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Study on the optimal time limit of frozen embryo transfer and the effect of a long-term frozen embryo on pregnancy outcome

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Abstract

In this retrospective study conducted at Sichuan Jinxin Xinan Women and Children's Hospital spanning January 2015 to December 2021, our objective was to investigate the impact of embryo cryopreservation duration on outcomes in frozen embryo transfer. Participants, totaling 47,006 cycles, were classified into 3 groups based on cryopreservation duration: ≤1 year (Group 1), 1 to 6 years (Group 2), and ≥6 years (Group 3). Employing various statistical analyses, including 1-way ANOVA, Kruskal–Wallis test, chi-square test, and a generalized estimating equation model, we rigorously adjusted for confounding factors. Primary outcomes encompassed clinical pregnancy rate and Live Birth Rate (LBR), while secondary outcomes included biochemical pregnancy rate, multiple pregnancy rate, ectopic pregnancy rate, early and late miscarriage rates, preterm birth rate, neonatal birth weight, weeks at birth, and newborn sex. Patient distribution across cryopreservation duration groups was as follows: Group 1 (40,461 cycles), Group 2 (6337 cycles), and Group 3 (208 cycles). Postcontrolling for confounding factors, Group 1 exhibited a decreased likelihood of achieving biochemical pregnancy rate, clinical pregnancy rate, and LBR (OR < 1, aOR < 1, $P < .05$). Furthermore, an elevated incidence of ectopic pregnancy was observed (OR > 1, aOR > 1), notably significant after 6 years of freezing time [aOR = 4.141, 95% confidence intervals (1.013–16.921), $P = .05$]. Cryopreservation exceeding 1 year was associated with an increased risk of early miscarriage and preterm birth (OR > 1, aOR > 1). No statistically significant differences were observed in birth weight or sex between groups. However, male infant birth rates were consistently higher than those of female infants across all groups. In conclusion, favorable pregnancy outcomes align with embryo cryopreservation durations within 1 year, while freezing for more than 1 year may diminish clinical pregnancy and LBRs, concurrently elevating the risk of ectopic pregnancy and preterm birth.

Abbreviations: aOR = adjusted odds ratios, BMI = body mass index, CI = confidence intervals, CPR = clinical pregnancy rate, FET = frozen embryo transfer, FSH = follicle-stimulating hormone, GEE = generalized estimation equations, GnRH-a = gonadotrophin-releasing hormone analogue, HCG = human chorionic gonadotropin, HMG = human menopausal gonadotropin, ICSI = intracytoplasmic sperm injection, IQR = interquartile range, IVF = in vitro fertilization, LBR = live birth rate, OPU = oocyte pick-up, OR = odds ratios.

Keywords: Embryo cryopreservation, embryo cryopreservation duration, frozen embryo transfer, pregnancy outcomes

X-J.W, M-X.C and L-L.R have contributed equally to this work.

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The Ethics Committee of the Sichuan Jinxin Xinan Women and Children's Hospital approved this study, and a waiver of informed consent was granted.

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1. Introduction

Embryo cryopreservation technology has gained widespread adoption in assisted reproduction.[1,2] Extensive clinical studies have consistently demonstrated that frozen embryo transfer (FET) carries fewer risky[3] and yields improved pregnancy outcomes.[4] Embryo cryopreservation offers a viable alternative for patients deemed unsuitable for fresh embryo transfer due to factors like the risk of ovarian hyperstimulation or intimal abnormalities during hyperstimulation during ovulation stimulation.[5,6] Additionally, For patients undergoing chemotherapy for conditions like breast cancer or leukemia, etc. preserving fertility through freezing embryos, eggs, and even ovarian tissue has become an indispensable approach.[7–9]

Although embryo cryopreservation technology is widely utilized, ensuring its safety has remained a significant concern. Currently, embryo cryopreservation is mainly done with open carriers, which means that embryos will be in contact with liquid nitrogen for a long time during the freezing process.[10] Consequently, it becomes crucial to investigate whether long-term exposure to high concentrations of cryoprotectants has adverse effects on embryos. This exploration is essential to address potential concerns associated with the preservation method.

