



Effects of Rederivation by Embryo Vitrification on Performance in a Rabbit Paternal Line

Jorge Daniel Juárez¹, Francisco Marco-Jiménez² and José Salvador Vicente^{2*}

¹ Facultad de Zootecnia, Universidad Nacional Agraria de la Selva, Tingo María, Peru, ² Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, Valencia, Spain

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*Correspondence:

José Salvador Vicente
jvicent@dca.upv.es

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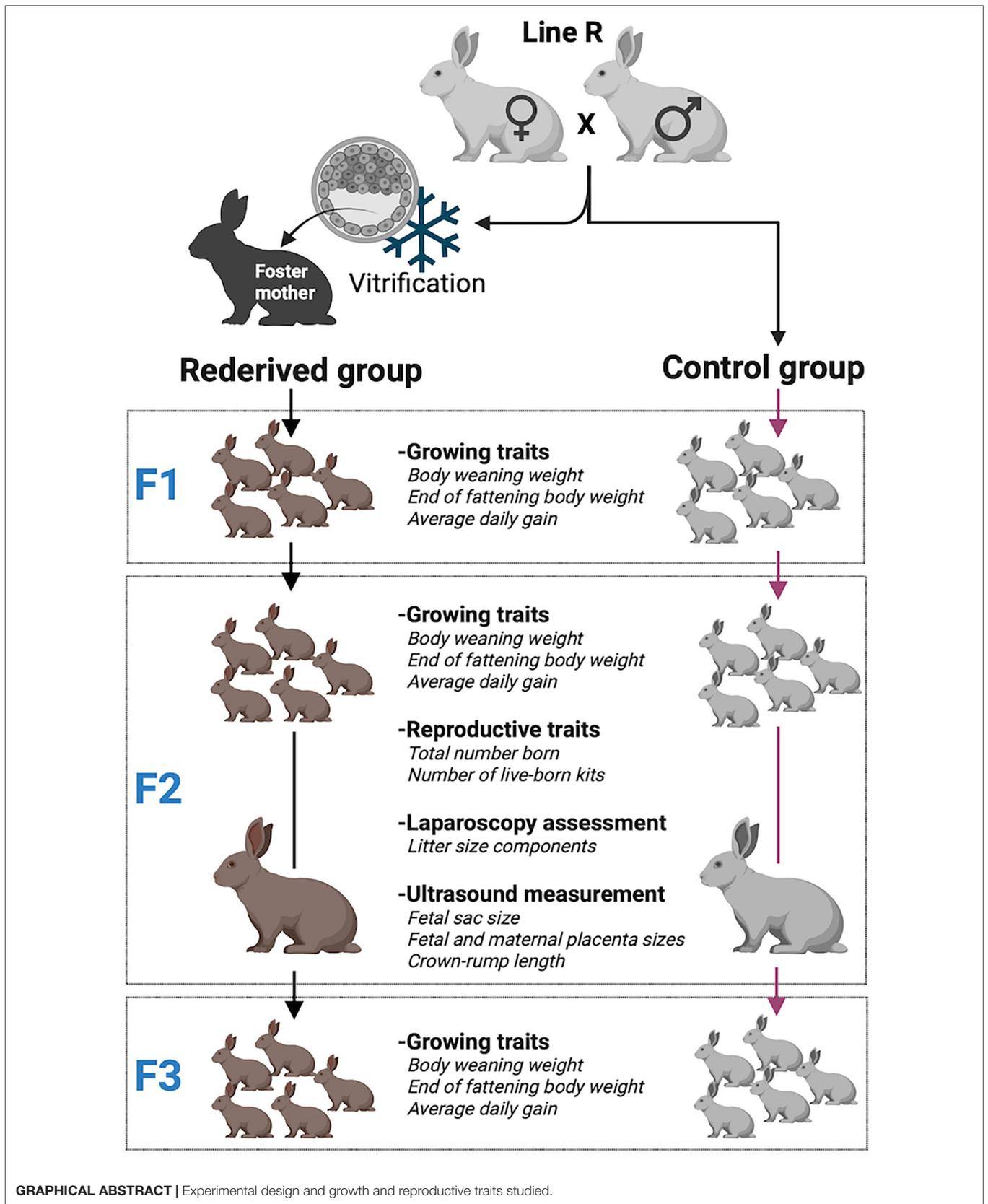
Embryo cryopreservation is a valuable tool for maintaining genetic variability and preserving breeds and lines, allowing to assess the response to selection and enabling genetic diffusion. This study aimed to evaluate the impact of rederivation by embryo vitrification and transfer procedures on the growth and reproductive traits in a paternal rabbit line selected for average daily gain from weaning (28 days old) to fattening (63 days old). The rederived population was bred over two generations at the same time as a control population of this paternal line and, growth trait parameters (weights at weaning, end of the fattening period, and average daily gain) and reproductive performance (kindling rate, litter size at birth and at weaning) were compared with three filial generations. Moreover, fetal growth and litter size components were assessed for the second generation by ultrasonography and laparoscopy. Differences in postnatal growth traits (end of fattening weight and average daily gain) were observed in the three generations assessed. However, fetal growth, litter size components, and reproductive traits did not show significant differences. In conclusion, cryopreservation and embryo transfer processes cause changes in growth traits of reconstituted populations that influence the following generations, without changes in reproductive traits in a paternal line of rabbits.

