarian stimulation, despite achieving a 2.5 times lower number of retrieved oocytes than non-POSEIDON patients. Logistic

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regression analyses revealed that female age, BMI, ovarian reserve, and a female infertility factor were relevant predictors of the POSEIDON condition.

Conclusions: The estimated prevalence of POSEIDON patients in the general population undergoing ART is significant. These patients differ in clinical characteristics compared with non-POSEIDON patients. The POSEIDON condition is associated with female age, ovarian reserve, BMI, and female infertility. Efforts in terms of diagnosis, counseling, and treatment are needed to reduce the prevalence of low-prognosis patients.

Keywords: assisted reproductive technology, POSEIDON criteria, real-world evidence, infertility, prevalence study

INTRODUCTION

The POSEIDON (**P**atient-**O**riented **S**trategies **E**ncompassing **I**ndividualize**D** **O**ocyte **N**umber) criteria were developed to help clinicians identify and classify 'low-prognosis' patients undergoing assisted reproductive technology (ART) (1, 2). The novel classification aims to capture subtle differences related to a poor treatment outcome, thereby creating homogenous patient groups, ultimately helping clinicians tailor ovarian stimulation strategies for these challenging patients (3).

Since its introduction, the number of clinical studies using the POSEIDON criteria has steadily increased (4–9). However, as yet, there are no global estimates of the real-world prevalence of low-prognosis patients defined according to the POSEIDON criteria.

Prevalence studies assess the burden of a disease or condition in a population and guide clinical practice, research, and resource allocation (10, 11). The accurate interpretation of prevalence studies requires an understanding of the input data on which estimates were based, including quality information, and an explanation of the methods used to derive the health estimates (12).

We investigated the prevalence of POSEIDON low-prognosis patients using big data analytics. Our primary objectives were (i) to determine the prevalence rate of POSEIDON patients in a general infertile population undergoing *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) and (ii) to identify clinical differences between POSEIDON and non-POSEIDON patients.

MATERIALS AND METHODS

Study Design

This prevalence study is based on retrospective data collected from consecutive infertile patients undergoing IVF-ICSI from October 2015 to November 2017 in three fertility centers (Androfert, Campinas, Brazil, Anatolia IVF and Women's Health Center, Ankara, Turkey, My Duc Hospital, Ho Chi Minh City, Vietnam). The following ethics committees approved the study: Instituto Investiga, Campinas, Brazil (CAAE 26429219.0.0000.5599), Hacettepe University, Ankara, Turkey (KA-180070), and My Duc Hospital, Ho Chi Minh City, Vietnam (05/18/DD-BVMD). The study complies with the guidelines for accurate and transparent health estimates reporting (GATHER) and standards for the reporting of observational studies (STROBE) (12, 13).

Study Population

Eligible patients were consecutive infertile women between 22 and 46 years undergoing their first IVF/ICSI cycle in each center with standard ovarian stimulation using exogenous gonadotropins. We included all patients who started treatment regardless of whether their cycle was canceled before oocyte collection. Only one cycle per patient was examined. We excluded patients undergoing IVF/ICSI for purposes other than infertility. We also excluded patients treated with mild or minimal stimulation protocols (i.e., <150 IU daily doses of exogenous gonadotropin, used alone or combined with oral compounds such as anti-estrogens or aromatase inhibitors) and those who underwent natural IVF (*i.e.*, no stimulation) (14). Notably, standard ovarian stimulation is a pre-requisite for classifying a patient according to the POSEIDON criteria (see the *Patient Classification* below).

Assessment of Ovarian Reserve

The ovarian reserve was determined before and no longer than three months before treatment initiation by measuring either antral follicle count (AFC) or anti-Müllerian hormone (AMH) serum levels, or both, using standardized protocols (15–17). Briefly, the AFC level was measured in the early follicular phase using a two-dimension ultrasound scan (16), whereas AMH serum values were obtained using the modified Beckman Coulter Generation II assay (17). At the ANDROFERT clinic and the My Duc Hospital, both AFC and AMH were routinely used to assess ovarian reserve during the study, whereas at the Anatolia IVF Center, AFC was the preferential method. AFC was determined in-house by the practicing physicians of each study center, whereas AMH values were extracted from reports provided by the reference laboratories partnered with each institution. Thus, AFC and AMH values, critical for the POSEIDON classification (1, 2), were determined by different operators and machines. In the attempt to mitigate this potential source of bias, in the study design phase, we selected study centers that shared similar standard operating procedures for AFC determination and using the same assay for AMH measurements.

Treatment Protocols

The choice of the ovarian stimulation regimen and gonadotropin dosage was based on each center's policies according to the ovarian reserve, female age, and history of previous ovarian stimulation (18–22). Patients underwent pituitary suppression with either a long GnRH agonist protocol (Lucrin; Abbott) or a

GnRH antagonist protocol (Cetrotide [Merck] or Orgalutran [MSD]). Daily subcutaneous injections of recombinant FSH monotherapy (Gonal-F [Merck] or Puregon [MSD]), recombinant FSH combined with recombinant LH (2:1 ratio, Pergoveris [Merck]), or recombinant FSH (Gonal-F, Merck) combined with either hMG (Menopur, Ferring] or recombinant LH (Luveris, Merck) or highly purified hMG (Menopur; Ferring) were used for ovarian stimulation. The initial daily gonadotropin doses varied between 150 IU and 450 IU.

Ovarian response was monitored primarily using serial transvaginal ultrasonography, and gonadotropin doses were adjusted as needed. Cycles were canceled when no follicles developed during ovarian stimulation. Final oocyte maturation was triggered by subcutaneous administration of either recombinant hCG (250 mcg; Ovitrelle, Merck) or 0.2 mg GnRH agonist (0.2 mg triptorelin [Decapeptyl; Ferring]) according to the policies of each center. Oocytes were retrieved under intravenous anesthesia using transvaginal ultrasound-guided puncture of follicles 35–37 h after triggering final oocyte maturation.

The follicular fluid collected was analyzed in the IVF laboratory, and the total number of retrieved oocytes was recorded. The metaphase II oocytes were inseminated *via* conventional IVF or ICSI, and embryos were cultured up to the cleavage or blastocyst stage. The resulting embryos were either transferred fresh or vitrified according to each center's policies. In this study, only data up to the number of collected oocytes were considered because this information—in addition to female age and ovarian marker results—is required to classify the patient according to the POSEIDON criteria.

Data Input

The participating centers used a case report form created for data collection. Data were extracted using each clinic's data management system: (Clinisys®, Brazil: Androfert; PostgreSQL version 10, USA: My Duc Hospital; a custom-made SPSS-derived database system: Anatolia). Codes replaced the records linking patients' identification.

The following demographic data were collected: female age, body mass index (BMI), infertility duration, infertility factor, and ovarian reserve markers AFC and/or AMH levels. Treatment data comprised the type of GnRH analog, gonadotropin regimen, total gonadotropin dose, duration of stimulation, and trigger type.

Anonymized individual-level data from the study's centers were sent to a third-party statistical service (Statistika Co., Campinas, Brazil) for compilation and analysis. Data validation was performed for implausible values due to data entry errors or missing values, and incongruencies were resolved with the principal investigators (SCE, HY and LNV). Individual-level measurements with non-resolved implausible values and/or critical missing values that would preclude the classification of a patient according to the POSEIDON criteria, namely, female age, ovarian biomarker result, and the number of oocytes retrieved (*Patient Classification*) were excluded.

After initial data cleaning, we observed that the number of participants with missing data for AMH was high (**Supplemental Table 1**). While AFC values were reported in virtually all cases by the Brazilian (n = 1,065; 100%), Turkish (n = 3,633; 100%), and Vietnamese (n = 8,448; 92.3%) centers, AMH values were markedly underreported in the Turkish center (n = 425; 11.6%). Therefore, given the aim of the present study, only AFC values were used to classify patients, as detailed in the *Patient Classification* section. We did not generate an indicator based on AFC and AMH when both were available to avoid the risk of having patients without a classification due to a discrepancy between the AFC and AMH results. An agreement analysis between AFC and AMH as a method with which to classify POSEIDON patients does not fall within the scope of the present study; the related data will be reported in a study specifically designed for the matter concerned. Treatment outcomes beyond the number of oocytes retrieved among POSEIDON *versus* non-POSEIDON patients are also beyond this study's aim and will be reported subsequently. No further data adjustments were made.

Patient Classification

We classified the patients into five groups based on the POSEIDON criteria (1, 2). Besides the four well-defined POSEIDON groups, patients who did not fit any POSEIDON group were classified into a fifth group designated the "non-POSEIDON" (group 5). The latter group constituted our control group of so-called 'normal prognosis' patients.

i. POSEIDON Group 1 (Group 1): Age <35 years, an adequate pre-stimulation ovarian reserve biomarker (AFC ≥5), and a previous conventional ovarian stimulation with <10 oocytes retrieved. This group is further divided into subgroup 1a, consisting of patients with fewer than four oocytes and subgroup 1b, consisting of patients with four to nine oocytes retrieved.
ii. POSEIDON Group 2 (Group 2): Age ≥35 years, an adequate pre-stimulation ovarian reserve biomarker (AFC ≥5), and a previous conventional ovarian stimulation with <10 oocytes retrieved. This group was further divided into subgroup 2a, consisting of patients with fewer than four oocytes and subgroup 2b, consisting of patients with four to nine oocytes retrieved.
iii. POSEIDON Group 3 (Group 3): Age <35 years and a poor pre-stimulation ovarian reserve biomarker (AFC <5).
iv. POSEIDON Group 4 (Group 4): Age ≥35 years and a poor pre-stimulation ovarian reserve (AFC <5).
v. Non-POSEIDON (Group 5): Patients with an adequate pre-stimulation ovarian reserve biomarker (AFC ≥5) and >9 oocytes retrieved.