A meta-analysis indicated that the duration of freezing did not have a noticeable impact on embryo viability, implantation, pregnancy, birth, or birth abnormalities.[11] However, some studies suggest that embryo cryopreservation duration for more than 6 years does not significantly affect its recovery and CPRs. But it does result in a reduced LBR, increased miscarriage rate, and negative impact on pregnancy outcomes.[12,13] According to the expert consensus of China in 2018,[14] embryo cryopreservation within 6 years does not affect the embryo survival rate, implantation rate, pregnancy rate, LBR, and offspring birth defect rate after embryo resuscitation. Nevertheless, conclusive evidence regarding the safety of freeze embryo transfer after a freezing period of more than 6 years impacts is still lacking.

More patients may opt for long-term embryo freezing due to changes in people's conceptions of fertility and the discontinuation of family planning in China. However, the impact of the embryo cryopreservation duration on the success of conception remains debatable. Therefore, this study aims to investigate the optimal window for FET and determine whether long-term embryo freezing affects conception through a retrospective study.

2. Materials and methods

2.1. Research on population and design

Patients who underwent FET at Sichuan Jinxin Xinan Women and Children's Hospital between January 2015 and December 2021 were divided into 3 groups based on the embryo cryopreservation duration: Group 1 (≤1 year), Group 2 (1–6 years), and Group 3 (≥6 years). The inclusion Criteria for the study were as follows: patients with frozen embryos and ready to FET; the endometrial preparation regimens were hormone replacement, natural, Gonadotrophin-Releasing Hormone Analogue (GnRH-a) downregulation, and ovulation stimulation cycles. Exclusion criteria: age of egg retrieval >40 years old; egg recipient patients; chromosomal abnormalities in either males or females; history of previous recurrent miscarriage; patients with more than 3 repeated transplant failures; loss to follow-up; incomplete data recording. A total of 47,006 cycles were included in the present study. This study was approved by the Ethics Committee of Sichuan Jinxin Xinan Women and Children's Hospital. All data were deidentified, and the institutional review board granted a waiver of informed consent.

2.2. Ovarian-controlled ovulation induction and embryo freezing strategies

This study employed ovarian-controlled ovulation induction regimens, including GnRH antagonist protocol, long GnRH agonist protocol, microstimulation protocol, etc. When the average diameter of the target follicle was ≥18 mm, trigger administration was done using human chorionic gonadotropin (HCG) or GnRH-a or HCG + GnRH-a. Egg retrieval was performed 34–36 hours after triggering administration. Depending on semen quality, in vitro fertilization or intracytoplasmic sperm injection (ICSI) was performed, with the selection of frozen cleavage and/or blastocyst stage embryos, considering the patient's embryo culture.

2.3. Endometrial preparation protocols and embryo transfer

Various endometrial preparation protocols were employed, including the hormone replacement cycle, natural cycle, GnRH-a downregulation cycle, and ovulation stimulation cycles. The allocation of optimal endometrial preparation protocol was determined based on the clinician's experience and patient characteristics. Natural cycles were typically utilized in patients with regular menstrual cycles. We monitored follicular development and hormonal changes, and cleavage embryos were transferred on the 3rd or blastocysts on the 5th day following ovulation. The hormone replacement cycle is employed for patients with irregular menstrual cycles, as estrogen is administered to stimulate endometrial growth. When the endometrial thickness is ≥8 mm, a progestogen is introduced to facilitate endometrial transformation. Following the endometrial transformation, the cleavage embryo or blastocyst is transferred on the 3rd or 5th day. Stimulation cycles are primarily used for patients who do not ovulate or those in whom the lining in the hormone replacement cycle does not meet the required criteria. In these cases, patients typically take oral tamoxifen or letrozole for 5 days, starting from the 2nd to 5th day of menstruation. If follicle growth is not ideal, human menopausal gonadotropin is added. Once the target follicle reaches a size larger than 18 mm, HCG is administered to induce ovulation. The embryo transfer occurs on either the 3rd or 5th day after ovulation, depending on the type of embryo selected for transplantation. The GnRH-a downregulation protocol is primarily employed in patients with ovarian dysfunction, endometriosis, and adenomyosis. It is common to transfer 1–2 embryos simultaneously during embryo transfer. Single blastocyst transfer is preferred when high-quality cleavage and blastocyst are available. After embryo transfer, luteal support is routinely provided. The main drugs utilized for luteal support include Dydrogesterone tablets (20–30 mg/d, abbott biologicals b.v. netherlands), progesterone vaginal sustained release gel (90 mg/d, Fleet Laboratories Ltd, Watford), progesterone softgel (0.2 g, tid Cyndea Pharma, S.L. Soria), and progesterone injection (60 mg/d, Zhejiang Xianju Pharmaceutical Co., Ltd., Zhejiang), etc.

