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IVF-conceived children outcome from birth to 3 years at a single IVF center in Indonesia: frozen versus fresh embryo transfer

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Abstract

Background: Frozen embryo transfer procedure is becoming more common in assisted reproduction technology. The effect of this technology is still not yet well studied in developing countries with limited resources, including Indonesia. This study explores the clinical outcomes between frozen and fresh embryo transfer-conceived children aged 0–3 years in Indonesia.

Results: The participants were divided into frozen embryo ($n = 30$) and fresh embryo ($n = 30$) transfer groups. There were no differences in growth and development. However, in vitro fertilization (IVF) children with frozen embryo transfer had a lower risk of developing low birth weight compared to the fresh embryo group with an OR of 0.17 (95% *CI*: 0.03–0.85). All children, both in the fresh and frozen embryos, have normal development.

Conclusions: Frozen versus fresh embryo transfer does not affect child growth and development.

Keywords: In vitro fertilization, Assisted reproductive techniques, Embryo transfer, Birth weight, Child growth, Child development

Background

Cryopreservation of sperm and embryos is an essential and routine thing to do in assisted reproductive technology. Pelkonen et al. have reported that embryo freezing does not affect perinatal outcomes [1]. A study by Wang et al. also reported an improved live birth rate and reduced rate of miscarriage in the frozen embryo transfer group [2]. However, these reports can not be extrapolated to Indonesia due to the difference in patients' race and the healthcare facility and quality. Moreover, mishandling of the cryopreservation procedure may pose several risks to the biological specimens, including cross-contamination of nitrogen, cross-infection between specimens, and reduced viability after thawing [3].

As the use of assisted reproductive technology continues to grow in Indonesia, its impact on the conceived offspring is still being debated. Furthermore, with the increased use of cryopreservation technology, it is important to understand the effect of this technology and its implementation in our country's setting. Therefore, this study explores the clinical outcomes between frozen and fresh embryo transfer-conceived children aged 0–3 years in Indonesia.

Materials and methods

Population and sample

We conducted a comparative analytical study that uses a cross-sectional research design. The single-center study was conducted from October 2018 until October 2019 in the Pediatric Clinic, RSCM. In this study, the target population was all pediatric patients conceived through in vitro fertilization (IVF) aged 0–3 years in Indonesia. The sample used in this study was pediatric patients conceived through IVF between 2016 and 2019 at the Yasmin Clinic, Cipto Mangunkusumo National Hospital (RSCM).

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The recruitment of participants in this study was carried out using consecutive sampling; all participants who came and met the selection criteria were included in the study until the required number of participants was met. Sample size calculation used two-sample parallel design formula, with a confidence level of 95% and a power of 80% [4]. The participants were divided into two groups: those who underwent fresh embryo transfer and those who underwent frozen embryo transfer (FET) in the other group. All fresh embryo transfers in our center use the intracytoplasmic sperm injection (ICSI) method. The inclusion criteria used in this study are pediatrics patients conceived through the IVF program at the Yasmin Clinic, RSCM Kencana, between 2016 and 2019. The participants were excluded from the study if the parents did not consent to participate in the study.

Potential participants were screened and listed from medical records data. Then, each potential participant's parents were invited to come to the Pediatrics Clinic in RSCM with their IVF-conceived children. The children who attended the invitation underwent development, growth, and physical examination. Examinations started at 1-month old, repeated once a month for 0–1-year old, once every 3 months for 1–2 years old, and once every 6 months for 2–3 years old.

Data analysis

Perinatal data, including gestational age at birth and birth weight, were taken from the medical records. Gestational age was categorized as term ($\geq$ 37 weeks) and preterm (< 37 weeks). Birth weight was categorized as low birth weight (< 2500 g) and normal ($\geq$ 2600 g). The WHO Child Growth Standards 2006 was used for growth assessment. Nutritional status was measured from weight for age, categorized as normal (standard deviation [SD] 0 to −1), underweight (SD < −2), and overweight (SD > +1). Stature was measured from height for age, categorized as normal (SD 0 to −1) and short stature (SD < −2) [5]. The Indonesian version of the Pre-Screening Developmental Questionnaire (Kuesioner Pra Skrining Perkembangan, KPSP) was used for development assessment. Development was categorized as appropriate, doubting, and deviation [6].