Main Outcome Measures

The primary outcome measure was the prevalence rate of POSEIDON patients (total and stratified by POSEIDON group and by study center) in the dataset (per period prevalence rate). The secondary outcomes were (i) the prevalence ratio of

POSEIDON patients among groups and according to study centers, and (ii) the influence of covariates on the probability that a patient be classified as "POSEIDON". We defined the prevalence rate as the proportion of patients fitting the POSEIDON criteria within the study period. The prevalence ratio was the ratio between the proportion of POSEIDON patients by group and study center.

Statistical Analysis

The prevalence rates and the simultaneous 95% confidence interval (CI) were computed by the Bonferroni-adjusted method of Goodman (22, 23). The prevalence ratios and associated 95% CI were calculated according to Altman's method (24). A formal sample size calculation for the estimation of POSEIDON prevalence rates was not carried out *a priori*. However, we included all consecutive patients who met the inclusion criteria and were treated in the study centers over a two-year period. Moreover, we computed CI to determine the statistical precision that was ultimately obtained. The population included in the current study represented 83.6% (13,146/15,728) of all patients treated in these institutions during the same period.

To investigate the relationship between covariates and the condition "POSEIDON", we performed nominal logistic regression analyses, including the patients' clinical characteristics (age, infertility duration, AFC, BMI, and infertility factor), and study center as independent variables, and "POSEIDON" [yes = patients fitting the POSEIDON criteria; no = Non-POSEIDON patients (group 5)] as the dependent variable. We explored the above relationships using the POSEIDON patients both as a single category and by subgroup.

For subgroup logistic regression analyses, we combined patients of groups 3 and 4 (poor ovarian reserve) and groups 1 and 2 (sufficient pre-stimulation ovarian reserve and an unexpected poor or suboptimal oocyte yield) into two separate categories. The binary response variables were "POSEIDON groups 3 or 4 = yes; Non-POSEIDON [group 5] = no" and "POSEIDON groups 1 or 2 = yes; Non-POSEIDON [group 5] = no". We excluded AFC as an independent variable in the model that examined the association between predictors and the condition "POSEIDON groups 3 or 4 = yes" because all patients classified within these groups had low AFC values. We included treatment characteristics (total gonadotropin dose, GnRH analog, type of gonadotropin, duration of ovarian stimulation) as independent variables in the model using "POSEIDON groups 1 or 2 = yes", because the number of oocytes retrieved—which is a critical variable to classify patients into groups 1 and 2—is affected by treatment. The covariate 'trigger type' was excluded from the latter model as the number of POSEIDON patients triggered with a GnRH agonist in the dataset was minimal (217/4,531 = 4.8%; **Supplemental Table 2**).

Categorical data are described by the number of cases, including numerator and denominator, and percentages. Continuous data are reported as median and interquartile range as none of the continuous variables followed a normal distribution. Categorical data were analyzed by Pearson chi-square, whereas continuous data were analyzed by non-parametric tests. The Wilcoxon rank test was used to compare continuous data between POSEIDON and non-POSEIDON patients, whereas the Kruskal-Wallis test was applied for the comparisons among study centers. Statistical significance was set at a p-value <0.05. Computations were carried out using JMP® PRO 13 and SAS 9.3 (SAS Institute, Cary, North Carolina, USA).

RESULTS

Participants

Of 13,853 eligible patients, 707 (5.1%) were excluded because AFC values were not reported. Hence, a total of 13,146 patients were included, all of whom had a complete IVF/ICSI record of the relevant covariates for the POSEIDON classification using AFC (**Figure 1**).

Table 1 shows the study population's characteristics. A total of 5,639 patients were classified as "POSEIDON", and 7,507 patients were classified as "non-POSEIDON". Cycle cancellation before oocyte pick-up was reported in 30 patients (0.23%), all of whom were "POSEIDON" (group 3: n = 14; group 4: n = 16).

Patient and treatment characteristics differed between POSEIDON and non-POSEIDON patients. Overall, POSEIDON patients were older, had a higher BMI, lower AFC, and a higher frequency of female factor as the primary indication for ART than non-POSEIDON patients. Moreover, POSEIDON patients had fewer oocytes retrieved than non-POSEIDON patients, despite using a higher total gonadotropin dose for ovarian stimulation. The GnRH antagonist protocol, an association between rec-FSH and HMG, and hCG trigger was the most commonly used ovarian stimulation regimen in POSEIDON and non-POSEIDON patients. Rec-FSH monotherapy and GnRH agonist trigger were more frequently used in non-POSEIDON patients than in POSEIDON patients (**Table 1**).

Main Outcome Measures

The prevalence rates of POSEIDON patients, both overall and according to POSEIDON groups and study centers, are reported in **Table 2**. POSEIDON patients represented approximately 43% of the overall studied population. Among POSEIDON patients, groups 1 and 2 (*i.e.*, younger and older patients, respectively, with sufficient pre-stimulation AFC values and unexpected low or suboptimal oocyte yield) had the highest prevalence rates (44.2 and 36.1%, respectively), followed by groups 4 and 3 (14.4 and 5.2%, *i.e.*, older and younger patients, respectively, with low AFC). Notably, most patients of groups 1 and 2 (>80%) had a suboptimal (4–9) oocyte yield (**Supplemental Table 2**).

The prevalence ratios of POSEIDON groups at each center and between centers are described in **Table 2**. The risk ratio of reporting a POSEIDON patient in study center 1 (ANDROFERT, Brazil) was 1.1 and 1.4 times higher than that of study center 2 (Anatolia IVF, Turkey) and study center 3 (My Duc Hospital, Vietnam), respectively, and it was about 1.3 times higher in study center 2 than in study center 3.

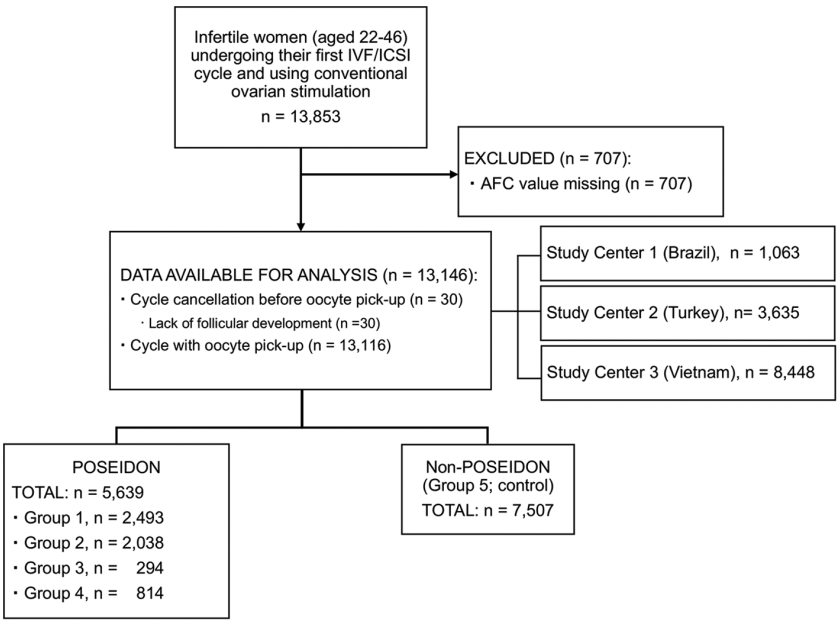

FIGURE 1 | Flow diagram showing total patient breakdown.

POSEIDON patients' characteristics stratified by groups are shown in **Supplemental Tables 3** and **4**. Center 1 POSEIDON patients were the oldest, had the highest frequency of having non-male factor as the primary indication for ART, the poorest ovarian reserve, the longest duration of stimulation, and the fewest number of oocytes retrieved. Study center 2 POSEIDON patients were the youngest, had the highest BMI and frequency of unexplained infertility, exhibited higher AFC, required the lowest total gonadotropin dose for ovarian stimulation, and had the highest number of oocytes retrieved. Lastly, study center 3 POSEIDON patients had the lowest BMI and the highest frequency of female factor as the primary indication for ART (**Supplemental Table 4**).

Patient and treatment characteristics differed among POSEIDON groups. Infertility lasted longer in groups 2 and 4 *versus* groups 1 and 3. Most patients had a female infertility factor as the primary indication for ART; however, this proportion was lower in groups 1 and 2 than groups 3 and 4. In group 1, the frequency of patients with unexplained infertility was higher than in the other groups. As expected, patients of groups 1 and 2 had a higher ovarian reserve and more oocytes retrieved than patients of groups 3 and 4. Overall, the GnRH antagonist protocol, recombinant FSH (alone or combined with HMG), and hCG trigger was the most frequently used ovarian stimulation regimen in POSEIDON patients, albeit practices varied across study centers (**Supplemental Table 3**).

Logistic Regression Analyses

To assess and quantify the relative importance of each independent variable for the "POSEIDON" condition, we entered our data into the logistic regression software and obtained the values reported in **Table 3** and **Supplemental Tables 5** and **6**.