Keywords: embryo vitrification, fetal growth, litter size, growth line, rabbit

INTRODUCTION

Estimation of response to selection in rabbit-breeding programs has been extensively described, and one alternative is the comparison between two different generations by rederivation of a control population cryopreserved generations ago (Khalil and Al-Saef, 2008), which could be stored for many years, achieving reasonable pregnancy rates and survival at birth (Lavara et al., 2011; Yao et al., 2012; Marco-Jiménez et al., 2018). The implementation of reproductive biotechnologies such as cryopreservation and embryo transfer in rabbits has been used in our research group for approximately three decades (Vicente and García-Ximénez, 1993; García-Dominguez et al., 2019). Vitrifying has proven to be one of the most cost-effective and optimal freezing technologies for rabbit embryos, achieving high-survival rates at birth and high-parturition rates (Kasai et al., 1992; Vicente and García-Ximenez, 1994), allowing the creation of an embryo bank (Vicente et al., 2003).

Rederivation of a control population has been used to estimate the response to selection in both maternal (García and Baselga, 2002a,b) and paternal lines (Piles and Blasco, 2003; Juárez et al., 2020a,b; Peiró et al., 2021). In some cases, without taking into account that the rederivation process



has phenotypic effects on the growth of the fetus and newborn during the prenatal (Mocé et al., 2010; Saenz-de-Juano et al., 2014), and in the postnatal period in organ weight, such as the liver (Lavara et al., 2015; García-Domínguez et al., 2020a,b). Moreover, Lavara et al. (2014) observed effects on reproductive traits such as litter size in the derived females (F1) and transgenerational effects on the female offspring (F2).

These phenotypic changes suggested that embryo cryopreservation is not neutral. Embryo cryopreservation itself presents specific issues, embryos are exposed to osmotic, chemical, and temperature stress, to which they have to respond. Consequently, developmental alterations emerge in the embryo, fetus, and postnatal life of vitrified-transferred embryos. The recent studies have demonstrated that vitrified-transferred embryos could respond to stressful procedures by altering gene expression or even incorporating lasting epigenetic marks with effects on the epigenome, transcriptome, proteome, and metabolome of adult rabbits (García-Domínguez et al., 2018, 2020a,b,c; García-Domínguez et al., 2021; Marco-Jiménez et al., 2020). These last studies provided evidence that embryo cryopreservation induced an adaptive response resulting in phenotypic plasticity without negative consequences on health. It has been observed that this adaptive response was still present in later generations through changes in organs such as the liver and heart or bodyweight; moreover, the liver epigenetic and metabolome variations have also been found (García-Domínguez et al., 2020a,b,c; García-Domínguez et al., 2021).

In mice, vitrification and slow freezing of embryos has been shown to increase the expression of genes associated with fat mass but did not alter physiological development, movement coordination function, or brain development, even though mice become heavier at 8 weeks of age (Wang et al., 2020). This could be associated with significant dysfunction of glucose metabolism in the liver due to increased insulin resistance reported in male mice (Qin et al., 2021). Even in humans, the freezing process might cause singletons to be born with a greater risk of high birth weight and larger gestational age than singletons after fresh embryo transfer and naturally conceived (Spijkers et al., 2017).

This study aimed to evaluate the impact of embryo vitrification and transfer procedures on the growth and reproductive traits of a paternal rabbit line selected for average daily gain from weaning (28 days old) to fattening (63 days old).

MATERIALS AND METHODS

Before the experimental phase began, all the animal experimentation protocols used were reviewed and approved by the Ethics and Animal Welfare Committee of the Universitat Politècnica de València (Code Number 2015/VSC/PEA/00061). Likewise, all the experiments followed the guidelines and regulations set forth in Directive 2010/63/EU/EEC. The facilities and cages used were adequate for experimentation on this species (Code: ES462500001091).