After the data was collected, verification, editing, and coding were carried out. The unpaired *t*-test or the appropriate nonparametric tests were used to analyze variables with numerical data output. Variables with categorical data output were analyzed using chi-square or Fisher exact test. All data analyses were done using SPSS version 20.0 (IBM, Armonk, NY, USA).

Result

A total of 1942 IVF cycles were conducted over 3 years (June 2016 to May 2019) at the Yasmin Clinic, RSCM, with 495 frozen embryo transfers (25.5%). Our center's biochemical, clinical, and ongoing pregnancy rates over the study period were 48.8%, 28.2%, and 13.8%, respectively. A total of 60 children from 42 parents, aged between 3 months to 36 months, were recruited in this study.

The mean age of fathers in both groups was 36 years old. Both in the fresh embryo group and the frozen embryo group, the largest proportion of the age group is 35–39 years. There was no statistically significant difference in the ratio of paternal age between the two groups ($p = 0.87$).

The mean age of mothers in the fresh embryo group was 35 years, while the frozen embryo group was 34 years. In both groups, the largest proportion of the age group is 35–39 years age group. There was no statistically significant difference in the ratio of maternal age between the two groups ($p = 0.76$). Characteristics of research participants are listed in Table 1.

The median duration of infertility in the fresh embryo group was 5 years. Meanwhile, the median length of infertility in the frozen embryo group was 4 years. However, the difference in infertility duration was not statistically significant ($p = 0.17$).

Based on the cause of infertility, more than half of infertility cases in the fresh and frozen embryo transfer groups occurred due to female factors. There was no statistically significant difference in the proportion of diagnoses causing infertility between the two groups ($p = 0.61$). Most of the embryo freezing was due to PGT (46.7%), followed by endometrial factor (30%), and ovarian hyperstimulation syndrome (OHSS) occurrence (23.3%).

All mothers in the fresh embryo group gave exclusive breastfeeding. Whereas in the frozen embryo group, 13.3% of mothers did not give exclusive breastfeeding. However, there was no statistically significant difference in the proportion of exclusive breastfeeding between the two groups ($p = 0.11$).

The median gestational age in the fresh and frozen embryos was 38 weeks, with the earliest birth occurring at 34 weeks and the latest at 39 weeks. In both study groups, 16.7% of participants experienced preterm labor. Three mothers had iatrogenic preterm labor, two (3.3%) due to preeclampsia with severe features and one (1.7%) due to fetal growth restriction. The other seven (11.7%) had spontaneous preterm labor due to multifetal gestation. There was no statistically significant difference in the proportion of preterm births between the two groups ($p = 1.00$).

Table 1 Participants' characteristics

No.	Patient characteristic	Frozen embryo transfer $n = 30$	Fresh embryo transfer $n = 30$	*p*-value
1	Father's age (years)*	36 (4)	36 (3)	0.87[1]
2	Mother's age (years)*	35 (3)	34 (3)	0.76[1]
3	Father's education (*n* [%])			
	Bachelor's degree	30 (100%)	23 (76.7%)	0.01[2]
	Master's degree	0 (0%)	7 (23.3%)	
4	Mother's education (*n* [%])			
	Bachelor's degree	29 (96.7%)	28 (93.3%)	1.00[2]
	Master's degree	1 (3.3%)	2 (6.7%)	
5	Duration of infertility (*n* [%])			
	1–2 years	3 (10%)	3 (10%)	0.95[2]
	3–4 years	10 (33.3%)	14 (46.7%)	
	2003≥ 5 years	17 (56.7%)	13 (43.3%)	
6	Infertility factor (*n* [%])			
	Female factor	15 (50%)	17 (56.7%)	0.61[2]
	Male factor	7 (23.3%)	4 (13.3%)	
	Both	8 (26.7%)	9 (30%)	
7	IVF indication^ (*n*)			
	Male factor	13	13	
	PCOS	6	9	
	Endometrial factor	6	7	
	Tubal factor	4	3	
	Diminished ovarian reserve	3	2	
	HIV/AIDS	1	0	
8	Freezing indication (*n*)			
	OHSS	7 (23.3%)		
	PGT	14 (46.7%)		
	Endometrial factor	9 (30%)		
9	Single/multifetal pregnancy (*n*)			
	Single pregnancy	23	19	
	Twin pregnancy	4	11	
	Triplet pregnancy	3	0	
10	Exclusive breastfeeding (*n* [%])			
	Yes	30 (100%)	26 (86.7%)	0.11[2]
	No	0 (0%)	4 (13.3%)	

[1] Mann-Whitney test

[2] Chi-square test

*Data presented as mean (SD). ^Some patients are siblings. Individual parents can have more than 1 condition

Low birth weight (LBW) cases were found to be higher in the fresh embryo transfer group (30%) compared to the frozen embryo group (6.7%). Statistical analysis showed the difference was significant (*OR* 0.17, 95% *CI*: 0.03–0.85; $p = 0.02$) (Table 2).