Table 3 shows the logistic regression values for demographic and clinical parameters using "POSEIDON" as a binary response variable in the whole population. A significant regression equation was found (ChiSquare = 4,225.79, df = 7; $p < 0.0001$), with an R^2 of 0.27. Female age, BMI, AFC, and presence of a female infertility factor were significant predictors of the POSEIDON condition. Overall, the probability that a patient was classified "POSEIDON" (versus non-POSEIDON) increased as a function of increased age, increased BMI, decreased AFC values and presence of a female infertility factor. A center effect was relevant, which indicates that the above probability varied across study centers.

A significant regression equation was also found when poor ovarian reserve POSEIDON patients (*i.e.*, groups 3 and 4) were combined (ChiSquare = 1,816.97, df = 6; $p < 0.0001$), with an R^2 of 0.45 (**Supplemental Table 5**). Female age, BMI, female infertility factor, and infertility duration were significant predictors of the POSEIDON condition among the expected poor ovarian responders. Accordingly, the probability of classifying a patient as POSEIDON group 3 or 4 (*versus* non-POSEIDON patients) increased with age, infertility duration, and presence of female factor infertility. A center effect was not evident in this model.

The logistic regression values associated with the binary response "POSEIDON groups 1 or 2" (*i.e.*, adequate ovarian reserve but low or suboptimal oocyte number) are shown in **Supplemental Table 6**. A significant regression equation was found (ChiSquare = 3,156.77, df = 13; $p < 0.0001$), with an R^2 of 0.23. This model retained the relevant predictors shown in the

TABLE 1 | Demographic, clinical, and treatment characteristics of the total studied population, stratified as POSEIDON and Non-POSEIDON patients.

	POSEIDON n = 5,639	Non-POSEIDON n = 7,507	P-value
Age (years)	34 [31-38]	31 [28-35]	<0.001[a]
BMI (kg/m^2)	22 [20.0-24.5]	21.3 [19.8-23.7]	<0.001[a]
Infertility duration (months)	48 [24-84]	48 [24-72]	<0.001[a]
Primary indication of ART:			<0.001[b]
Male	1,734/5,639 (30.7)	2,759 /7,507 (36.8)	
Endometriosis	763/5,639 (13.6)	279/7,507 (3.7)	
Ovulatory	731/5,639 (13.0)	1,105/7,507 (14.7)	
Tubal	627/5,639 (11.1)	1,041/7,507 (13.9)	
Unexplained	1,784/5,639 (31.6)	2,323/7,507 (30.9)	
Ovarian reserve:			
AFC (n)	8 [5-12]	17 [13-23]	<0.001[a]
AMH (ng/mL)	1.5 [0.87-3.0]	4.9 [3.0-7.8]	<0.001 [a]
Duration of stimulation (days)	9 [8-10]	9 [8-10]	0.48
GnRH analogue:			<0.001[b]
Antagonist	4,545/5,639 (80.6)	6,629/7,507 (88.3)	
Agonist	1,094/5,639 (19.4)	878/7,507 (11.7)	
Total gonadotropin dose (IU)	2,700 (1,100-5,100)	2,300 [1,050-4,465]	<0.001[a]
Gonadotropin:			<0.001[b]
HMG	232/5,639 (4.1)	90 /7,507 (1.2)	
Rec-FSH	1,579/5,639 (28.0)	3,431/7,507 (45.7)	
Rec-FSH+HMG	3,263/5,639 (57.9)	3,599/7,507 (47.9)	
Rec-FSH+recLH	565/5,639 (10.0)	387/7,507 (5.2)	
Trigger:			<0.001[b]
hCG	5,264/5,639 (93.3)	5,667/7,507 (75.5)	
GnRH agonist	375 /5,639 (6.7)	1,840/7,507 (24.5)	
Number of oocytes retrieved	6 [4-8]	15 [12-19]	<0.001[a]

[a]*Wilcoxon test; values are median and 25%-75% interquartile range.*

[b]*Pearson $\chi 2$ test. Values are number (percentage).*

BMI, body mass index; AFC, antral follicle count; AMH, anti-Müllerian hormone; ART, assisted reproductive technology; GnRH, gonadotropin-releasing hormone; IU, international units; HMG, human menopausal gonadotropin; rec-FSH, recombinant follicle-stimulating hormone; rec-LH, recombinant luteinizing hormone; hCG, human chorionic gonadotropin.

total population model (**Table 3**) and included the type of GnRH analogue, duration of stimulation, and type of gonadotropin as significant predictors of the POSEIDON condition. Accordingly, older age and higher BMI, lower AFC values, and female factor infertility increased the probability of classifying a patient as groups 1 or 2 POSEIDON. Moreover, shorter stimulation duration, use of the GnRH antagonist protocol, and HMG or rec-FSH+HMG (*versus* rec-FSH alone) for ovarian stimulation was associated with an increased probability of a patient being classified as POSEIDON groups 1 or 2 (*versus* non-POSEIDON counterparts). A center effect was relevant in this model, indicating that the above probability varied across study centers.

DISCUSSION

Main Findings

We report POSEIDON patients' per period prevalence rates using real-world data of three fertility centers in Brazil, Turkey, and Vietnam. Approximately 40% of patients undergoing IVF/ICSI with standard ovarian stimulation were classified as "low-prognosis" according to the POSEIDON criteria. Among them, patients with sufficient pre-stimulation AFC values and an unexpected low or suboptimal oocyte number (groups 1 and 2) constituted about 80% of the POSEIDON individuals while patients with a poor ovarian reserve (groups 3 and 4) comprised the remaining 20%. Despite varying across study centers, the prevalence rates were consistent, thus confirming the perception that the low-prognosis patient accounts for a relevant proportion of individuals undergoing ART. In general, we found that the older the patient population and the lower the ovarian reserve, the higher the proportion of POSEIDON patients.

Clinical and treatment characteristics differed between POSEIDON and non-POSEIDON patients. In general, the former patients were older, slightly heavier, and had a lower ovarian reserve. Moreover, they required larger doses of gonadotropin for ovarian stimulation, which, however, were unable to compensate for the low or suboptimal oocyte yield ultimately obtained. On average, the number of oocytes retrieved was 2.5 times lower in POSEIDON patients than in non-POSEIDON patients. In the former patients, these numbers were twice as high in patients of groups 1 and 2 than in groups 3 and 4. Lastly, a known female infertility factor (*e.g.*, endometriosis) or unexplained infertility was more frequent in POSEIDON patients than in non-POSEIDON counterparts. In general, POSEIDON patients were treated with the GnRH antagonist protocol and a stimulation regimen consisting of recombinant FSH combined with LH-activity provided by hMG. The most common trigger method was the hCG trigger, and most embryo transfers were fresh transfers.

Interpretation

The main aim of this study was to describe the magnitude and distribution of the so-called "low-prognosis" patient in the routine IVF practice. We determined the number of individuals who had "low-prognosis" as defined by the

TABLE 2 | Prevalence rates of POSEIDON patients in total population, overall and according to POSEIDON groups and study's center.

	POSEIDON N (%)	Prevalence Rate	95% Confidence Interval
Overall Population:			
Total	**5,639/13,146**	**42.9**	**42.0-43.7**
Group 1:	2,493/5,639	44.2	42.6-45.9
Group 2:	2,038/5,639	36.1	34.6-37.7
Group 3	294/5,639	5.2	4.5-6.0
Group 4	814/5,639	14.4	13.3-15.6
Study Center 1:			
Total	**592/1,063**	**55.7**	**52.7-58.6**
Group 1:	126/592	21.3	17.4-25.8
Group 2:	292/592	49.3	44.2-54.4
Group 3	40/592	6.7	4.6-9.8
Group 4	134/592	22.6	18.6-27.2
Study Center 2:			
Total	**1,783/3,635**	**49.1**	**47.4-50.7**
Group 1:	1,051/1,783	58.9	56.0-61.8
Group 2:	532/1,783	29.8	27.2-32.6
Group 3:	51/1,783	2.9	2.0-4.0
Group 4:	149/1,783	8.3	6.9-10.1
Study Center 3:			
Total:	**3,264/8,448**	**38.6**	**37.6-39.7**
Group 1:	1,316/3,264	40.3	38.2-42.5
Group 2:	1,214/3,264	37.2	35.1-39.3
Group 3:	203/3,264	6.2	5.2-7.4
Group 4:	531/3,264	16.3	14.7-17.9
Prevalence ratio of POSEIDON (total) [95% Confidence Interval] among study centers:			
1.13 [1.07-1.21]^{SC1xSC2}			
1.44 [1.35-1.53]^{SC1xSC3}			
1.27 [1.21-1.32]^{SC2xSC3}			
Prevalence ratio [95% Confidence Interval] of POSEIDON (by group) among study centers:			
Group 1:			
0.36 (0.31-0.42]^{SC1xSC2}			
0.53 [0.45-0.62]^{SC1xSC3}			
1.46 [1.38-1.54]^{SC2xSC3}			
Group 2:			
1.65 [1.48-1.84]^{SC1xSC2}			
1.32 [1.21-1.45]^{SC1xSC3}			
0.80 [0.74-0.87]^{SC2xSC3}			
Group 3:			
2.36 [1.58-3.53]^{SC1xSC2}			
1.09 [0.78-1.50]^{SC1xSC3}			
0.46 [0.34-0.62]^{SC2xSC3}			
Group 4:			
2.70 [2.19-3.35]^{SC1xSC2}			
1.39 [1.18-1.64]^{SC1xSC3}			
0.51 [0.43-0.61]^{SC2xSC3}			

Study Center (SC) 1: ANDROFERT (Brazil); SC2: Anatolia IVF (Turkey); SC3: My Duc Hospital (Vietnam).