Animals and Experimental Design

A paternal rabbit line (R) selected at the Universitat Politècnica de Valencia was used. This line was founded in 1989 from two

closed paternal lines (Estany et al., 1992). Since then, the line has been selected for individual weight daily gain from 28 days (weaning) to 63 days old (end of fattening). The rederivation procedure of the population was described by Marco-Jiménez et al. (2018) from embryos of 28 donors belonging to 15 different sire families of the 36th generation. Sire families were established to avoid matings between relatives sharing a grandparent. The control population was the offspring of the 36th generation born by artificial insemination at the same time as the rederived population. Offspring from both populations were bred over two generations. The offspring of vitrified embryos were named rederived-F1 and inseminated conceived named control-F1 and the following filial generations (rederived-F2 and F3 and control-F2 and F3, **Graphical Abstract** and **Table 1**).

The animals were housed at the Universitat Politècnica de València experimental farm in flat deck indoor cages (75 × 50 × 30 cm), with free access to water and commercial pelleted diets (Cunilap, NANTA S.A., Spain, minimum of 15 g of crude protein per kg of dry matter (DM), 15 g of crude fiber per kg of DM, and 10.2 MJ of digestible energy (DE) per kg of DM). The photoperiod was 16 h of light and 8 h of dark, with a regulated room temperature between 14 and 28°C.

Growing Traits Analysis

Individual weaning weight (WW, 28 days old), individual weight at the end of the fattening period (EFW, 63 days old), and average daily weight gain (weight gained from day 28 to 63 divided by 33, ADG) during the fattening period were recorded for all the generations.

Female Reproductive Management

Reproductive management of both populations (rederived and control) and their offspring avoided mating among relatives with common grandparents, achieving non-overlapping generations. The first reproductive cycle took place at ~5 months of age, and after kindling, the new insemination was tried 10–12 days later. Briefly, two ejaculates per male were collected in each replica using an artificial vagina. Only ejaculates with total motility higher than 70% and <30% of the abnormal sperm were used. After semen evaluation, optimal ejaculates from each male were extended to 40 million/ml. All the females were synchronized by intramuscular injection with 15UI eCG (Cuniser, HIPRA S.A., Spain) 48 h before being inseminated with 0.5 ml of extended semen using a curved plastic pipette. Females were induced to ovulate by intramuscular injection of 1 µg of buserelin acetate (Suprefact, Hoechst Marion Roussel, S.A., Spain) at insemination time. Pregnancy was checked at 14 days from insemination and non-pregnant does were inseminated again at 21 days after the previous insemination. In addition, it was noted whether rabbits underwent a lactation–gestation overlap, and the dams were classified into four groups, according to the reproductive status: offspring from primiparous does without overlapping (PD), offspring from primiparous lactating does (females that were pregnant while suckling their first litter, PLD), multiparous lactating does (females with more than one parturition that were pregnant while suckling their litter, MLD) and multiparous non-lactating does (females from more than one parturition that were

TABLE 1 | The total number of parents used to generate the offspring.

Population	Filial generation	Generation	Females	Males	Total
37th	F1	R37	77	16	93
	F2	R38	49	9	58
	F3	R39	78	27	105
		Total	204	52	256
37th vitrified	F1	R37V	11	10	21
	F2	R38V	26	13	39
	F3	R39V	78	18	96
		Total	115	41	156

pregnant after lactation, MD). Total litter size, liveborn, and litter size at weaning were recorded for each female.

Evaluation of Litter Size Components

A total of 139 laparoscopies were carried out on females from the fourth and fifth parity (80 does from control-F2 and 59 does from rederived-F2). Females were sedated according to the procedure described by Juárez et al. (2021). In brief, the females were sedated with an intramuscular injection of 5 mg xylazine/kg (Rompun, Bayer AG, Leverkusen, Germany) and 3 mg/kg morphine chloride. Five minutes later, 35 mg/kg of ketamine hydrochloride (Imalgene, Merial, S.A., France) was administered intravenously. After laparoscopy, does were treated with antibiotics (200,000 IU procaine penicillin and 250 mg streptomycin, Duphaphen Strep, Pfizer, S.L., Spain), 0.03 mg/kg of buprenorphine hydrochloride every 12 h and 0.2 mg/kg of meloxicam every 24 h for 3 days. The number of corpora lutea, the number of implanted embryos at 12 days (IE), and litter size at birth (LS) were recorded per female. The following variables were calculated using the aforementioned data. Ovulation rate (OR), defined as the number of corpora lutea, embryonic loss rate (ELR), estimated as $(OR-IE)/OR$, and fetal loss rate (FLR), estimated as $(IE-LS)/IE$.