2.4. Outcome measurements

The primary outcomes examined were clinical pregnancy rate (CPR) and live birth rate (LBR). Secondary outcomes included the biochemical pregnancy rate, multiple pregnancy rate, ectopic pregnancy rate, early miscarriage rate, late miscarriage rate, preterm birth rate, neonatal birth weight, weeks at birth, and newborn sex.

Clinical pregnancy was defined as the presence of an ultrasonically visible gestational sac, including ectopic pregnancy. A successful birth after 28 weeks of gestation was classified as a live birth. Biochemical pregnancy was identified by elevated blood HCG approximately 14 days after embryo transfer.

Ectopic pregnancy is diagnosed through ultrasound or laparoscopic observation of at least 1 ectopic pregnancy sac. Early miscarriage refers to pregnancy loss within 12 weeks of gestation, while late miscarriage denotes loss between 12 and 28 weeks.

2.5. Statistical analysis

Continuous variables conforming to normal distributions were analyzed using 1-way ANOVA and expressed as mean ± standard deviation; nonnormally distributed continuous variables were analyzed using the Kruskal–Wallis *t* test, and their values were reported as the median and interquartile range (median [interquartile range, IQR]); for multiple comparisons, the Bonferroni multiple comparison method was used; categorical variables were presented as the number of cases and percentages, and the chi-square test was used for group comparisons.

Given the baseline characteristics differences among groups, generalized estimation equations were employed to investigate the association between embryo freezing time and pregnancy outcome. Odds ratios (OR) and their corresponding 95% confidence intervals (CI) were calculated. Additionally, adjusted odds ratios (aOR) were calculated to account for potential confounding factors, namely FET age, oocyte pick-up (OPU) age, body mass index, basal follicle-stimulating hormone (FSH), basal estradiol, basal progesterone, intimal thickness, duration of infertility, infertility type, infertility factors, FET preparation protocol, number of embryos transferred, embryo type, and other confounding factors. To analyze the relationship between embryo cryopreservation duration and pregnancy and neonatal outcomes. Statistical significance was determined by evaluating the statistically significant difference between the 2 sides of $P < .05$. All statistical analyses were performed using SPSS (version 25.0, IBM US).

3. Results

In this retrospective study, we ultimately included 47,006 cycles for analysis. Patients were grouped according to embryo cryopreservation duration: Group 1 (≤1 year) consisted of 40,461 cycles, Group 2 (1–6 years) included 6337 cycles, and Group 3 (≥6 years) comprised 208 cycles.

There were some statistical differences in the baseline characteristics of the study participants. Patients in Groups 2 and 3 had higher maternal age during FET than those in Group 1. Group 3 had the longest duration of infertility (4 years) despite being the youngest at the time of egg retrieval. This could be attributed to the possibility that individuals younger at egg retrieval are more inclined to pursue a second child, leading to a relatively longer duration of embryo frozen.