POSEIDON criteria (1, 2) at a particular time ("per period prevalence").

The narrow prevalence rate confidence intervals support the certainty of our estimates. However, caution should be exercised in generalizing our results because prevalence rates may be influenced by patient characteristics, clinical practices, and diagnosis criteria (11). Our evaluation relied solely on AFC as the ovarian marker criterion with which to classify the POSEIDON patients. Moreover, the profiles of treated patients and treatment practices varied among centers.

We found that advanced female age, decreased ovarian reserve, increased BMI and the presence of a female infertility factor were the POSEIDON population's main traits. These predictors were deemed relevant to the "POSEIDON" condition in our logistic regression models. We chose models that introduced all covariates at once because it is biologically plausible that interactions among covariates might better explain the associations ultimately observed (3, 20, 25). We also report the adjusted odds ratio of continuous variables per unit change in regression. This method allows a better understanding of the effect magnitude of explanatory variables on the probability of classifying a patient as POSEIDON. For example, according to the regression coefficients obtained with our models, the probability that an exemplary 33-year-old patient, with a BMI of 23 kg/m^2, AFC equal to 10 and no female infertility factor be classified "POSEIDON" after a standard ovarian stimulation is 42%. This probability increases to 67% in a patient of similar age with a BMI of 27, AFC of 7, and a female infertility factor.

TABLE 3 | Association of patient characteristics and the condition 'POSEIDON'.

Term (unit)	Estimate	Std Error	P value	Odds ratio*	Lower 95%	Upper 95%
Intercept	0.3970	0.2474	0.1086			
Female age (year)	0.0291	0.0053	<0.0001	1.0279	1.0172	1.0386
BMI (Kg/m2)	0.0364	0.0070	<0.0001	1.0373	1.0231	1.0517
Infertility duration (month)	0.0002	0.0005	0.6245	1.0002	0.9992	1.0013
AFC (n)	-0.1944	0.0045	<0.0001	0.8230	0.8157	0.8304
Primary treatment indication (Female factor)	0.3090	0.0342	<0.0001	1.6376[1]	1.4683	1.8265
Study Center (1-3)	-0.4003	0.1160	0.0006	0.3715[2]	0.2617	0.5273
Study Center (2-3)	0.5944	0.0675	<0.0001	2.1975[3]	1.9427	2.4857
Response: POSEIDON=yes				BIC: 11134.3		
Distribution: binomial				AICc: 11068.4		
Estimation method: nominal logistic				RSquare: 0.2667		
Number of Parameters: 7				Area under the curve ROC curve: 0.84		
Whole model effect: ChiSquare=4225.79; p<0.0001				Lack of fit test: 0.85		

Study Center (SC) 1: ANDROFERT (Brazil); SC2: Anatolia IVF (Turkey); SC3: My Duc Hospital (Vietnam).

**Per unit change in regressor (independent variable).*

[1]Odds ratio for female factor vs. no female factor (unexplained or male factor).

[2]Odds ratio for Study Center 1 vs. Study Center 2.

[3]Odds ratio for Study Center 2 vs. Study Center 3.

The POSEIDON patients' prevalence rates were overall high and varied across study centers, both when the population was analyzed as a whole and after subgrouping. We hypothesize that these findings are mainly related to the characteristics of the treated population. For instance, the median female age was the highest in study center 1, whereas ovarian reserve was the lowest, translating into its highest prevalence rates among the three centers. However, other variables may have influenced the overall and per-center prevalence rates. It is plausible that treatment factors (*e.g.*, gonadotropin total dose and stimulation regimen) and the presence of genetic polymorphisms affecting gonadotropins or their receptors might have accounted for the overall high prevalence of groups 1 and 2 patients. Moreover, we did not investigate smoking habits and socioeconomic factors (*e.g.*, income, education, or occupation). In particular, socioeconomic factors might influence the patient's decision to seek medical treatment. Also, we did we control for patients' and doctors' preferences concerning the gonadotropin regimens used for ovarian stimulation. Besides, it has been suggested that ethnicity might be an independent factor that affects the baseline ovarian reserve (26, 27). However, it remains to be established whether the differences in ovarian reserve observed in POSEIDON patients of different ethnic backgrounds are attributable to genetic, nutrition, infertility causes, or lifestyle factors. Consequently, uncertainty in our estimates may be larger than the statistical uncertainty reflected in confidence intervals and logistic regression models.

Notably, our study concerns a retrospective analysis of a large IVF population dataset; patients were stratified according to the POSEIDON criteria *a posteriori*, that is, after finishing the IVF cycle. Thus, the protocols utilized were based on the prevailing practices during the study period, which have not considered the POSEIDON criteria to guide patient management. In these lines, genetic factors, including polymorphisms affecting endogenous and/or their receptors, may also play a role in ovarian response to exogenous gonadotropin stimulation and ovarian reserve (28). Indeed, hypo-response to gonadotropin therapy is still highly undervalued (29). It is therefore possible that we do not adequately stimulate these patients. However, in routine practice, clinicians only detect the hypo-response during or after treatment, unless there is an evident history of hypo-response in previous cycles. POSEIDON patients, particularly those within groups 1 and 2, may harbor genetic variants potentially contributing to the hypo-response. Genotyping before COS could help better personalize treatment protocols (30), but the frequency and impact of specific genotype profiles on ovarian reserve and reproductive outcomes of POSEIDON patients have not yet been studied. Once these gaps in knowledge are filled, the pharmacogenomic-based COS may become a reality for these patients. Nonetheless, further research is needed to clarify the clinical utility of genotyping in low-prognosis patients.

Clinical Importance

Estimating the prevalence of low-prognosis patients in real-world settings using unified criteria, such as the POSEIDON classification, has relevant clinical implications. Firstly, it may provide information about the frequency of this condition. Secondly, it may reveal possible causal associations between patient characteristics and the low-prognosis status, with implications for research on infertility etiology, clinical practices, and public health policies.

This study is the first to report global prevalence estimates of POSEIDON patients. Herein we show that "low-prognosis" defined according to the POSEIDON criteria is a common condition, albeit with some geographic variations. Overall, our POSEIDON population was characterized by older patients with a lower ovarian reserve than non-POSEIDON counterparts. Furthermore, we found that, on average, POSEIDON patients had a higher BMI and, more often, a female infertility factor associated with this condition (versus non-POSEIDON). The relationship between female age, ovarian markers and ART reproductive success is well-established (31–33), and BMI has been shown to influence the number of oocytes retrieved and embryos obtained, as well as pregnancy outcomes (34).

The association between increased BMI and the condition 'POSEIDON' is a novel and interesting finding of our study. We found that BMI was an independent predictor of 'low-prognosis' both in the overall POSEIDON population and its subgroups. The regression coefficients obtained from our models indicated that women with expected low ovarian response (groups 3 and 4) were those mostly affected by increased BMI. However, after adjustment for other relevant covariates, the odds ratios obtained for patients of groups 1–2 and groups 3–4 were essentially the same (**Supplemental Tables 5** and **6**). These findings indicate that BMI has a significant effect, albeit of small magnitude, on the risk of 'low-prognosis', which seems to affect POSEIDON subgroups similarly and interact with other risk factors.

Our findings may help decision-makers and practitioners implement measures to mitigate the risk of low-prognosis and optimize reproductive planning. Awareness campaigns highlighting the adverse impact of advanced female age and impaired ovarian reserve on reproductive success (35, 36), the effect of lifestyle changes (37–40), and the role of treatment strategies to improve treatment success could be explored (41–46). Although the impact of the above interventions on POSEIDON prevalence rates remains mostly unknown, our data suggest that the "low-prognosis" burden could be prevented at least partially by treating patients earlier.

To date, only two single-center studies have looked at how often POSEIDON patients account for the overall treated population. In one large retrospective study from China, approximately 20% of IVF cycles fit the POSEIDON criteria (9). However, the authors studied cycles rather than patients, thereby precluding an accurate analysis of the real prevalence rates per treated patient population. In another study, also from China, Li and co-workers reported a POSEIDON prevalence rate of 31.5% over a 3-year period (4), which is consistent with the prevalence rate of the Vietnamese center (38.6%) included in our study. Nevertheless, an in-depth evaluation of the features that characterize POSEIDON and non-POSEIDON patients was not possible as the study by Li et al. lacked a control group.

Limitations

The AFC was determined by different operators using different machines. Although the reported AFC inter-observer variability is low (47), we cannot exclude that variations in technique and reporting have influenced patient classification. Besides, we did not assess prevalence rates using AMH due to excessive missing data. Different prevalence rates might have been obtained if AMH had been used. Another limitation is that referral for ART treatment may differ among study centers, thus affecting the prevalence rates of POSEIDON patients potentially. Besides medical factors, economic and social factors may impact access to treatment. We were unable to control for this potential source of bias and recognize that POSEIDON prevalence rates might differ, for instance, in studies conducted in countries/regions where IVF is publicly reimbursed or recommended earlier. Lastly, our study included only patients who initiated the treatment cycle.

Notwithstanding the above limitations, we are confident that our analysis of a large patient cohort provides a fair representation of real-world IVF practices and that our methods may guide future data collection. Our sample size is large enough to analyze the effect of candidate factors on the occurrence of "low-prognosis". However, due to the intrinsic limitations of cohort studies like ours to establish causal relationships, readers should interpret the impact of explanatory variables on the POSEIDON condition as associations rather than causation. Our study should be viewed as contributing to the literature and not as a stand-alone basis for inference and action.