However, we also found 36.7% of participants in the fresh embryo group were multiple pregnancy, compared to 23.3% in the frozen embryo group. Multiple pregnancies could be a confounding factor that affects birth weight. Therefore, it is necessary to analyze the stratification of the relationship between LBW and embryo transfer types in IVF based on the type of pregnancy that occurred (Table 3).

From our analysis for both strata of single pregnancy and multiple pregnancy, there was no statistically significant correlation between LBW and embryo types ($p = 0.20$ and 0.33 for single and multiple pregnancy, respectively) (Table 3).

As many as 10% ($n = 3$) of the fresh embryo group children and 6.7% ($n = 2$) of the frozen embryo group were overweight. None of our participants were assessed as underweight. There was no significant relationship

between the type of embryo and the nutritional status of children ($p = 1.00$). As many as 16.7% ($n = 5$) of the fresh embryo group children and 6.7% ($n = 2$) of the frozen embryo group had short stature. There was no relationship between the types of embryos and the stature of children ($p = 0.42$). All children in both groups have normal development (Table 4).

Discussion

Based on the level of education, both groups have a minimum educational background of bachelor's degree students. According to previous studies, higher parents' education levels are associated with nutritional status in children. In a study conducted by Vollmer et al. (2016), the prevalence of children with underweight nutritional status was 23.65% in groups which both parents did not have or not completed primary education, whereas in those whose both parents completed secondary education or above, the prevalence of underweight was only 16.76% [7].

All mothers in the frozen embryo group gave exclusive breastfeeding. Whereas in the fresh embryo group, 13.3% of mothers did not give exclusive breastfeeding. However, the difference in the proportion of exclusive breastfeeding between the two groups was not statistically significant ($p = 0.11$). Breast milk contains 3 to 5% fat, 0.8 to

Table 2 Perinatal outcome

Birth outcome	Fresh embryo $n = 30$	Frozen embryo $n = 30$	*p*-value	*OR* (95% *CI*)
Preterm (*n* [%])	5 (16.7%)	5 (16.7%)	1.00[2]	1 (0.26–3.89)
Term (*n* [%])	25 (83.3%)	25 (83.3%)		
Low birth weight (*n* [%])	9 (30%)	2 (6.7%)	0.02[2]	0.17 (0.03–0.85)
Normal (*n* [%])	21 (70%)	28 (93.3%)		

[2] Chi-square tests

Table 3 Stratification analysis of relationship between LBW and embryo transfer types based on pregnancy

Birth weight	Fresh embryo	Frozen embryo	*p*-value	*OR* (95% *CI*)
Single pregnancy	$n = 19$	$n = 23$		
LBW	2 (10.5%)	0 (0%)	0.20[1]	Cannot be calculated[2]
Normal birth weight	17 (89.5%)	23 (100%)		
Multiple pregnancy	$n = 11$	$n = 7$		
LBW	7 (63.6%)	2 (28.6%)	0.33[1]	0.23 (0.03–1.77)
Normal birth weight	4 (36.4%)	5 (71.4%)		

LBW low birth weight

[1] Chi-square test

[2] Cannot be assessed because there are cells that have zero values

Table 4 Growth and development of children at the age of 0 to 3 years

Variable	Fresh embryo	Frozen embryo	*p*-value	*OR* (95% *CI*)
Nutritional status				
Normal	27 (90%)	28 (93.3%)	1.00[a]	
Overweight	3 (10%)	2 (6.7%)		0.64 (0.10–4.15)
Stature				
Normal	25 (83.3%)	28 (93.3%)	0.42[a]	
Short	5 (16.7%)	2 (6.7%)		0.36 (0.06–2.01)
Development				
Appropriate	30 (100%)	30 (100%)	1.00[a]	
Doubting	0	0		
Possible deviation	0	0		