Secondary infertility was more prevalent than primary infertility in all groups, with tubal factors being the leading cause of infertility. The endometrial thickness at embryo resuscitation transplantation was 9.60 ± 1.87 vs 9.27 ± 2.02 vs 9.92 ± 1.76, and Group 2 had a slightly thinner endometrial lining compared to Groups 1 and 3. The hormone replacement cycle is the primary FET endometrial preparation plan. Due to the lack of coverage by medical insurance and the traditional concept in China, most patients preferred to transfer 2 embryos to increase the success rate.

In Group 2, both the proportion of 2 embryos transferred and the blastocyst transfer rate were significantly lower compared to Groups 1 and 3. Furthermore, the rate of high-quality embryos transferred in Group 1 surpassed that in Groups 2 and 3. The baseline characteristics of the patient are shown in Table 1.

The primary outcome measures revealed statistically significant differences among the groups. The CPR was (59.95% vs 52.60% vs 55.77%, $P < .001$), and the LBR was (49.29% vs 42.05% vs 43.75%, $P < .001$). The secondary outcomes also exhibited significant differences in biochemical pregnancy rate, multiple pregnancy rate, early miscarriage rate, and late miscarriage rate ($P < .05$). However, the groups had no significant difference in the ectopic pregnancy rate.

Multiple comparisons revealed significant findings in the CPR between Groups 1 and 2 (59.95% vs 52.60%), indicating a statistically significant difference. However, no statistically significant differences were observed between Groups 1 and 3 (59.95% vs 55.77%) or between Groups 2 and 3 (52.60% vs 55.77%).

Regarding comparing LBRs between groups, the results suggest a statistically significant difference between Groups 1 and 2 (49.29% vs 42.05%). However, no statistically significant differences were found between Groups 1 and 3 (49.29% vs 43.75%) or between Groups 2 and 3 (42.05% vs 43.75%). Multiple comparisons for secondary outcomes indicate that the data outcomes of biochemical pregnancy rate, early miscarriage rates, and late miscarriage rate were similar to the primary outcome. However, no statistically significant differences were observed in ectopic and multiple pregnancy rates (Table 2).

When the embryo cryopreservation duration was longer than 1 year, the incidence of preterm birth was statistically increased (16.19% vs 18.57% vs 19.72%, $P < .05$). The multiple comparisons of the preterm birth rate showed a statistically significant difference between Groups 1 and 2, but no statistical difference was observed between Group 3 and Group 1 and Group 2. The sex ratio at birth and birth weight in the 3 groups were similar, with no significant differences. However, the birth rate of male infants was higher than that of female infants in all groups (Table 3).

After adjusting for influential factors (FET age, OPU age, body mass index, basal follicle-stimulating hormone, basal estradiol, basal progesterone, intimal thickness, duration of infertility, infertility type, infertility factors, FET preparation protocol, number of embryos transferred, embryo type), the results consistently demonstrated that a freezing embryo duration exceeding 1 year had a detrimental impact on pregnancy outcomes. After accounting for confounding variables, both Group 2 [aOR = 0.791, 95% CI (0.736, 0.850), $P < .001$] and Group 3 [aOR = 0.624, 95% CI (0.445, 0.875), $P = .006$] were found to have lower chances of achieving clinical pregnancy compared to Group 1. These differences were statistically significant. Furthermore, when compared to Group 1, both Group 2 [aOR = 0.801, 95% CI (0.745, 0.861), $P < .001$] and Group 3 [aOR = 0.615, 95% CI (0.437, 0.866), $P = .005$] were also less likely to achieve a live birth, with the differences being statistically significant.

Similarly, when the embryo freezing time was >6 years, the probability of ectopic pregnancy increased significantly [aOR = 4.141, 95% CI (1.013, 16.921), $P = .048$]. Additionally, longer embryo freezing time (>1 year) was associated with a lower incidence of multiple pregnancies and full-term deliveries and a higher risk of early miscarriage and preterm birth. Notably, Group 2 had the highest risk of late miscarriage [aOR = 1.423, 95% CI (1.059, 1.910), $P = .019$] (Table 4).