Fetal Growth and Ultrasound Measurement

A total of twenty-nine pregnant does from laparoscoped females (13 from control-F2 and 16 from rederived-F2) were examined on days 12, 19, and 26 of gestation using a portable color Doppler ultrasound device (Esaote, Spain) with a 7.5 MHz linear probe (4–12 MHz range). Does were sedated according to the procedure described aforementioned and placed in a polystyrene cage where they were prevented from moving. The ultrasound examination was performed according to the procedure described by Juárez et al. (2021). After localization of different fetal sacs, 4–6 whole fetal sacs were examined per doe. The identifiable structures (fetal sac, fetus, and fetal and maternal placenta) were measured from the frozen frame pictures on the monitor, using the Esaote 16 ultrasound software.

TABLE 2 | Effect of rederivation by embryo cryopreservation on weaning weight (WW, kg), weight at end of fattening (EFW, kg), and average daily gain (ADG, g/day) in the paternal rabbit line.

Population	<i>n</i>	WW (kg)	EFW (kg)	ADG (g/day)
Control (F1 + F2 + F3)	1,117	0.73 ± 0.01	2.34 ± 0.01 ^a	49.24 ± 0.30 ^a
Rederived (F1 + F2 + F3)	1,209	0.73 ± 0.01	2.26 ± 0.01 ^b	46.61 ± 0.34 ^b
Covariate effect		0.015 ± 0.001 ^{***}	0.11 ± 0.001 ^{***}	9.77 ± 1.21 ^{***}

n, number of young rabbits.

^{a,b}Values with different superscripts in column differ significantly ($P < 0.05$). Significance of estimated covariate effect (^{***} $P < 0.001$).

Statistical Analyses

Growth Traits

The first analysis of growth traits was carried out to assess the effects of cryopreservation procedures on the reconstituted population:

$$Y_{ijklm} = \mu + P_i + R_j + MY_k + PR_{ij} + CO_l + Cov X_m + \epsilon_{ijklm}, \quad (1)$$

where Y_{ijklm} was the trait to analyze, μ was the general mean, P_i was the fixed effect of rederivation (control population vs. rederived), R_j was the fixed effect of reproductive status of the doe used in the WW analysis (PD, PLD, MLD, and MD), MY_k was the fixed effect of month-year in which the fattening period ended (39 levels), PR_{ij} was the effect of interaction between rederived population and reproductive status of the mothers used for WW analysis, CO_l was the random effect of common litter, $Cov X_m$ was the covariate of the number born alive (BA) used for the WW trait or the covariate of WW used for weight at the end of the fattening period (EFW) and ADG traits and ϵ_{ijklm} was the error term of the model.

A second analysis of growth traits to evaluate the effect of rederivation in each filial generation (F1, F2, and F3: control vs. rederived) was performed using the mixed linear model described aforementioned:

$$Y_{ijklm} = \mu + P_i + R_j + MY_k + PR_{ij} + CO_l + Cov X_m + \epsilon_{ijklm}, \quad (2)$$

where P_i was the fixed effect of filial generation (control-F1 and rederived-F1 or control-F2 and rederived-F2 or control-F3 and rederived-F3). In the WW analysis of the first filial generation, the fixed effect month-year (MK_k) had 33 levels, for the filial generation 2 had 31 levels and filial generation 3, had 32 levels.

Reproductive Traits

The general linear model was used to assess reproductive performance, including as fixed effects the rederived generation group (P_i , control-F2 and rederived-F2), reproductive status of does (R_j , nulliparous, primiparous lactating, multiparous lactating, and non-lactating does), month-year in which insemination was done (MK_k , 9 levels) and the interaction

between generation group and reproductive status of the mothers (PR_{ij}).

$$Y_{ijklm} = \mu + P_i + R_j + MY_k + PR_{ij} + e_{ijklm} \quad (3)$$

Litter size components (ovulation rate, implanted embryos, litter size, liveborn, and rates of the embryo and fetal losses) were analyzed by a generalized linear model, including as fixed effects the rederived generation group (P_i , control-F2 and rederived-F2) and lactating or non-lactating status (L_j) and their interactions (PL_{ij}).

$$Y_{ijklm} = \mu + P_i + L_j + PL_{ij} + e_{ijklm} \quad (4)$$

To analyze fetal sac area, CRL of the fetus, fetal and maternal placenta areas at days 12, 19, and 26 of gestation, and the weight of liveborn kits, a mixed linear model was used:

$$Y_{ijklm} = \mu + P_i + R_j + PR_{ij} + CO_k + CovX_l + e_{ijklm}, \quad (5)$$

where Y_{ijkl} was the trait to analyze, μ was the general media, P_i was the fixed effect of the rederived generation group (control-F2 and rederived-F2), R_j was the fixed effect of reproductive status of the doe used for analysis of weaning weight (lactating and non-lactating doe); PR_{ij} was the effect of interaction between rederived population and reproductive status of the mothers used to analyze the weaning weight, CO_k was the random effect of common litter, $Cov X_l$ was the covariate of the number of implanted embryos and e_{ijklm} was the error term of the model. Values were considered statistically different at $P < 0.05$. Results were reported as least-square means with a standard error of the mean (SEM). All the analyses were performed with SPSS 26.0 software package (SPSS Inc., Chicago, IL, United States, 2012).