Future Research

Additional real-world studies and pragmatic trials should be carried out to confirm (or refute) our observations, particularly those concerning the contribution of relevant covariates affecting the probability of the condition "POSEIDON". Moreover, prospective trials looking into the relationship between low-prognosis and gonadotropin receptor polymorphisms, and lifestyle and treatment regimens to mitigate its occurrence may be prioritized.

CONCLUSIONS

The estimated prevalence of POSEIDON patients in the general population undergoing IVF/ICSI is significant. The 'low-prognosis' patient defined according to the POSEIDON criteria has distinct clinical characteristics compared to the non-POSEIDON counterpart. The 'POSEIDON' condition is associated with female age, ovarian reserve, BMI, female infertility, and possibly ovarian stimulation regimens. Efforts are needed to reduce the prevalence of 'low-prognosis' patients by adequate diagnosis, counseling, and treatment.

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.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the following ethics committees: Instituto Investiga, Campinas, Brazil (CAAE 26429219.0.0000.5599), Hacettepe University, Ankara, Turkey (KA-180070), and My Duc Hospital, Ho Chi Minh City, Vietnam (05/18/DD-BVMD). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SE designed and coordinated the study and helped with the data acquisition, analysis, and interpretation. HY and LV designed the study, helped with the data acquisition, analysis, and interpretation. JC helped in designing the study, carried out the statistical analyses, and helped with the data interpretation.

İÖ, MP, HL, TP, and TH participated in the data acquisition and article's revision for critical intellectual content. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.630550/full#supplementary-material

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Improving Reporting of Clinical Studies Using the POSEIDON Criteria: POSORT Guidelines

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The POSEIDON (**P**atient-**O**riented **S**trategies **E**ncompassing **I**ndividualize**D** **O**ocyte **N**umber) criteria were developed to help clinicians identify and classify low-prognosis patients undergoing assisted reproductive technology (ART) and provide guidance for possible therapeutic strategies to overcome infertility. Since its introduction, the number of published studies using the POSEIDON criteria has increased steadily. However, a critical analysis of existing evidence indicates inconsistent and incomplete reporting of critical outcomes. Therefore, we developed guidelines to help researchers improve the quality of reporting in studies applying the POSEIDON criteria. We also discuss the advantages of using the POSEIDON criteria in ART clinical studies and elaborate on possible study designs and critical endpoints. Our ultimate goal is to advance the knowledge concerning the clinical use of the POSEIDON criteria to patients, clinicians, and the infertility community.

Keywords: Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number (POSEIDON) criteria, ovarian stimulation, low prognosis, poor response, infertility, assisted reproductive technology, ART calculator, guidelines

INTRODUCTION

The POSEIDON (**P**atient-**O**riented **S**trategies **E**ncompassing **I**ndividualize**D** **O**ocyte **N**umber) criteria were developed to identify and classify the low-prognosis patient undergoing assisted reproductive technology (ART) treatments (1–3). The new criteria' primary objectives were to help clinicians delineate subtle differences in patients' characteristics and provide guidance for possible stimulation strategies for these challenging patients classified as low prognosis (4, 5).

Women with low prognosis undergoing ART have defied clinicians for a long time, as no clear treatment strategies exist to improve outcomes significantly (6, 7). These women are characterized by a reduced chance of live birth after ART for at least two main issues: 1) reduced number of

oocytes and, consequently, embryos; and 2) poor oocyte/embryo quality resulting from advanced female reproductive age (8–11).

Based on female age, ovarian biomarkers, and the number of oocytes retrieved, the low-prognosis patient is identified and further classified into four POSEIDON groups (**Figure 1**) (1, 4). Outside POSEIDON, patients without a low prognosis can be categorized based on their expected response to ovarian stimulation and hence their prognosis as a non-POSEIDON group. An important outcome that would set the POSEIDON and non-POSEIDON groups apart is the cumulative delivery rate (CDR) (4). In 2017, the International Committee for Monitoring Assisted Reproductive Technology (ICMART) defined the term as *'the number of deliveries with at least one live birth resulting from one initiated or aspirated ART cycle, including all cycles in which fresh and/or frozen embryos are transferred, until one delivery with a live birth occurs or until all embryos are used, whichever occurs first, expressed per 100 cycles (initiated or aspirated)'* (12). On this basis, POSEIDON patients are expected to have lower CDR than non-POSEIDON patients overall. Moreover, CDRs are likely to differ across the four low-prognosis POSEIDON groups (4, 13, 14).

A critical backbone of the POSEIDON criteria is the number of oocytes retrieved –or expected to be retrieved– after a conventional ovarian stimulation with exogenous gonadotropins (1, 2, 4). The importance of oocyte numbers relates to its strong and independent association with the CDR (9, 11). Given that each oocyte has pregnancy potential, increased oocyte numbers may logically lead to higher CDR (8). The reason stems from the overall positive correlation between the numbers of oocytes retrieved and the resulting embryos obtained in *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) treatment (15). Thus, the higher the embryo cohort, the higher the CDR, as more opportunities are available to achieve a pregnancy after transferring fresh and cryopreserved embryos (13, 14).

Ovarian markers, particularly antral follicle count (AFC) and anti-Müllerian hormone (AMH) levels, constitute another backbone of the POSEIDON criteria (16–19). These markers have been widely used in routine clinical practice to estimate ovarian response to gonadotropin stimulation in women undergoing ART. Despite their acceptability and overall good accuracy to predict poor and high ovarian responses, they cannot correctly uncover the so-called hypo-responder patient, who, despite having normal ovarian reserve markers like AFC and AMH, finish with an unexpected suboptimal low oocyte yield after conventional ovarian stimulation (20–22). These patients are included in the POSEIDON criteria (Groups 1 and 2), as the hypo-response decreases the number of oocytes retrieved, consequently impacting the CDRs (9).

To assess the ovarian response to stimulation, the POSEIDON group developed the 'Follicle to-Oocyte Index' (FOI). This index calculates the ratio between the total number

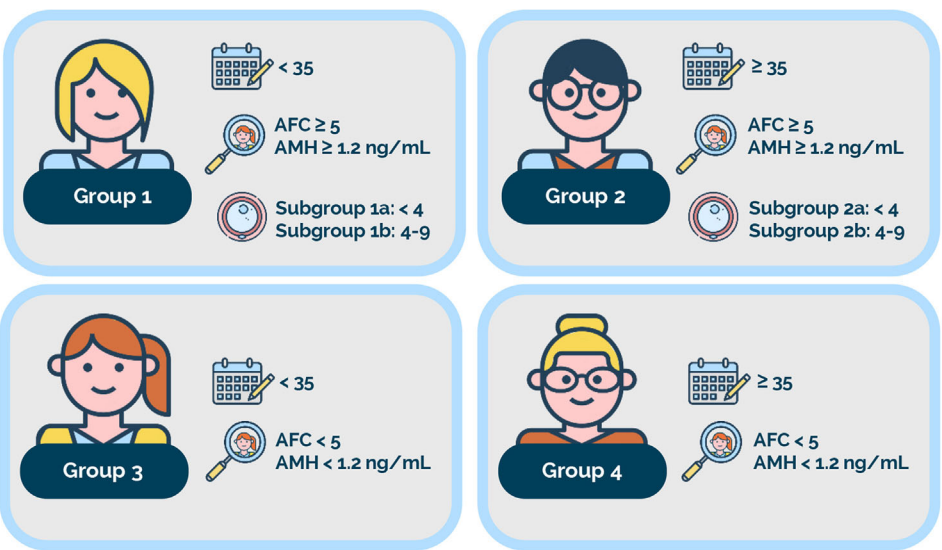

FIGURE 1 | The POSEIDON criteria. Four distinct groups of low-prognosis patients can be established based on quantitative and qualitative parameters, namely: 1. The age of the patient and its related embryo aneuploidy rate; 2. Ovarian biomarkers [antral follicle count (AFC) and/or anti-Müllerian hormone (AMH)], and 3. The ovarian response in terms of oocyte quantity (if a previous cycle of conventional ovarian stimulation was carried out). Group 1: Patients <35 years with sufficient prestimulation ovarian reserve parameters (AFC ≥ 5, AMH ≥1.2 ng/ml) and with an unexpected poor (<4 oocytes) or suboptimal (four to nine oocytes) ovarian response. This group is further divided into subgroup 1a, constituted by patients with fewer than four oocytes; and subgroup 1b, constituted by patients with four to nine oocytes retrieved after standard ovarian stimulation, who, at any age, have a lower live birth rate than age-matched normal responders. Group 2: Patients ≥35 years with sufficient prestimulation ovarian reserve parameters (AFC ≥ 5, AMH ≥ 1.2 ng/ml) and with an unexpected poor or suboptimal ovarian response. This group is further divided into subgroup 2a, constituted by patients with fewer than four oocytes; and subgroup 2b, constituted by patients with four to nine oocytes retrieved after standard ovarian stimulation, who, at any age, have a lower live birth rate than age matched normal responders. Group 3: Patients <35 years with poor ovarian reserve prestimulation parameters (AFC < 5, AMH < 1.2 ng/ml). Group 4: Patients ≥35 years with poor ovarian reserve prestimulation parameters (AFC < 5, AMH < 1.2 ng/ml). Art drawing courtesy of Chloé Xilinas, Med.E.A., Rome, Italy.

of oocytes retrieved following conventional ovarian stimulation and the number of antral follicles at the start of stimulation (20). This new parameter better reflects the dynamic nature of follicular recruitment and might be adopted to assess the response to gonadotropin stimulation in all patients undergoing ART. This parameter is particularly informative to identify the patient with a suboptimal response to exogenous gonadotropin stimulation, typically observed in hypo-responders who usually have low FOIs. Accordingly, treatments aimed at increasing the FOI can be tested in interventional trials.