Table 5 shows the neonatal outcomes for the 3 groups before and after adjusting for confounding factors. No significant difference in birth weight was observed between Group 1, serving as the reference group, and the remaining experimental groups.

However, the gestational weeks at birth were shorter in Groups 2 and 3. It is worth noting that the difference between Group 2 and Group 1 was statistically significant ($P < .05$) (Table 5).

4. Discussion

Our study analyzed 47,006 FET cycles and identified a strong association between embryo cryopreservation duration, pregnancy outcomes, and neonatal outcomes. Prolonged freezing time beyond 1 year significantly reduced biochemical pregnancy

Table 1

Baseline characteristics of frozen embryo resuscitation transfer cycles.

	Group 1 (≤1 year)	Group 2 (1 < group < 6 years)	Group 3 (≥6 years)	*P* value
Number of cycles, *n*	40,461	6337	208	NA
Age (FET), mean (SD), y	31.29 ± 4.10	32.71 ± 3.83	34.88 ± 3.04	<.001
Age (OPU), mean (SD), y	30.78 ± 4.11	30.44 ± 3.92	27.97 ± 2.95	<.001
BMI, mean (SD)	21.89 ± 3.07	21.86 ± 2.99	21.24 ± 2.63	.002
FSH, mean (SD)	7.73 ± 3.32	7.40 ± 3.40	6.20 ± 2.21	<.001
P median (IQR)	0.62 (0.42,0.91)	0.63 (0.43,0.94)	0.64 (0.40,0.95)	.01
E2, median (IQR)	46 (34,61)	47 (35,63)	52 (39,67)	<.001
LH, median (IQR)	4.25 (3.11,5.96)	4.25 (3.08,5.98)	4.51 (3.25,6.67)	.24
FET Endometrial thickness, mean (SD)	9.60 ± 1.87	9.27 ± 2.02	9.92 ± 1.76	<.001
Duration of infertility, median (IQR), y	3 (2,5)	3 (2,5)	4 (2,5)	<.001
Infertility type, *n* (%)				
Primary	20,080 (49.63)	3035 (47.89)	95 (45.67)	.02
Secondary	20,381 (50.37)	3302 (52.11)	113 (54.33)	
Infertility factors, *n* (%)				
Tubal factors	31,427 (77.67)	4146 (65.43)	134 (64.42)	<.001
Ovulation disorders	431 (1.07)	94 (1.48)	0	
The male factor	5662 (13.99)	1260 (19.88)	48 (23.08)	
Others	2941 (7.27)	837 (13.21)	26 (12.50)	
FET scheme, *n* (%)				
Hormone replacement	26,565(65.66)	3697 (58.34)	139 (66.83)	<.001
GnRH-a downregulation	3716 (9.18)	704 (11.11)	10 (4.81)	
Stimulation	5191 (12.83)	1057 (16.68)	26 (12.50)	
Natural	4989 (12.33)	879 (13.87)	33 (15.87)	
Number of embryos transferred, *n* (%)				
1	12,433 (30.73)	2152 (33.96)	56 (26.92)	<.001
2	28,028 (69.27)	4185 (66.04)	152 (73.08)	
Type of embryo transfer, *n* (%)				
Cleavage embryo	10,612 (26.23)	2185 (34.48)	9 (4.33)	<.001
Blastocyst	29,849 (73.77)	4152 (65.52)	199 (95.67)	
Number of high-quality embryo transfers, *n* (%)				
0	13,171 (32.55)	2356 (37.18)	139 (66.83)	<.001
1	13,296 (32.86)	2211 (34.89)	51 (24.52)	
2	13,994 (34.59)	1770 (27.93)	18 (8.65)	

IQR = Interquartile range, NA = Not applicable, Opu = Oocyte Pick-Up.

Table 2

Pregnancy outcomes.