RESULTS

Growing Traits

Growing Traits and Rederived Effect

Cryopreservation and embryo transfer procedure significantly affected EFW and ADG, but not for WW (Table 2). However, reproductive status of does affected WW, showing that young and adult mothers without lactation–gestation overlap (ND and MD, respectively) had the lowest WW (0.67 ± 0.01 and 0.68 ± 0.01 vs. 0.78 ± 0.02 and 0.78 ± 0.01 kg to ND, MD, PLD, and MLD, respectively, $P < 0.05$, data not shown in tables). MY fixed and common litter random effects were significant for all analyses.

Evolution of Growth Traits by Filial Generation

Differences in WW were greater for the control-F1 than rederived-F1, but in subsequent generations were greater for offspring of the rederived population (F2 and F3) than control (F2 and F3). Otherwise, for EFW and ADG, in the first generation (F1) the rederived population had higher values, but in the following generations (F2 and F3), it was greater to the control population in both the traits (Figure 1).

Reproductive Traits

Reproductive Performance of F2 Females

No differences in litter size at birth and at weaning, perinatal and lactation mortalities, and kindling interval were found between the populations assessed (control-F2 and rederived-F2, Table 3). Likewise, no significant differences in the reproductive status of does were registered, except for the kindling interval, registering a higher KI in multiparous non-lactating does (MD), almost twice the interval (Table 3).

Similarly, no effects of interaction between population and reproductive status of doe were reported for all the reproductive parameters evaluated.

Litter Size Components of F2 Females

In all the litter size components assessed, no significant differences were found between populations (control-F2 and rederived-F2) or between lactation status at insemination, and the interaction between generation and lactation status of the doe was not significantly different (Table 4).

Fetal Growth During Gestation in F2 Females

No differences were observed in the size of fetal sac, maternal placenta, fetal placenta, and CRL of the fetus on any day of gestation assessed between both populations (Figure 2). Differences in the fetal sac size were observed relative to the lactational status of the doe only at 12 days of gestation, being larger in the non-lactating does. The bodyweight of the liveborn kits was no different between populations and was not affected by reproductive status (66.5 ± 2.43 , data not shown in tables).

DISCUSSION

Growth Traits

The selection objective of paternal line R is the average daily gain at fattening (Estany et al., 1992). ADG has been estimated to increase from 0.45 to 1.23 g/d per generation (Baselga, 2005), having a moderate heritability (0.17) ideal for the improvement. These estimates could be refined using an unselected, divergently selected, or a population rederived by cryopreservation as control. The latter offers a more economical tool and avoids the genetic drift of the population. However, recently, there are more and more studies showing genetic and phenotypic effects in rederivative populations that could question their use as control populations. Our study shows effects on the end of fattening weight (EFW) and average daily gain (ADG), which indicates some effect derived from the cryopreservation and transfer process performed. Both populations were coetaneous, received the same feed, and were placed at the same facilities, avoiding the environmental effect on traits under evaluation. Differences in each trait become more evident when each generation is compared, showing that growth traits are affected in all the generations. Direct or short-term effects of embryo manipulation (production, cryopreservation, and transfer) have been reported in the previous studies (Vicente et al., 2013; Saenz-de-Juano et al., 2014, 2015; Lavara et al., 2015) and by other authors in other animal species (Leme et al., 2020; López-Damián et al., 2020; Cuello et al., 2021), and even humans (Barsky et al., 2016; Beyer