[a] Fisher exact test

0.9% protein, 6.9 to 7.2% carbohydrates, and minerals of 0.2% constituents, including sodium, potassium, calcium, magnesium, phosphorus, and chloride. Protein in breast milk also includes immunoglobulin A, lysozyme, and albumin, which are related to the immune function of children [8]. However, Kramer et al. (2007) conducted a randomized trial on groups of children given exclusive breastfeeding for more than 1 year and those who were only given breast milk for 1 month. The study found no significant difference between the two groups at the age of 6.5 years. Moreover, the group that did not give exclusive breastfeeding had a higher BMI [9]. Our study also did not find any significant difference between the two groups, even though the fresh embryo group was not entirely exclusively breastfed.

We also conducted an analysis based on the birth weight of babies. As many as 30% of infants in the fresh embryo group were born with LBW, compared to only 6.7% in the frozen embryo group. The OR value of 0.17 (95% *CI*: 0.03–0.85) indicates that the frozen embryo group had a lower risk of developing LBW than the fresh embryo group. Previous studies also reported that children conceived from frozen embryo transfer have a lower risk of LBW (*OR* 0.63, 95% *CI* 0.45–0.87) and premature birth (*OR* 0.70, 95% *CI*, 0.53–0.92) compared with those conceived from fresh embryo transfers [10, 11].

Factors that influence infant birth weight were not examined in this study. Other research conducted on Asian populations also states that the risk of low birth weight is significantly reduced in infants conceived from frozen embryo transfers compared to fresh embryos. Still, the reasons why infants conceived from frozen embryos were heavier than fresh embryos are unknown [12]. The difference in embryos' quality and the side effects of hormone stimulation in fresh cycles were presumed to cause this difference [13].

The growth and development of both groups showed the same results. There is no correlation between the type of embryo with nutritional status, stature, and development of children conceived from IVF with either fresh or frozen embryo transfer. The same result was also reported by research conducted by Ainsworth et al., which followed the growth of children up to the age of 5 years. They also found that children's growth, including age and weight by sex and body mass index percentile, were not significantly different between the two groups ($p = 0.26$ for weight, $p = 0.48$ for height, and $p = 0.44$ for weight BMI) [14].

We did not find any significant difference in child development between the two groups. Koivurora et al., who compared the development of IVF-conceived children up to 3 years with those from natural pregnancy, also did not find any significant impact of IVF on child development [15]. However, we used a relatively simpler instrument for assessing child development. Pre-Screening Developmental Questionnaire (KPSP) is one of the instruments often used in Indonesia due to its simple and practical use. We recommend using a more sophisticated and accurate instrument in research or academic settings in the future.

This study is a preliminary study and a novel study in our country. There were no data on the pediatric outcomes of those conceived through ART in Indonesia. Nevertheless, due to that nature, it leads to the weakness of our study. Our study has a small sample size, despite meeting the minimum sample requirement. Nonclinical factors that might affect the growth and development of other children, such as socioeconomic, nutrition, parenting, and environment, other than embryo transfer factors, were not analyzed. We also did not analyze obstetrics problems that occurred, which might affect perinatal outcomes. Multicenter and more comprehensive studies are needed in the future.

Conclusion

Frozen embryo transfer resulted in no difference in child growth and development compared to ones conceived from fresh embryos. However, it decreases the risk of low birth weight.

Abbreviations

ART: Assisted reproductive technology; BMI: Body mass index; IVF: In vitro fertilization; KPSP: Kuesioner Pra Skrining Perkembangan (Pre-Screening Developmental Questionnaire); LBW: Low birth weight; OHSS: Ovarian hyperstimulation syndrome; RSCM: Cipto Mangunkusumo National Hospital; SD: Standard deviation; SPSS: Statistical Package for the Social Sciences; WHO: World Health Organization.

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Authors' contributions

RM analyzed and interpreted the data and was a major contributor in writing the manuscript. EU performed the clinical examination and contributed to writing the manuscript. HG designed and supervised the study. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Signed informed consent was obtained from all of the participants' parents. The study was approved by the Health Research Ethics Committee — University of Indonesia and Cipto Mangunkusumo Hospital (code: 708/UN2.F1/ETIK/2017).

Consent for publication

Not applicable.

Competing interests
The authors declare that they have no competing interests.

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