	Group 1 (≤1 year)	Group 2 (1 < group < 6 years)	Group 3 (≥6 years)	*P* value
BPR, *n* (%)	27,640 (68.31)[a]	3862 (60.94)[b]	129 (62.02)[a,b]	<.001
CPR, *n* (%)	24,256 (59.95)[a]	3333 (52.60)[b]	116 (55.77)[a,b]	<.001
LBR, *n* (%)	19,943 (49.29)[a]	2665 (42.05)[b]	91 (43.75)[a,b]	<.001
EPR, *n* (%)	394 (1.62)[a]	56 (1.68)[a]	3 (2.59)[a]	.70
MPR, *n* (%)	7747 (31.94)[a]	885 (26.55)[a]	37 (31.90)[a]	<.001
EMR, *n* (%)	3188 (13.14)[a]	490 (14.70)[b]	19 (16.38)[a,b]	.03
LMR, *n* (%)	516 (2.13)[a]	101 (3.03)[b]	2 (1.72)[a,b]	.004

The Chi-square test is used for between group comparison, where "a" and "b" denote the outcomes of pair-by-pair comparisons after adjusting the *P* value. The abbreviations used are as follows: BPR = Biochemical pregnancy rate, CPR = Clinical pregnancy rate, EMR = Early miscarriage rates, EPR = Ectopic pregnancy rate, LBR = Live birth rate, LMR = Late miscarriage rate, MPR = Multiple pregnancy rate.

rate, CPR, and LBR. Additionally, there was a considerable increase in early miscarriage and preterm birth rates with prolonged freezing. Notably, when embryo cryopreservation duration exceeded 6 years, the risk of ectopic pregnancy was approximately 4 times higher than in periods under 1 year (aOR 4.141, 95% CI 1.013, 16.921 $P < .05$). In conclusion, FET within 1 year after cryopreservation may lead to better pregnancy outcomes.

In assisted reproduction, the embryo cryopreservation duration has been a topic of concern. While some studies have reported successful pregnancies and healthy deliveries after long-term cryopreservation,[15–19] these anecdotal reports lack sufficient evidence on the impact of prolonged cryopreservation duration. Theoretically, freezing embryos at −196°C in liquid nitrogen inhibits enzyme activity and cell metabolism, allowing embryos to be frozen for a long time. In our research, all embryos are frozen by vitrification, ensuring consistent freezing techniques and a higher survival rate than slow freezing.[20,21] However, vitrification, characterized by high cryoprotectant concentrations and ultra-rapid cooling, can stress gametes, embryos, and histiocytes, potentially causing adverse effects.[22] Recent research on mouse embryos suggests that slow freezing and vitrification can impact mitochondrial distribution, activate apoptosis, impair embryonic development potential, and alter epigenetic markers.[23] Similar harm may occur in human embryos.[24]

Antioxidants may help mitigate cell damage caused by freezing,[25,26] but the mechanisms underlying molecular damage after embryo freezing are not extensively studied. Our study demonstrates a decrease in clinical pregnancy and LBRs when the embryo freezing time exceeds 1 year. It is plausible that these changes are associated with molecular alterations occurring during embryo freezing. However, the mechanisms underlying molecular damage after embryo freezing have not been extensively studied.

Consequently, paying attention to these potential damage mechanisms is crucial, particularly regarding whether the damage to embryos intensifies with prolonged freezing time. Additionally, age is an essential factor affecting fertility.[27] The American College of Obstetricians and Gynecologists acknowledged that women's fertility gradually declines after age 32.[28] In this study, patients with longer cryopreserved embryos were older at resuscitation transfer, possibly contributing to the adverse impact on pregnancy outcomes.

Table 3

Neonatal outcomes.