Lastly, female age, which has consistently shown to be the most predictive parameter for live birth in ART, is also included in the POSEIDON criteria. In ART, the older the woman, the lower the chances of reproductive success (23, 24). Thus, female age can be regarded as a proxy for oocyte/embryo genetic competence, given the well-established association between age and oocyte/embryo ploidy status (25, 26). Female age in the POSEIDON criteria is used to stratify the low-prognosis patients accordingly (**Figure 1**).

The decline in reproductive success is mainly attributed to higher oocyte aneuploidy rates in older women. However, the availability of euploid embryos for transfer increases the chances of having a baby, as sustained implantation rates after transfer of euploid embryos are about 50% and relatively independent of maternal age (27, 28). While blastocyst morphology and development speed (i.e., day of trophectoderm biopsy) do seem to impact the implantation potential of euploid embryos, and thus LBdR, maternal age has no apparent influence (29). In practical terms, the current evidence indicates that older women's euploid embryos have similar implantation, live birth, and miscarriage rates than those of younger counterparts.

Accordingly, the POSEIDON group introduced a metric of success in ART, namely, the ability to retrieve the number of oocytes needed to obtain at least one euploid blastocyst for transfer in the specific patient (1, 2, 4). This number can be estimated without preimplantation genetic testing for aneuploidy (PGT-A), as embryo euploidy rates per age strata are well established (10, 25). The estimation can be performed manually using data from the literature or a dataset from an individual clinic or automatically using predictive models (30). According to the estimation, patient-oriented strategies can be elaborated to achieve the number of oocytes needed to obtain one euploid embryo and potentially increase success prospects (31–35).

THE NEED TO IMPROVE THE QUALITY OF CLINICAL STUDIES USING THE POSEIDON CRITERIA

After introducing the POSEIDON criteria in 2015, several studies have explored its potential benefit in clinical practice (14, 34–46). However, a discrepancy has been noticed concerning the reporting of critical outcomes (36, 39, 40, 44–46). Failure to recognize the critical pillars of the POSEIDON criteria, as mentioned above, might limit the clinical utility of such studies, notably when the essential endpoints are incompletely reported or not reported at all (4, 13).

Studies looking at live birth rates in fresh cycles have shown that increased oocyte numbers are associated with increased live birth rates (8). However, reporting reproductive endpoints like clinical pregnancy, ongoing pregnancy, and even live birth may not necessarily reflect the impact of an enlarged oocyte or embryo cohort as a way to potentially increase the probability of pregnancy, particularly in the low prognosis patient (9, 11). Logically, having more embryos to transfer potentially increases the CDR. Along these lines, comparing two ovarian stimulation regimens that result in a similar number of oocytes retrieved might still reveal that one protocol is more efficient than the other for a specific low-prognosis patient group if an endpoint like the FOI was included in the study design. Lastly, a given ovarian stimulation strategy might result in more patients achieving the estimated oocyte number required to obtain at least one euploid embryo for transfer, thus indicating a better efficacy, which will only be recognized if this particular endpoint is included in the study design and analyzed accordingly.

Given the low-prognosis patient's particularities and the steady increase in infertility studies using the POSEIDON criteria, we feel a need to clarify what to report and how to report. Therefore, to improve the quality of studies using the POSEIDON criteria, we developed a guideline based on the best evidence and expert judgment.

METHOD

Guideline Development

We developed the current guideline on behalf of the POSEIDON group (www.groupposeidon.com.br). The coordinators (SCE, CA, AC) assembled a guideline development group (GDG) composed of clinicians and researchers with experience developing and/or participating in infertility clinical trials. The group included both POSEIDON group members and non-members. It also included the editors-in-chief of two leading journals in reproductive medicine, 'Frontiers in Endocrinology (Reproduction)' and 'Reproductive Biology and Endocrinology'.

The participants were given access to the relevant literature concerning the POSEIDON criteria and their related content. For this, a literature search was performed in PUBMED/MEDLINE from inception up to 20th July 2020, based on defined keywords ('POSEIDON', 'Low-prognosis', 'Assisted Reproductive Technology'). A total of 41 articles were retrieved, including 11 review articles, 13 retrospective cohort studies, nine opinion/commentary/editorial articles, three articles concerning development and validation of predictive models, two prospective cohort studies, two letters to the editor, and one randomized controlled trial (RCT) (see **Supplementary Table 1** for a summary of published literature). The vast majority of articles were published in Frontiers in Endocrinology (23 articles), followed by Reproductive Biology and Endocrinology (three articles), Human Reproduction (three articles), and PLoS One (two articles) (1–5, 13, 14, 19, 20, 25, 30–60). The intention was to provide participants with the POSEIDON criteria' conceptual features and the existing evidence on its clinical use.

The coordinators used the published CONSORT (Consolidated Standards of Reporting Trials), IMPRINT (Improving the Reporting of Clinical Trials of Infertility Treatments), STROBE (Strengthening the Reporting of Observational Studies in Epidemiology), and GRACE (Good Research for Comparative Effectiveness) statements as guidance to elaborate a list of items –with definitions– relevant to POSEIDON trials (61–64). The new statement was named **POSORT** (**PO**SEIDON **S**tatement **O**f **R**eporting **T**rials) guidelines. The document was circulated among participants, and a consensus was achieved on items to be reported and how. The group also achieved a consensus concerning the endpoints to be included in POSEIDON trials.

RESULTS

POSEIDON Statement Of Reporting Trials (POSORT) Guidelines

The POSORT guidelines incorporate items on relevant quality dimensions of infertility care, including effectiveness, safety, and patient-centeredness (**Table 1**), which served as the basis for a 20-item checklist to be used by investigators in infertility trials using the POSEIDON criteria (**Supplementary Table 2**).

A list of endpoints is provided in **Table 2**. The GDG considered that CDR, as defined by ICMART (12), should be the preferred primary endpoint in intervention trials using the POSEIDON criteria. The recommended secondary endpoints include the number of oocytes retrieved (both overall and metaphase II oocytes), the number of embryos generated, the FOI (20), and how effective a specific intervention was in achieving the number of oocytes estimated by the ART calculator (30). Time to live birth (TTLB) is an additional outcome that should be considered, given that a shorter time in achieving a live birth is a reflection of the clinical and cost-effectiveness of any intervention (65). Also, in observational studies, particularly those involving big analytics, the frequency of patients fitting each POSEIDON group should be reported, including –if possible– a control group of non-POSEIDON patients for comparison. Other endpoints can be included but must be justified. A list of additional endpoints that may merit reporting is provided in **Table 3**.

TABLE 1 | Information to include when reporting studies using the POSEIDON criteria*.

Title and abstract	Identification as an observational study or randomized trial using the POSEIDON criteria.
Introduction	Explanation of rationale, specific objectives or hypotheses, and how the study may help to advance knowledge concerning the POSEIDON concept.
Methods	
Participants	• Inclusion and exclusion criteria must be clearly defined; • Characterize how infertility factors in participants were evaluated, describe the definitions used, and the settings where the data were collected; • Define which ovarian marker, AFC or AMH or both, was used to classify the patients as per the POSEIDON criteria, and describe the methods for AFC/AMH measurements; • In POSEIDON groups 1 and 2 studies, previous ovarian stimulation should be characterized; • The preferred unit of analysis is 'patient' rather than 'cycle'.
Interventions	• Characterize the intervention (if applicable) and state the duration of the intervention noting when the treatment started and concluded. State the temporal relation of the intervention to pregnancy.
Outcomes	• Clearly define the primary outcome. When more than one embryo transfer cycle occurs, the preferred outcome is cumulative delivery rate per initiated or aspiration cycle; • Both male and female outcomes, other than cumulative delivery rate, could be the primary outcome and should be justified. However, when cumulative delivery rate is not the primary endpoint and embryos are transferred, reproductive outcomes (e.g., live birth rate, ongoing pregnancy rate, miscarriage rate, time to delivery rate) should be reported; • Efforts should be made to include live birth data, including gestational age, birthweight, and sex of infant; • Clearly define predictors, potential confounders, and effect modifiers. Describe how confounders were adjusted for.
Data collection and analysis	• In observational studies, particularly the ones using real-world data, explain features of electronic medical records utilized, including how data quality was verified (e.g., completeness of data, availability of data on exposure, outcomes, and covariates); • Describe statistical methods, including those used to control for confounders, sensitivity analyses, and how the sample size was determined.
Results	• State the duration of infertility (including whether it is primary or secondary), relevant infertility treatment history, and cause of infertility in women and men. • Report the numbers of couples/patients who were screened and eligible, and describe (in observational studies) the proportion of patients fitting each POSEIDON group and those classified as non-POSEIDON; • Report numbers of individuals completing the follow-up and analyzed, and consider the use of a flow diagram; • Provide unadjusted and confounder-adjusted estimates with precision (e.g., 95% confidence interval), and other analyses carried out (e.g., subgroup and sensitivity analyses) • Report harms¶ or unintended effects in each group (men, women, infants) during treatment (including both male and female partners), during pregnancy, and around birth, and in infants after birth.
Discussion	• Discuss generalizability of the study findings and how the results compare to other studies using the POSEIDON concept; • Discuss trial limitations, including, but not limited to potential bias and imprecision (factors & interventions affecting endpoints should be discussed as 'associations' rather than 'causation' in observational studies).

