ORIGINAL RESEARCH ARTICLE

Effects of the first frozen-thawed embryo transfer time on pregnancy outcomes after progestin primed ovarian stimulation regimen

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Abstract

This was an original article, mainly explored the effects of the first frozen-thawed embryo transfer (FET) time on pregnancy outcomes after progestin primed ovarian stimulation (PPOS) regimen. Our study implemented a retrospective analysis of 315 infertile patients who underwent in vitro fertilization and embryo transfer (IVF-ET) treatment from January 2021 to June 2023. Patients were divided into three groups based on their first FET time. Group A prepared FET at the first menstrual period after oocyte retrieval; Group B prepared FET at the second menstrual period,; while Group C prepared FET at the third menstrual period. The general information of patients, clinical data of PPOS cycle, clinical data of FET cycle, along with pregnancy outcomes were compared in three groups. The results showed no significant differences in general information of patients, clinical data of PPOS cycle, clinical data of FET cycle and pregnancy outcomes among three groups (P>0.05). The results showed that the choice of the first FET time for whole embryo freezing patients had no significant effect on the outcome of assisted pregnancy. We conclude that the first menstrual cycle after the oocyte retrieval cycle can be considered to select the appropriate FET scheme and performance of the FET. *(Afr J Reprod Health 2024; 28 [11]: 39-45).*

Keywords: Frozen-thawed embryo transfer, pregnancy outcomes, progestin primed ovarian stimulation, time

Résumé

Il s'agissait d'un article original, explorant principalement les effets du premier transfert d'embryons congelés-dégelés (FET) sur l'issue de la grossesse après un régime de stimulation ovarienne amorcée par un progestatif (PPOS). Notre étude a mis en œuvre une analyse rétrospective de 315 patientes infertiles ayant subi un traitement de fécondation in vitro et de transfert d'embryons (FIV-ET) de janvier 2021 à juin 2023. Les patientes ont été divisées en trois groupes en fonction de leur première période de FET. Le groupe A a préparé le FET lors de la première période menstruelle après le prélèvement des ovocytes ; Le groupe B a préparé le FET à la deuxième période menstruelle ; tandis que le groupe C préparait le FET à la troisième période menstruelle. Les informations générales des patients, les données cliniques du cycle PPOS, les données cliniques du cycle FET, ainsi que les issues de grossesse ont été comparées en trois groupes. Les résultats n'ont montré aucune différence significative dans les informations générales des patientes, les données cliniques du cycle PPOS, les données cliniques du cycle FET et les issues de grossesse entre trois groupes (P > 0,05). Les résultats ont montré que le choix du premier moment FET pour les patientes congelées d'embryons entiers n'avait pas d'effet significatif sur l'issue de la grossesse assistée. Nous concluons que le premier cycle menstruel après le cycle de récupération des ovocytes peut être pris en compte pour sélectionner le schéma FET approprié et les performances du FET. *(Afr J Reprod Health 2024; 28 [11]: 39-45).*

Mots-clés: Transfert d'embryons congelés-décongelés, issues de la grossesse, stimulation ovarienne amorcée par un progestatif, temps

Introduction

Recently, with the change of living conditions and social environment, the group of "infertility" patients is increasing[1]. Since the world's first "test tube baby" was born in the UK, human assisted reproductive technology has used worldwide for more than 30 years[2]. With the development of embryo cryopreservation technology, the clinical pregnancy rate of embryo frozen-thawed cycle has gradually increased, and has even exceeded that of the fresh cycle[3].

At a time when ovulation stimulation in luteal phase and whole embryo frozen transplantation

strategies are becoming increasingly mature, progestin primed ovarian stimulation (PPOS) regimen in follicular phase is now widely used. It implies the continuous application of progesterone to inhibit endogenous luteinizing hormone (LH) peaks in early follicular phase/ When combined with gonadotropin as a mode of ovulation stimulation, this has become an important new line of research[4].