	Group 1 (≤1year)	Group 2 (1 < group < 6 year)	Group 3 (≥6 years)	*P* value
PBR, *n* (%)	2282 (16.19)[a]	378 (18.57)[b]	14 (19.72)[a,b]	.02
Sex ratio, *n* (%)				
Male	7827 (55.51)[a]	1104 (54.22)[a]	38 (53.52)[a]	.52
Female	6272 (44.49)[a]	932 (45.78)[a]	33 (46.48)[a]	
BW, mean(SD), g	3251.18 ± 511.81[a]	3238.66 ± 529.43[a]	3296.76 ± 515.29[a]	.45
GW, mean(SD), wk	36.09 ± 1.85[a]	35.86 ± 1.92[b]	35.71 ± 1.38[a,b]	<.001

One-way ANOVA was used for continuous variables conforming to a normal distribution, expressed as mean ± standard deviation. The Bonferroni method facilitated pair-by-pair comparisons between groups. For categorical variables, the number of cases (expressed as a percentage) underwent between group comparisons using chi-square tests, and chi-square splitting was utilized for 2-by-component comparisons. The results of pair-by-pair comparisons between groups after *P* value adjustment are denoted as "a" and "b." The abbreviations used are as follows: BW = Birth weight, GW = Gestational week, PBR = Preterm birth rate.

Table 4

Analysis of pregnancy outcomes before and after adjusting for confounders.

	OR (95%CI)	*P* value	aOR (95%CI)	*P* value
BPR				
≤1 year	Ref	–	Ref	N.A.
1 < group < 6 years	0.712 (0.674,0.753)	<.001	0.796 (0.739,0.858)	<.001
≥6 years	0.757 (0.563,1.018)	.07	0.586 (0.412,0.836)	.003
CPR				
≤1 year	Ref	–	Ref	N.A.
1 < group < 6years	0.723 (0.685,0.763)	<.001	0.791 (0.736,0.850)	<.001
≥6 years	0.832 (0.625,1.108)	.21	0.624 (0.445,0.875)	.006
LBR				
≤1 year	Ref	–	Ref	N.A.
1 < group < 6 years	0.732 (0.693,0.772)	<.001	0.801 (0.745,0.861)	<.001
≥6 years	0.794 (0.596,1.057)	.11	0.615 (0.437,0.866)	.005
EPR				
≤1 year	Ref	–	Ref	N.A.
1 < group < 6 years	1.061 (0.797,1.412)	.69	1.149 (0.810,1.629)	.44
≥6 years	1.693 (0.535,5.352)	.37	4.141 (1.013,16.921)	.05
MPR				
≤1year	Ref	–	Ref	N.A.
1 < group < 6 years	0.767 (0.707,0.832)	<.001	0.770 (0.693,0.856)	<.001
≥ 6 years	0.993 (0.672,1.467)	.97	0.895 (0.573,1.400)	.63
EMR				
≤1 year	Ref	–	Ref	N.A.
1 < group < 6 years	1.172 (1.057,1.300)	.003	1.143 (0.996,1.311)	.06
≥6 years	1.331 (0.816,2.172)	.25	1.583 (0.875,2.865)	.13
LMR				
≤1 year	Ref	–	Ref	N.A.
1 < group < 6 year	1.439 (1.159,1.787)	.001	1.423 (1.059,1.910)	.02
≥6 years	0.810 (0.199,3.288)	.77	0.771 (0.153,3.882)	.75
Preterm birth rate				
≤1 year	Ref	–	Ref	N.A.
1 < group < 6 years	1.182 (1.048,1.334)	.006	1.203 (1.089,1.329)	<.001
≥6 years	1.273 (0.708,2.289)	.42	1.389 (0.880,2.192)	.16
Full-term birth rate				
≤ 1 year	Ref	–	Ref	N.A.
1 < group < 6 years	0.837 (0.744,0.942)	.003	0.738 (0.625,0.871)	<.001
≥ 6 years	0.833 (0.464,1.498)	.54	0.712 (0.323,1.572)	.40

The abbreviations used are as follows: BPR = Biochemical pregnancy rate, CPR = Clinical pregnancy rate, EMR = Early miscarriage rates, EPR = Ectopic pregnancy rate, LBR = Live birth rate, LMR = Late miscarriage rate, MPR = Multiple pregnancy rate. Adjustment factors: FET age, OPUage, BMI, basal FSH, basal estradiol, basal progesterone, intimal thickness, duration of infertility, infertility type, infertility factors, FET preparation protocol, number of embryos transferred, embryo type; NA: Not applicable.