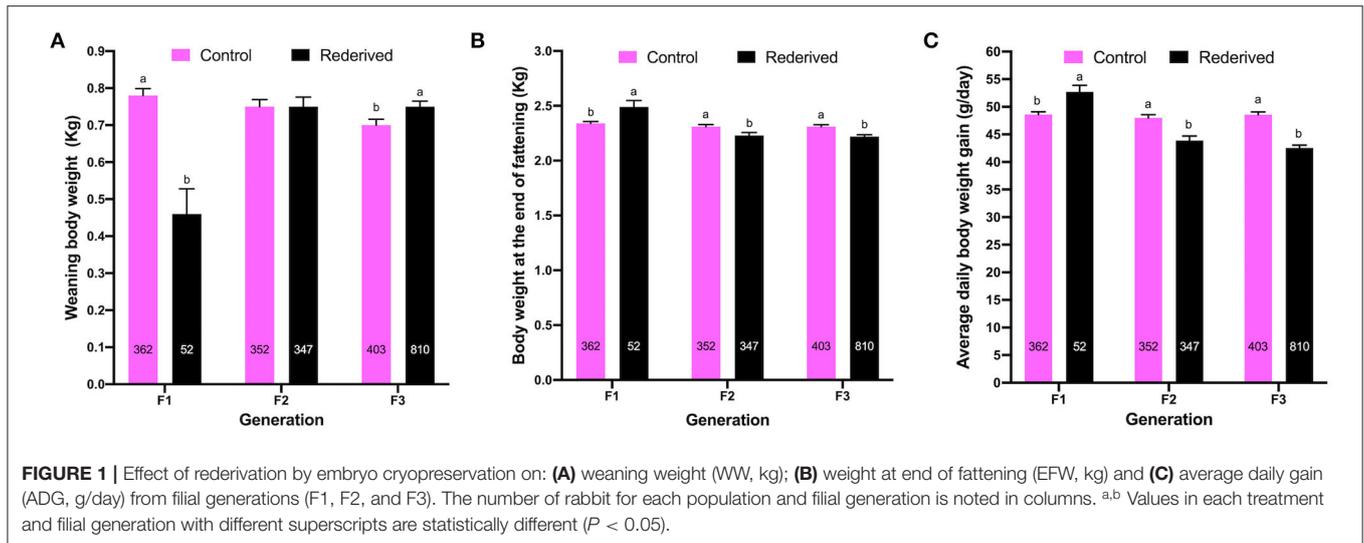


TABLE 3 | Effect of rederivation by embryo cryopreservation on total litter size, liveborn, litter size at weaning, perinatal, and lactation mortality rates of F2 females.

Type	n	Litter size			Mortality rate		KI	
		Total	Liveborn	At weaning	Perinatal	Lactation		
Population	Control-F2	98	6.38 ± 0.37	5.50 ± 0.40	4.00 ± 0.37	15.56 ± 3.28	36.50 ± 4.33	56.41 ± 1.62
	Rederived-F2	172	7.21 ± 0.45	5.86 ± 0.50	3.55 ± 0.46	21.36 ± 4.06	46.55 ± 5.42	58.35 ± 2.01
Lactation status	Nulliparous	115	6.29 ± 0.42	4.84 ± 0.46	2.97 ± 0.43	25.33 ± 3.79	51.39 ± 5.27	
	Primiparous Lactating	50	7.20 ± 0.62	6.34 ± 0.68	4.08 ± 0.62	12.09 ± 5.53	35.35 ± 7.36	46.33 ± 2.41 ^a
	Multiparous lactating	103	6.81 ± 0.47	5.79 ± 0.51	4.16 ± 0.48	17.43 ± 4.20	38.85 ± 5.69	42.08 ± 1.89 ^a
	Non-lactating	75	6.88 ± 0.49	5.74 ± 0.54	3.87 ± 0.50	19.00 ± 4.39	40.51 ± 5.85	83.73 ± 1.88 ^b

n, Number of births. Data are expressed as least squared mean ± standard error of means. KI, Kindling Interval. ^{a,b} Values in the same column and factor with different superscripts are statistically different ($P < 0.05$).