Studies have proved that PPOS program not only inhibits the early LH peak, and decrease the incidence of ovarian hyperstimulation syndrome (OHSS), but also is also convenient and economical[5]. However, PPOS regimen cannot transfer fresh embryos, and it is necessary to freeze and thaw embryos after freezing the whole embryo for a period of time[6]. Currently, there is no relevant research at home and abroad on the influence of the first frozen-thawed embryo transfer (FET) time after PPOS program on pregnancy outcome. Domestic reproductive centres generally start to prepare for FET only after the second or even the third menstrual period after oocyte retrieval, which greatly prolongs the time required for pregnancy in infertile couples[7].

The objective of this study was to compare the effects of preparation for FET at the first, second, and third menstrual periods after oocyte retrieval on pregnancy outcomes. We believe that the results will enable clinicians to decide whether clinical preparation for freeze-thaw embryo transfer could be considered at the first menstrual period after oocyte retrieval cycle, so as to greatly reduce the time required for pregnancy among infertile couples

Methods

Materials

A retrospective analysis was implemented on 315 infertile patients who underwent in vitro fertilization and embryo transfer (IVF-ET) treatment in Huizhou Central People's Hospital from January 2021 to June 2023. Inclusion criteria included: (1) patients adopting the PPOS scheme to improve ovulation; (2) first FET after oocyte retrieval; (3) age 38 years or lessold; (4) anti-mullerian hormone (AMH) >1.2 ng/ml; and (5) number of ovaobtained more than 5. The exclusion criteria were: (1) uterine and ovarian factors: uterine malformation, uterine adenomyosis, uterine adhesions, unilateral or bilateral ovarian space occupying lesions; (2) hydrosalpinx on one or both sides; (3) previous history of adverse pregnancy; (4) patients with uncontrolled hyperthyroidism or hypothyroidism; (5) Any of the couples with chromosomal abnormalities.

PPOS scheme

From the 2nd to 5th day of the menstrual cycle, patients took medroprogesterone acetate (MPA) table (Zhejiang Xianju Pharmaceutical Co., LTD.) orally, 4 mg/time, twice a day. From the 2nd to 5th day of the menstrual cycle, patients began to receive injections of follicle-stimulating hormone (FSH, Lizon Pharmaceutical Group Co., LTD.), 150 ~ 300 IU/d. From the 6th to 7th day of the menstrual cycle, B-ultrasound was used to monitor follicle condition, blood LH, estradiol level and progesterone level and FSH dosage was adjusted until the injection day of human chorionic gonadotrophin (HCG, Merck Serono, Switzerland). Thirty-six hours later, vaginal ultrasound-guided oocyte retrieval was performed. Fertilization was observed 18 h after routine IVF.

Embryo assessment

Embryo culture was performed by microdrop hypoxia (CO_2 concentration was 6%, O_2 concentration was 5%, N_2 concentration was 89%). Blastocysts were evaluated according to the Istanbul Consensus and Gardner scoring system. High quality blastocysts refer to embryos with blastocyst cavity expansion of grade 3 or above, inner cell mass and trophoblast rating of grade B or above, and the rest are non-high quality blastocysts. Blastocysts rated above 3BC were cryopreserved by vitrification.

Embryo cryopreservation

Vitrification of embryos was performed using commercial reagents (Vitrolife, Sweden) according to the reagent instructions. Before freezing, the reagent was balanced in a 37 the reagent was he reagent instr When freezing, the embryos to be frozen were transferred to freeze solution 1 for 5 to 10 min, and then transferred to freeze solution 2 for 2 min, and then transferred to freeze solution 3 for 30 to 45 s, and finally loaded onto frozen wheat tube and quickly inserted into liquid nitrogen. The

frozen embryos were stored in a liquid nitrogen tank at -196°C where the level of liquid nitrogen was measured weekly and the liquid nitrogen was filled weekly.