The embryo cryopreservation duration and its impact on pregnancy outcomes remain debated. Some studies[13,29–31] suggest that the embryo cryopreservation duration does not significantly affect pregnancy outcomes, while others[32,33] indicate decreased survival and pregnancy rates with prolonged vitrification freezing. Our study shows that embryo cryopreservation duration for over 1 year specifically impairs pregnancy outcomes, consistent with the negative effects of extended freezing periods found in other studies.

Furthermore, some researchers[34,35] and a meta-analysis have suggested[36] that immediate FET in the subsequent menstrual cycle following whole embryo freezing (i.e., immediate transfer) yields higher clinical pregnancy and LBRs than delayed FET. This indirectly supports the notion that shorter periods of embryo cryopreservation are associated with improved pregnancy outcomes. Therefore, proceeding with FET as early as possible is advisable unless patients require long-term fertility preservation, which may lead to better pregnancy outcomes.

The health of the offspring born through assisted reproductive techniques has raised significant concern. Some studies showed an increased risk of preeclampsia and a higher birth weight among children conceived through FET.[37,38] Additionally,

Table 5

Results of neonatal outcome analysis before and after adjusting for confounders.

	β	Standard error	*P*	β	Standard error	Adjusted *P*
Birth weight, g						
≤1 year	Ref	–	N.A.	Ref	–	NA
1 < group < 6 years	−12.057	12.525	.34	−116.534	110.51	.29
≥6 years	45.474	60.876	.46	−448.695	438.58	.31
Gestational age, wk						
≤1 year	Ref	–	N.A.	Ref	–	NA
1 < group < 6 years	−0.227	0.045	<.001	−0.188	0.06	.002
≥6 years	−0.376	0.164	.02	−0.207	0.209	.32

Adjustment factors: the female age, egg retrieval age, BMI, basal FSH, basal estradiol, basal progesterone, intimal thickness, duration of infertility, infertility type, infertility factors, FET preparation protocol, number of embryos transferred, embryo type; NA: Not applicable.

reports suggest an elevated risk of childhood cancer compared to natural conception.[39] Another study indicated that delayed FET could increase the risk of macrosomia.[40] However, it has also been published[41] that long-term cryopreservation does not affect the risk of low birth weight, macrosomia, small-for-gestational-age, or large-for-gestational-age infants in singleton or multiple births. The embryo cryopreservation duration was deemed to have no apparent effect on newborn birth weight in this study. However, the preterm birth rate also increased as the cryopreservation duration increased. There was no statistically significant difference in the proportion of male and female births, but the birth rate of male infants was higher than that of female infants across all groups. This disparity could be attributed to the higher number of blastocysts transferred in each group than the number of cleavage embryos in this study. Some studies have reported[42,43] that male embryos tend to develop more rapidly in vitro, reaching the blastocyst stage more quickly than female embryos.[44] Consequently, male embryos may appear more robust in the blastocyst stage, making them more likely to be classified as high-quality embryos and prioritized for transfer.

In conclusion, this large retrospective study provides evidence that embryo cryopreservation duration for more than 1 year has a negative effect on pregnancy outcomes. Combining these findings with a literature review, immediate FET following embryo freezing may achieve better pregnancy outcomes and reduce the risk of neonatal birth. Furthermore, ongoing studies investigate the possibility of preserving embryos and germ cells at ambient temperatures.[45] This potential advancement could revolutionize the maintenance and management of valuable biological materials. However, there is still much to explore in this field. Achieving long-term preservation of embryos with minimum harm may become a reality, benefiting a broader range of patients needing fertility preservation.

5. Conclusions

In conclusion, this large retrospective study proves that embryo cryopreservation followed by FET within 1 year improves pregnancy outcomes. However, embryo cryopreservation duration exceeding 1 year decreases clinical pregnancy and LBRs and increases the risk of ectopic pregnancy and preterm birth.

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