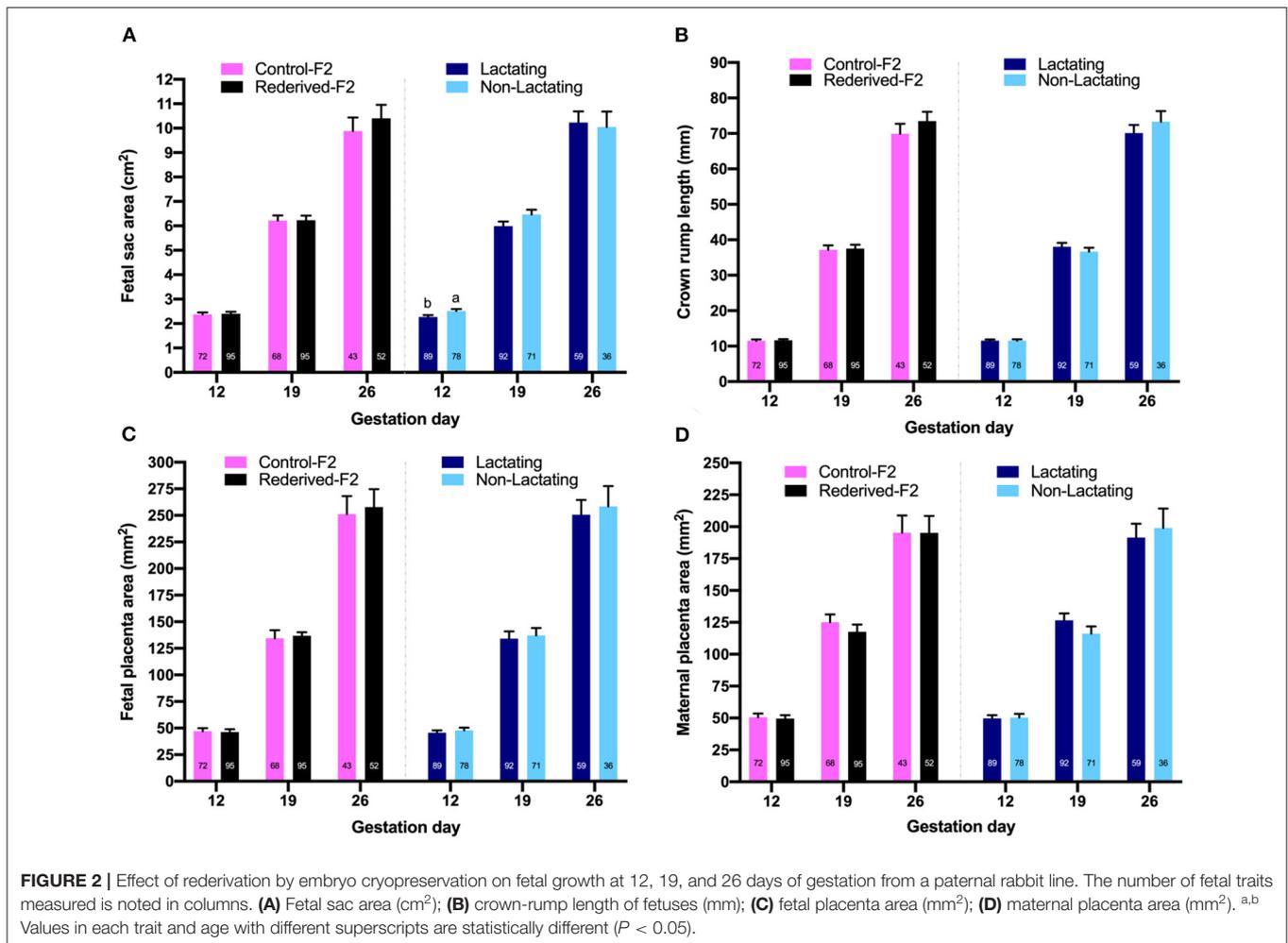
TABLE 4 | Effect of rederivation by embryo cryopreservation on the litter size components of F2 females.

	Ovulation rate ¹	Implanted embryo		Loss rate		Litter size	
		Implanted embryo	Implanted embryo ²	Embryonic ¹	Fetal		
Population	Control-F2	12.11 ± 0.32	6.87 ± 0.57	9.43 ± 0.46	24.01 ± 3.46	41.84 ± 4.84	6.02 ± 0.54
	(n)	80	80	59	59	59	55
	Rederived-F2	12.72 ± 0.40	6.79 ± 0.70	8.83 ± 0.56	32.71 ± 4.10	31.43 ± 5.76	6.70 ± 0.62
	(n)	59	59	45	45	44	44
Lactation status	Non-lactating	13.01 ± 0.39	7.08 ± 0.69	8.89 ± 0.55	31.62 ± 3.98	40.53 ± 5.57	6.07 ± 0.61
	(n)	64	64	51	51	51	48
	Lactating	11.81 ± 0.33	6.58 ± 0.58	8.89 ± 0.49	25.10 ± 3.59	32.74 ± 5.06	6.65 ± 0.55
	(n)	75	75	53	53	52	51

Data are expressed as least squared mean ± standard error of means. n: number of laparoscopies.

¹Determined as the number of corpora lutea.

²Implanted embryos in pregnant does.



and Griesinger, 2016; Chen et al., 2020; Heber and Ptak, 2021), which may be involving epigenetic changes at the embryonic level (Chatterjee et al., 2017) that could cause post-implantation losses, changes in fetal and maternal placental tissues, and higher birth weight in rabbits (Saenz-de-Juano et al., 2014, 2015). Long-term (transgenerational) effects have also been reported in maternal lines (selected for litter size, Lavara et al., 2014; García-Domínguez et al., 2020d) and recently in the paternal lines (selected for growth rate, García-Domínguez et al., 2020c), where growth traits are affected more than the reproductive traits.