Methods

Patients were randomly selected into three groups. Each group had 105 patients. The random allocation method was as follows: 315 numbers were selected successively from the random number table for numbering, and the numbers from 1 to 315 corresponded to 315 random numbers, which were put into sealed envelopes. The numbers in an envelope were randomly selected when the study participants were admitted to the hospital and grouped according with 1-105 as group A, 106-210 as group B, and 211-315 as group C. Group A prepared FET at the first menstrual period after oocyte retrieval, group B prepared FET at the second menstrual period, and group C prepared FET at the third menstrual period.

Endometrial preparation regimen took hormone replacement cycle. From the 3rd to 5th day of the menstrual cycle, patients took estradiol valerate tablets (Bayer, Germany), 2 mg, twice/day. Five days later, vaginal B-ultrasonography was reviewed, and if the endometrial thickness was ≥8 mm, patients were intramuscularly injected with progesterone injection (Zhejiang Xianju Pharmaceutical Co., LTD.), 60 mg, once a day, or treated with progesterone vaginal sustained release gel (Merck Serono Co., Ltd., UK), 90 mg, once a day, vaginal use. The cleavage embryos were transplanted on day 3 and the blastocysts on day 5 after endometrial transformation. Patients in each group were allowed to transfer three embryos at a time.

Embryo grading

Based on the embryo prokaryotic score, the morphology and number of blastomere, the proportion of cytoplasmic fragments and the development rate, the embryo quality score was divided into 4 grades: Grade I: the blastomere was uniform in size, the cytoplasm was homogeneous and transparent, and there were no fragments; Grade II: the blastomere was uniform in size, with fragments < 20%; Grade III: the blastomere was not uniform in size, with fragments between 20% and 50%; Grade IV: the blastomere was not uniform in size, with fragments > 50%. A morphological rating of grade I or Grade II indicated that the embryo was of good quality and was classified as a high-quality embryo. Patients were transferred the same grade of embryo in three groups.

Observed indicators

(1) General information of patients including age, years of infertility, types of infertility, causes of infertility, basal FSH, basal LH, body mass index (BMI) along with AMH were compared in 3 groups.
(2) Clinical data of PPOS cycle containing number of retrieved oocytes, fertility rate, number of viable embryos and number of high-quality embryos were compared in 3 groups.
(3) Clinical data of FET cycle including number of transplanted embryos, type of transplanted embryos and thickness of endometrium on transfer day were compared in 3 groups.
(4) Pregnancy outcomes included ratio of biochemical pregnancy, clinical pregnancy, embryo implantation, spontaneous abortion, ectopic pregnancy, along with multiple birth were compared in 3 groups. Quantitative HCG detection was performed 14 days after FET to determine whether there was biochemical pregnancy, and vaginal B-ultrasonography was carried out 30 days after FET to determine clinical pregnancy.

Statistical analysis

SPSS 24.0 statistical software was employed for data analysis. Measurement data were exhibited as (x±s), followed by comparison using one-way ANOVA with Bonferroni's post-test. Count data were exhibited as (n, %), and χ^2 test was employed for comparison. $P<0.05$ meant statistical significance.

Ethical consideration

Our study was approved by the Ethics Committee of Huizhou Central People's Hospital.

Results

General information of patients in 3 groups

As Table 1 displayed, no differences were discovered in age, years of infertility, types of infertility, causes of infertility, basal FSH, basal LH, BMI and AMH among 3 groups ($P>0.05$), indicating comparability.