**We recommend application of these guidelines in conjunction with the CONSORT, IMPRINT, STROBE, and GRADE guidelines as appropriate (see http://www.consort-statement.org/; https://strobe-statement.org/; https://www.graceprinciples.org/).*

¶Reportable harms include OHSS, infection, bleeding, multiple pregnancy and maternal pregnancy complications, and harms or unintended effects on the fetus/newborn, including congenital abnormalities, and major neonatal complications as well as infant developmental delays or medical problems.

AFC, antral follicle count; AMH, anti-Müllerian hormone.

TABLE 2 | POSEIDON endpoints.

Endpoint	Definition
Cumulative delivery rate (CDR)*	Number of deliveries with at least one live birth resulting from one initiated, aspirated, or embryo transfer ART cycle, including all cycles in which fresh and/or frozen embryos are transferred, until one delivery with a live birth occurs or until all embryos are used, whichever occurs first, expressed per 100 cycles (the denominator must be specified. i.e., initiated or aspirated cycles)
Time to pregnancy/Time to live birth (TTP/TTLB)	The time taken to establish a clinical pregnancy or live birth, measured in days or in number of treatment cycles
Follicle-to-oocyte index (FOI)	Ratio between the number of oocytes retrieved at oocyte pick-up and the number of antral follicles (AFC) at the start of stimulation
Number of oocytes retrieved	Total number of oocytes retrieved after oocyte pick-up
Number of metaphase II oocytes	Total number of metaphase II oocytes retrieved after oocyte pick-up
Number of embryos generated	Total number of viable embryos‡ generated after an IVF or ICSI cycle
Percentage of patients who achieved the minimum number of metaphase II oocytes estimated by the ART calculator	The ART calculator is a clinical predictive model that estimates, prior to treatment, the minimum number of metaphase II oocytes (MIImin) (and the 95% confidence interval of that number) needed to obtain at least one euploid blastocyst¶
Prevalence of low prognosis (POSEIDON) and non-low prognosis (Non-POSEIDON)	Frequency (%) of POSEIDON patients (by subgroup) and non-POSEIDON patients in the cohort§

**Live birth: any delivery of a live infant ≥22 weeks' gestation (fetus exiting the body with signs of life: movement, breathing, heartbeat).*
‡The embryo stage must be specified (cleavage, blastocyst).
¶The probability of success (e.g., 70%, 80%, and 90%) used for the estimation should be specified.
§Observational studies, including real-world data analysis.
AFC, antral follicle count; ART, assisted reproductive technology; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection.

The justifications and discussion on the key elements of the POSORT guidelines are provided in the next sections.

DISCUSSION

Advantages of POSEIDON Criteria in Clinical ART Trials

The likelihood of delivering a live born decreases progressively with female age (23, 24). Although this effect may be partially modulated by ovarian reserve, paternal factors, and the number of oocytes and embryos obtained after ovarian stimulation, the impaired reproductive outcome in the aging woman is primarily related to the increased oocyte/embryo aneuploidy rate (8–11, 13, 25, 26, 51). Indeed, the probability of having euploid embryos decreases progressively with age, being ≥50% and <50% overall, when a threshold of 35 years is used (25). Despite this given fact, an increased oocyte yield might lead to more embryos available for transfer, which would provide the patient with a better prospect when the transfer of fresh and frozen-thawed embryos is considered. Indeed, existing data indicate that the number of oocytes is strongly and independently associated with CDR (11, 14).

POSEIDON patients have an overall lower CDR than non-POSEIDON patients (13, 14, 42). However, the prognosis varies according to subgroup. In a recent large retrospective analysis involving 18,455 cycles, the authors showed a progressive decrease in CDR across POSEIDON groups (42). In this study, the CDR was 44.6% in Group 1, 35.5% in Group 3, 24.5% in Group 2, and 12.7% in Group 4. Notably, a significantly higher CDR was observed in women who did not fulfill the POSEIDON criteria (non-POSEIDON) than those who did. These findings are consistent with a recent Dutch multicenter observational cohort study in which differences in pregnancy rates among POSEIDON groups were also observed (40). In both studies, the female age emerged as impacting the reproductive prognosis more than the ovarian reserve and the number of oocytes retrieved. Nonetheless, these and other studies (13, 14) indicate that CDR in the POSEIDON patient is affected not only by oocyte/embryo quality (i.e., female age) but also by oocyte quantity.

The existing evidence, albeit limited, collectively suggest that the POSEIDON criteria are overall useful to prognosticate

TABLE 3 | Other endpoints that merit reporting.

Endpoint	Definition and formula
Live birth* delivery rate (LBdR)	Number of deliveries that resulted in at least one live birth, expressed per 100 cycle attempts (initiated, aspirated, transfer cycles).
Ongoing Pregnancy rate (OPR)	Number of viable intrauterine pregnancies of at least 12 weeks duration confirmed on ultrasound scan per 100 clinical pregnancies
Time-to-live birth	The time taken to achieve a live birth, measured in days or in number of treatment cycles, start time point from oocyte retrieval and end time point the day of delivery.
Multiple birth rate	Number of multiple births, defined by the complete expulsion or extraction of ≥1 fetus, after ≥ 22 wks. gestational age (e.g., twin delivery = two births) per 100 deliveries
Miscarriage rates	Number of spontaneous losses of clinical pregnancies before 22 completed weeks of gestational age per 100 clinical pregnancies

**Live birth, any delivery of a live infant ≥22 weeks' gestation (fetus exiting the body with signs of life: movement, breathing, heartbeat).*

reproductive outcomes among women undergoing ART, in which each group might demand specific treatment strategies (4, 5, 21, 31–35, 38, 39, 45, 54–57, 60). Thus, besides providing a counseling tool, the POSEIDON criteria may guide clinical management to optimize the FOI. Improving oocyte yield with a consequent higher number of embryos may result in a higher chance of having a euploid embryo transferred (10, 25). Transfer of a euploid embryo potentially results in an increased implantation rate and shortened TTLB. Given each POSEIDON subgroup is characterized by a more homogenous population with specific prognostic characteristics, we encourage clinicians to move from the existing heterogeneous definitions of poor responders to the low-prognosis notion proposed by the POSEIDON group.

Biomarkers' Considerations

The POSEIDON criteria are simple and straightforward as regards thresholds to defining each subgroup. For instance, unlike other criteria that apply an ill-defined ovarian reserve threshold (66), the POSEIDON classification uses objective thresholds of antral follicle count (AFC) and Anti-Müllerian hormone (AMH) values. According to the POSEIDON stratification, a rigorous and precise assessment of AFC and/or AMH is necessary before starting a clinical trial. For an adequate AFC evaluation, the criteria proposed by Broekmans and co-workers in 2010 (16) and Coelho Neto and co-workers in 2017 (67) seem appropriate. These practical guidelines summarize the main technical aspects for performing AFC, including the optimal machine settings, time of menstrual cycle (e.g., early follicular phase, which follicles to measure and how, and clinical considerations. However, inter-observer and intra-observer variability in AFC determination has been reported (68), and the use of two-dimensional transvaginal sonography may yield different results even by experienced operators (69). The adoption of automated ultrasonographic assessments could also be considered (70). Manual and automated methods did not differ in terms of fertility outcome (71); however, the automatized method seems to offer a lower intra- and inter-observed variability than standard 2D methods (70).

Along these lines, several enzyme-linked immunosorbent assays (ELISA) have been developed for AMH assessments (72), and manual assays were recently replaced by fully automatized assays (73, 74). Despite this, the reliability of some assays has been questioned due to technical issues, and it has been suggested that the existing automated methods cannot be used interchangeably as their results do not necessarily line up (75). For example, automated assays generate lower values than ELISA, and POSEIDON thresholds were based on Gen II ELISA. Therefore, POSEIDON AMH thresholds must be converted if an automated assay (e.g., Elecsys) is utilized (76). Nonetheless, a recent multicenter study showed that the area under the curve (AUC) for predicting poor response, using an AMH automated assay, was 0.929, compared to previous data of 0.78 (73).

Clinicians relying on AMH to assess ovarian reserve must understand the existing assays' technical limitations. The AMH assay used should be standardized, and if possible, calibrated against other assays. At this point, however, it might be advisable to use a single assay in the clinic with precise thresholds to distinguish between patients expected to have a poor, normal, or high response to ovarian stimulation. Apart from this, factors potentially affecting AMH results should be considered, including oral contraceptives used for cycle synchronization before OS in GnRH antagonist regimens (17, 77).

Collectively, the POSEIDON criteria underline the importance of correctly classifying infertility patients undergoing ART. The classification system emphasizes the impact of female age and its related oocyte and embryo's aneuploidy rates, and the number of oocytes retrieved for ART success. It also underlines that treatment delays should be avoided in the low-prognosis infertility patient.

Study Design Considerations

Rigorous planning and strict execution are critical parameters in performing high-quality studies. The time invested in planning usually pays off in the end. Having acknowledged the heterogeneity of the low prognosis group of patients undergoing ART, researchers need to focus on well-defined subgroups to test specific interventions. In this regard, the POSEIDON criteria are advantageous in terms of providing a more homogeneous patient grouping.