Differences observed from the first generation (F1) were significant in the three growth traits assessed (WW, EFW, and ADG). A higher EFW achieved by the rederived population has been previously reported associated with disturbances in the liver and adrenal gland size and lipid and fatty acid metabolism (García-Domínguez et al., 2020b; Marco-Jiménez et al., 2020). Direct effects could be due to the stress to which the embryos are subjected during recovery, vitrification, warming and transfer, in addition to the maternal effects derived from the host rabbits. Thus, in F1 WW was higher for the control population, while for EFW and ADG it was higher for the rederived population. This differentiated and lower growth before weaning could

be related to the metabolism of Zn and Fe, which has been reduced in children born from ART (Xia et al., 2019), and which could influence the lower weight achieved at weaning by the rederived-F1 population, but apparently, these differences are reversed at the end of fattening where this population has the highest results. These disturbances should be taken into account when selecting animals from populations rederived by vitrification to be used as a control group, even in the subsequent generations.

It was demonstrated that cryopreservation and embryo transfer processes in lines selected for daily weight gain cause changes in the reconstituted population in weaning weight that stabilize in the following generations, but the changes in pubertal and adult weight, and liver and heart weight, are maintained, and also in some seminal characteristics and changes in the transcriptome and metabolome profile of the liver of adult rabbits (García-Domínguez et al., 2020e). These findings would indicate that the change caused in the first generation influences the following generations in the characteristics studied (WW, EFW, and ADG), causing higher WW, lower EFW, and therefore lower ADG in the descendants of the rederived population.

Reproductive Traits

Reproductive traits, such as litter size and live births, seem not to be influenced by the effects of cryopreservation either by vitrification or by slow freezing, but by the seasons and the physiological state of the dam (Cifre et al., 1996, 1999). However, Lavara et al. (2014) showed that cryopreservation and embryo transfer increased litter size, live births, and postnatal survival in the cryopreservation-born females (F1) and their female offspring (F2), whereas, in our study, no differences are observed in any of the reproductive traits examined in F2 females; differences are only reported based on the lactation status of does, being higher in non-lactating does. These discordant results could be due to the genotype of the populations studied.

Similarly, fetal sac size was smaller in lactating rabbits than in non-lactating rabbits at 12 days of gestation, but as gestation progresses these differences disappear. As in primiparous rabbits, lactation could be detrimental to receptivity, conception and ovulation rate, embryo and fetal survival, and fetal growth (Fortun-Lamothe and Prunier, 1999), possibly due to the nutritional deficit that occurs during lactation, which induces competition between the mammary glands and the pregnant uterus for the supply of nutrients (Fortun-Lamothe et al., 1999).

The effects of vitrification and embryo transfer processes may not affect the reproductive characteristics evaluated in this line because they showed lower reproductive performance than maternal lines (Baselga, 2002; García and Baselga, 2002b; Vicente et al., 2003, 2013; Naturil-Alfonso et al., 2015).

Embryo cryopreservation in rabbits has been used to estimate genetic gain by rederivation of the frozen populations from previous generations (García and Baselga, 2002a,b; Pascual et al., 2008), either in maternal lines selected for their reproductive traits or in paternal lines selected for their growth traits. It has also been found to be a valuable technique to re-establish populations cryopreserved 15 years ago (Marco-Jiménez et al., 2018). Likewise, the transgenerational effect on some reproductive traits have been noted (Lavara et al., 2014), as has the effect on birth weight, transcriptomic and metabolomic changes, and liver size of offspring of individuals born by these reproductive techniques (García-Dominguez et al., 2020e). However, our work shows for the first time the impact of these biotechnologies on the traits under selection in the paternal lines without detriment or changes in their reproductive traits two generations later, evidencing possible transgenerational epigenetic changes, suggesting that when estimating genetic gain

using former generations cryopreserved years ago, this should be done with a current population subjected to the cryopreservation and embryo transfer processes.

CONCLUSIONS

Embryo vitrification and embryo transfer processes cause changes in growth traits of reconstituted populations that influence the following generations, without changes in female reproductive traits in a paternal line of rabbits. This finding should be taken into account when these populations are used as controls to estimate genetic changes in these characters. A possible solution would be a double control, rederiving the contemporary population with the same procedure.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal study was reviewed and approved by Ethics and Animal Welfare Committee of the Universitat Politècnica de València (Code Number, 2015/VSC/PEA/00061).

AUTHOR CONTRIBUTIONS

FM-J and JV conceptualized, designed, and obtained the financial support for this study. JD, FM-J, and JV participated in data collection and analysis and developed and discussed the manuscript. All authors contributed to the article and approved the submitted version.

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