Table 1: General information of patients among 3 groups

Groups	Group A (n=105)	Group B (n=105)	Group C (n=105)	χ^2/F	P
Average age (years)	35.62±3.56	35.51±3.50	35.43±3.54	0.076	0.926
Years of infertility (years)	3.12±0.32	3.08±0.31	3.14±0.36	0.896	0.409
Basal FSH (miu/ml)	5.87±0.58	6.01±0.62	5.96±0.65	1.387	0.251
Basal LH (miu/ml)	8.42±0.83	8.67±0.88	8.51±0.85	2.311	0.100
BMI (kg/m^2)	23.54±2.38	23.21±2.31	23.36±2.35	0.520	0.594
AMH (ng/ml)	7.68±0.78	7.72±0.76	7.81±0.79	0.771	0.463
Types of infertility				0.330	0.847
High ovarian response	52 (49.52%)	55 (52.38%)	51 (48.57%)		
Poor ovarian response	53 (50.48%)	50 (47.62%)	54 (51.43%)		
Causes of infertility				0.367	0.985
Obstruction of fallopian tubes	45 (42.86%)	42 (40.00%)	43 (40.95%)		
Polycystic ovary syndrome	32 (30.47%)	36 (34.29%)	34 (32.38%)		
Endometriosis	28 (26.67%)	27 (25.71%)	28 (26.67%)		

Clinical data of POSS cycle in 3 groups

As Figure 1A-1D revealed, no differences were observed in the number of retrieved oocytes, fertility rate, number of viable embryos as well as number of high-quality embryos among three groups (P>0.05).

Clinical data of FET cycle in 3 groups

As shown in Figure 2A-2C, no difference was seen in the number of transplanted embryos, type of transplanted embryos as well as thickness of endometrium on transfer day among the 3 groups (P>0.05).

Pregnancy outcome in 3 groups

Table 2 displayed no differences in the ratio of biochemical pregnancy, clinical pregnancy, embryo implantation, spontaneous abortion, ectopic pregnancy, along with multiple birth among 3 groups (P>0.05)

Discussion

With the development and maturity of IVF-ET technology, FET has also become an indispensable link in the process of IVF-ET to improve the cumulative pregnancy rate[8]. During IVF treatment, hyperovulation induced polyfollicular development can lead to physiologically high estrogen levels and early increase of progesterone, thus altering endometrial receptivity and reducing pregnancy rate[9]. A multicenter meta-analysis by Roque and his colleague[10] pointed out that FET had a higher clinical pregnancy rate than fresh embryo transfer, which confirmed that FET has a satisfactory clinical outcome. In addition, there may be some complications during IVF, such as ovarian hyperstimulation syndrome (OHSS), surgical bleeding, infection, endometrial abnormalities, which may not enable a fresh embryo transfer[11]. In the past, due to the restriction of frozen embryo technology, the embryo quality of frozen-thawed embryos decreased after thawing, which affected the implantation rate[12]. However, with the comprehensive application of vitrification frozen technology, the frozen recovery rate and integrity rate have become high, providing reliable laboratory technical support for FET after whole embryo freezing[13]. At present, studies have proved that FET has fewer perinatal complications after pregnancy than fresh embryo transfer[14]. Hence, some scholars believe that the whole embryo freezing strategy is a more reasonable choice in IVF-ET treatment.

At present, PPOS is mainly used in elderly patients with declined ovarian reserve, who have fewer basal follicles and are more likely to reach an early LH peak than those possessing normal ovarian function[15]. Studies have suggested that PPOS can effectively inhibit LH peak, increase follicle output rate, increase the number of retrieved oocytes, reduce cycle cancellation rate as well as increase the available embryo rate of these patients[16]. Additionally, the available embryo rate presents higher than the conventional regimen group or micro-stimulation group[17].