Among different study designs, it is widely recognized that RCTs represent the optimal way to verify the clinical efficacy of specific interventions (59, 60). In RCTs, participants are prospectively and randomly allocated to either intervention or another, following strict inclusion and exclusion criteria. The CONSORT and IMPRINT statements have provided useful guidance to increase the quality of infertility trials (61, 62). These guidelines also served as the basis for the development of the current POSORT guidelines.

Although RCTs remain the backbone of high-quality evidence (78), an overwhelming majority of infertility patients are treated outside the scope of such studies. Besides, most patients treated in routine clinical practice do not necessarily meet the inclusion and exclusion criteria adopted in RCTs (79). Importantly, valuable information can be obtained from data generated during routine clinical practice using pragmatic clinical trials and observational studies (63, 64). These study designs may provide valid information on how therapy affects a heterogeneous infertility population (e.g., those who are older and those with concomitant medical problems, impaired ovarian function, and diverse ethnicities/races). Observational studies can also generate hypotheses for testing in RCTs, assess trial practicability by assessing the impact of planned inclusion/exclusion criteria in the pertinent population, informing about probability distributions to be used in statistical analyses, and identifying prognostic factors or patient baseline attributes for improvement or stratification.

If well conducted, observational studies may generate real-world evidence, which refers to evidence generated from clinically relevant data gathered outside of the conditions imposed by conventional RCTs (78, 80). These data can be collected from various sources, including registries (at a country or region level) and electronic health records (at a site level). Such studies are less time-consuming and less expensive than

RCTs and allow individual fertility centers to contribute their specific experience on how treatments work in real-world settings. However, minimum standards should be followed to secure the quality of observational studies. Given treatment decisions might be driven by many factors (performance bias), and real-world patients can have complex clinical conditions (selection bias), studies must address unbalanced groups, confounders, differential follow-ups, and missing data (64). Thus, our guidelines have also taken into consideration the STROBE and GRACE recommendations.

Endpoints in POSEIDON Criteria Clinical Studies

Several preclinical (e.g., cumulative gonadotropin dose, number of oocytes retrieved, metaphase II oocyte rate, 2PN rate, blastulation rate; post-ICSI degeneration rate, survival rates (embryo/oocyte/sperm) post-warming) and clinical endpoints (e.g., clinical pregnancy rate, ongoing pregnancy rate, live birth delivery rate [LBdR], CDR, TTLB, multiple birth rate, OHSS rates) are used in ART clinical trials. As mentioned above, the number of oocytes retrieved is strictly related to live births. Thus, this parameter represents an important surrogate endpoint that should be pursued in clinical trials devoted to POSEIDON patients. Moreover, the POSEIDON group proposed an innovative method to assess ovarian sensitivity by introducing the FOI, which measures the efficiency of the ovarian stimulation protocol and the ovarian resistance to gonadotropin stimulation. The FOI is defined by the ratio between the number of oocytes retrieved at the end of the ovarian stimulation in relation to the AFC at the beginning of stimulation (20). The FOI may be informative, especially in patients with unexpected suboptimal or poor responses to ovarian stimulation (i.e., POSEIDON groups 1 and 2). In these patients, the primary aim of interventional trials would be to identify strategies to overcome suboptimal response to ovarian stimulation, like personalizing FSH starting dosage based on specific genotype characteristics, supplementing with recombinant luteinizing hormone, or modifying the trigger strategy (21, 22, 31, 34, 47, 54, 60). On this basis, the FOI may serve as a marker to identify patients with a relative FSH/LH deficiency who could benefit from individualized ovarian stimulation.

As for reproductive endpoints, the LBdR –defined as the number of deliveries that resulted in at least one live birth obtained after 22 weeks' gestation, expressed per 100 cycle attempts (initiated, aspirated, or embryo transfer cycles)–, and more recently, the CDR represent essential endpoints for patients, clinicians, and the public when evaluating the effects of treatment (12). Among these, the CDR following the transfer of fresh and/or frozen embryos obtained from a single initiated/aspiration cycle represents the best way to evaluate ART success in POSEIDON studies.

We recognize that the CDR might be difficult to obtain because this implies that all useful embryos should be transferred and allowed to have a chance to develop into a live born. Indeed, some patients will end up not using all their embryos, and if they do, it will often take a considerable period to complete the trial. However, the number of oocytes and embryos in POSEIDON patients is overall low, thus allowing an account of the outcome of all embryos and therefore generating a true CDR.

Live birth endpoints could also be challenging in low responders and advanced age patients, given the noticed age-dependent miscarriage rate (23). A significant treatment discontinuation rate before delivery may also be noted during trials, making the sample size required to analyze such endpoints less practical. For instance, intrauterine fetal death is observed in about 5% of ongoing IVF pregnancies after a 12-week gestation period, a risk that is further increased in older women (81). Consequently, large RCTs have opted to use other primary endpoints than live births (81). Clinical endpoints such as implantation rates, ongoing pregnancy rates, and miscarriage rates are also clinically significant as they represent intermediate outcomes reflecting the continuum of the ART process (82, 83). However, the use of such endpoints in preference over CDR in POSEIDON studies should always be justified. When considering time-to-pregnancy (TTP) or TTLB as an outcome, the start time point should be oocyte retrieval and the end time point the clinical pregnancy (TTP) or live birth (TTLB) (65). As POSEIDON interventions should aim to increase the oocyte yield for the low prognosis patients, this justifies the start point from oocyte retrieval for TTP and TTLB outcomes. Lastly, we recommend a more comprehensive reporting of outcomes in POSEIDON trials, including potential harms and health of the resulting offspring (**Table 1**).

The ART Calculator

To establish a valuable working plan for low prognosis patients and improve patient counseling, the POSEIDON group, as previously mentioned, proposed a novel metric of success in ART, namely, the retrieval of a sufficient number of oocytes to achieve at least one euploid embryo for transfer (1, 2, 4). In this context, Esteves and co-workers, on behalf of the POSEIDON group, developed a predictive model to determine the minimum number of oocytes required to obtain at least one blastocyst for transfer (30). In their study, female age, sperm source for IVF/ICSI (ejaculated vs. testicular sperm), and the number of oocytes retrieved were the main predictors affecting the blastocyst euploid probability. In practical terms, the predictive model estimates the optimal average number of metaphase II (MII) oocytes (and the 95% confidence interval), which increases progressively with aging and is magnified further by the use of testicular sperm from patients with nonobstructive azoospermia (30).

The ART calculator estimations may be adopted in POSEIDON clinical trials as a novel endpoint to determine the effectiveness of the interventions used. For example, the proportion of POSEIDON patients reaching the target number of MII oocytes as per the ART calculator could be determined and compared, and results analyzed in terms of how they translated to pregnancy success. Besides estimating the number of MII oocytes for at least one euploid blastocyst, the calculator also estimates the chance of having a euploid blastocyst based on

the real number of oocytes retrieved. Thus, even if the ideal number of MII oocytes is not achieved, the probability of having a euploid blastocyst could be compared according to the interventions investigated. The latter might be of particular relevance to the advanced age POSEIDON patient, in whom the calculated ideal number of oocytes is more challenging to achieve.

The ART calculator was recently validated in a multicenter study (51). In detail, clinical and embryological data of 1,464 consecutive infertile couples subjected to IVF/ICSI and PGT-A were assessed. The authors demonstrated that the estimations provided by the ART calculator were strongly correlated with the actual probability of blastocyst euploidy per MII oocyte ($r = 0.91$) and the minimum number of MII oocytes to obtain at least one euploid blastocyst ($r = 0.88$).

In summary, besides being a new tool to be used both in clinical practice for counseling and treatment planning, the ART calculator could be a useful tool in POSEIDON clinical trials to compare treatments and strategies between study and control groups balancing both quantity (number of oocytes collected) and quality (euploidy of embryos).

Strengths and Limitations

The POSORT guidelines have several strengths. They were developed by an international panel of experts in reproductive medicine, many of which are members of the POSEIDON group. The group reached a consensus on the minimum standards for relevant clinical studies using the POSEIDON criteria. The consensus was based mainly on a detailed and critical analysis of the available literature concerning the POSEIDON criteria.

However, our guidelines have some limitations. First, the number of published studies on POSEIDON criteria is still limited. Therefore, evidence from other relevant studies and expert experience were also considered, and the current version may not represent an exhaustive list of statements. Additionally, the guidelines only represent the opinion of the expert included. Along these lines, patient representatives were not included. Despite these limitations, the POSORT guidelines are the first of their kind to provide an expert opinion on specific approaches to be considered in POSEIDON studies. As with all guidelines, ours is an evolving document that should be revised periodically as new evidence emerges. The perspectives provided in this consensus complement existing guidelines and may help advance knowledge, potentially improving treatment outcomes.

CONCLUSIONS

We developed guidelines to improve the quality of reporting in clinical infertility studies using the POSEIDON criteria. Our aims are to help researchers better characterize the study participants and report critical endpoints relevant to the POSEIDON framework. The ultimate goal is to promote complete and consistent reporting to advance knowledge concerning the POSEIDON criteria's clinical utility.

AUTHOR CONTRIBUTIONS

SE and AC coordinated the GDG and had a leading role in collecting the evidence, drafting the manuscript, and handling the GDG's comments. All participants contributed to the guideline development, evidence summary and recommendations, and writing sections of the manuscript. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.587051/full#supplementary-material

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