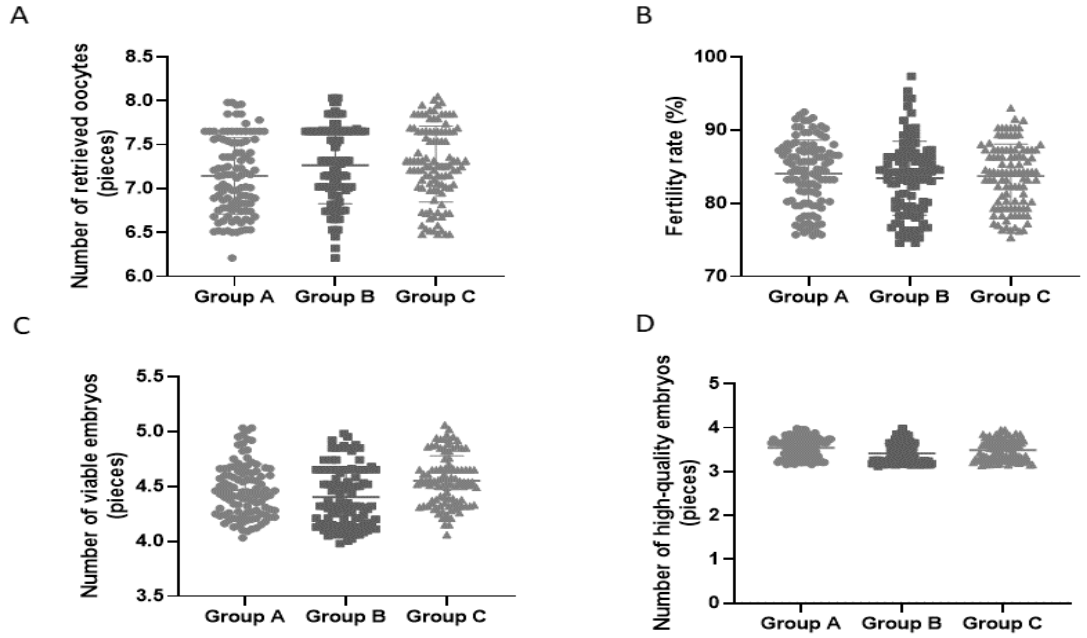

Figure 1: Clinical data of POSS cycle in 3 groups. A. Number of retrieved oocytes. B. Fertility rate. C. Number of viable embryos. D. Number of high-quality

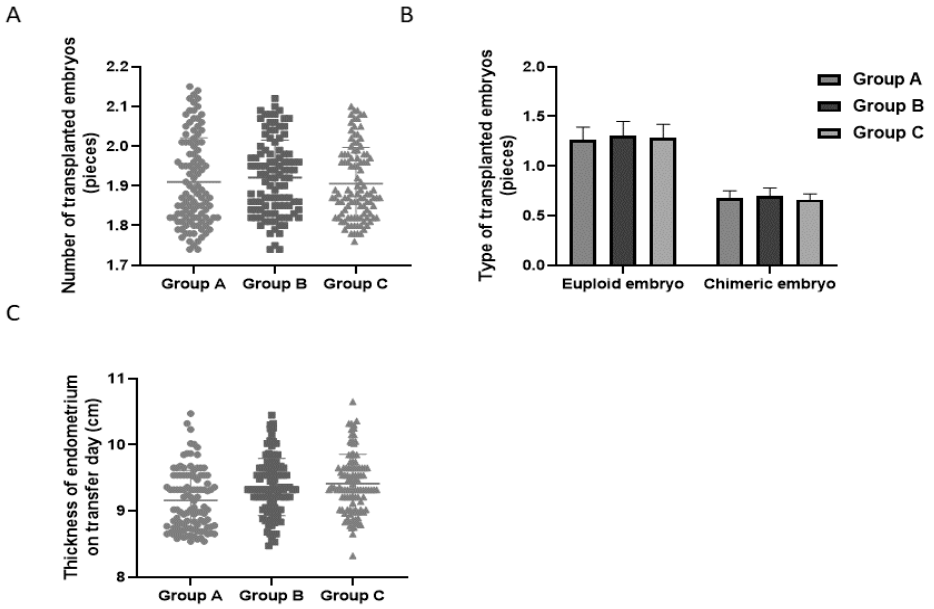

Figure 2: Clinical data of FET cycle in 3 groups. A. Number of transplanted embryos. B. Type of transplanted embryos. C. Thickness of endometrium on transfer day

Simultaneously, because of the short administration time of PPOS program, ovulation can be promoted several times in a short period of time, so that these patients with poor ovarian function can maximize the chance of conception in the limited ovarian cycle[18]. The FET cycle can effectively reduce the stimulation of a large amount of gonadotropin, and can reduce the influence of high estrogen on endometrial susceptibility in patients undergoing ovarian hyperresponse[19]. Nevertheless, there are few studies on the first time of FET at home and abroad, and there are many controversies.

Table 2: Pregnancy outcome in 3 groups

Groups	N	Biochemical pregnancy rate	Clinical pregnancy rate	Embryo implantation rate	Spontaneous abortion rate	Ectopic pregnancy rate	Multiple birth rate
Group A	105	51.43% (54/105)	47.62% (50/105)	36.00% (108/300)	6.00% (3/50)	4.00% (2/50)	4.00% (2/50)
Group B	105	49.52% (51/105)	48.57% (51/105)	35.08% (107/305)	5.88% (3/51)	5.88% (3/51)	3.92% (2/51)
Group C	105	50.48% (53/105)	46.67% (49/105)	34.19% (106/310)	6.12% (3/49)	4.08% (2/49)	4.08% (2/49)
χ^2		0.177	0.076	0.218	0.002	0.257	0.001
T		0.914	0.962	0.896	0.998	0.879	0.999

In previous clinical experience, in order to avoid the influence of controlled hyperovulation on ovarian and endometrial receptivity in patients with ovarian hyperresponse, the FET time is often scheduled to 2 ~ 3 months after oocyte retrieval[20]. However, IVF-ET assisted pregnancy patients often require urgent transfer of embryos the waiting process will increase the anxiety of patients and their families, which is not conducive to the assisted pregnancy process[21]. Volodarsky-Perel et al.[22] proposed through retrospective analysis that after the fresh embryo transfer cycle failed, the ratio of implantation, clinical pregnancy along with live birth in the delayed FET group presented higher than the non-delayed group, suggesting that high estrogen produced during control ovary hyperstimulation had a persistent adverse effect on the intima. The first FET after a failed fresh embryo transfer will delay at least one menstrual cycle. However, Song et al. suggested that in women whose previous IVF-ET attempts had failed, immediate FET had higher rates of sustained pregnancy and live birth than delayed FET[23]. Moreover, some studies have demonstrated that whether fresh embryo transfer fails or the first FET after whole embryo freezing, different FET timing has similar clinical pregnancy rate as well as live birth rate, implying that endometrial tolerance has returned to normal after a menstruation, and whether delayed transplantation has no effect on pregnancy outcome[24,25]

Study strengths and limitations

Strengths include the novelty of the subject, well-designed and randomized trial, and the administration of PPOS scheme. This study was conducted with a small sample size and short follow-up time; therefore, interpretation of the results requires great caution. Our study provides a clinical reference for pregnant patients undergoing frozen embryo transfer.

Conclusion

Different time choices had no impact on pregnancy outcome. In order to alleviate the anxiety of expectant pregnant patients waiting for transplantation, it is advisable to select a suitable FET program in the first menstrual cycle after the oocyte extraction cycle and perform frozen embryo transfer as soon as possible.

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Contribution of authors

Zha QJ and Chen ZY: conceived and designed the study, as well as collected and analysed the data. Ji Y and Liu X: prepared the manuscript. All authors mentioned in the article approved the manuscript.

References

1.Inhorn MC and Patrizio P. Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. Hum Reprod Update. 2015; 21(4):411-26.

2.Cyranoski D, Contreras JL and Carrington VT. Intellectual property and assisted reproductive technology. Nat Biotechnol. 2023; 41(1):14-20.

3.Von Versen-Höynck F and Griesinger G. Should any use of artificial cycle regimen for frozen-thawed embryo transfer in women capable of ovulation be abandoned: yes, but what's next for FET cycle practice and research? Hum Reprod. 2022; 37(8):1697-1703.

4.Matevossian K and Sauerbrun-Cutler MT. The progestin-primed ovarian stimulation protocol: more economical, but at what cost? Fertil Steril. 2022; 118(4):713-714.
5.Pai AH, Sung YJ, Li CJ, Lin CY and Chang CL. Progestin Primed Ovarian Stimulation (PPOS) protocol yields lower euploidy rate in older patients undergoing IVF. Reprod Biol Endocrinol. 2023; 21(1):72.
6.Yang AM, Feng TF, Han Y, Zhao ZM, Wang W, Wang YZ, Zuo XQ, Xu X, Shi BJ, Li L, Hao GM and Cui N. Progestin-Primed Ovarian Stimulation Protocol for Patients With Endometrioma. Front Endocrinol (Lausanne). 2022; 13:798434.
7.Tu YA, Yang PK, Chen SU and Yang JH. Optimal time interval between hysteroscopic polypectomy and frozen-thawed blastocyst transfer: A retrospective study. PLoS One. 2020; 15(10):e0240882.
8.Maheshwari A, Pandey S, Amalraj Raja E, Shetty A, Hamilton M and Bhattacharya S. Is frozen embryo transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer? Hum Reprod Update. 2018; 24(1):35-58.
9.Lafontaine S, Labrecque R, Blondin P, Cue RI and Sirard MA. Comparison of cattle derived from in vitro fertilization, multiple ovulation embryo transfer, and artificial insemination for milk production and fertility traits. Journal of dairy science. 2023; 106(6):4380-4396.
10.Roque M, Lattes K, Serra S, Solà I, Geber S, Carreras R and Checa MA. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. Fertil Steril. 2013; 99(1):156-162.
11.Roque M, Haahr T, Geber S, Esteves SC and Humaidan P. Fresh versus elective frozen embryo transfer in IVF/ICSI cycles: a systematic review and meta-analysis of reproductive outcomes. Hum Reprod Update. 2019; 25(1):2-14.
12.Fang L, He J, Yan Y, Jia Q, Yu Y, Zhang R, Cheng JC and Sun YP. Blastocyst-stage embryos provide better frozen-thawed embryo transfer outcomes for young patients with previous fresh embryo transfer failure. Aging (Albany NY). 2020; 12(8):6981-6989.
13.Casciani V, Monseur B, Cimadomo D, Alvero R and Rienzi L. Oocyte and embryo cryopreservation in assisted reproductive technology: past achievements and current challenges. Fertil Steril. 2023; 120(3 Pt 1):506-520.
14. Ainsworth AJ, Wyatt MA, Shenoy CC, Hathcock M and Coddington CC. Fresh versus frozen embryo transfer has no effect on childhood weight. Fertil Steril. 2019; 112(4):684-690.e1.
15. Guan S, Feng Y, Huang Y and Huang J. Progestin-Primed Ovarian Stimulation Protocol for Patients in Assisted Reproductive Technology: A Meta-Analysis of Randomized Controlled Trials. Front Endocrinol (Lausanne). 2021; 12:702558.
16. Cui L, Lin Y, Wang F and Chen C. Effectiveness of progesterone-primed ovarian stimulation in assisted reproductive technology: a systematic review and meta-analysis. Arch Gynecol Obstet. 2021; 303(3):615-630.
17.Khurana RK, Rao V, Nayak C, Pranesh GT and Rao KA. Comparing Progesterone Primed Ovarian Stimulation (PPOS) to GnRH Antagonist Protocol in Oocyte Donation Cycles. Journal of human reproductive sciences. 2022; 15(3):278-283.
18.Xi Q, Tao Y, Qiu M, Wang Y and Kuang Y. Comparison Between PPOS and GnRHa-Long Protocol in Clinical Outcome with the First IVF/ICSI Cycle: A Randomized Clinical Trial. Clinical epidemiology. 2020; 12:261-272.
19.Busnelli A, Di Simone N and Levi-Setti PE. Artificial cycle frozen embryo transfer and obstetric adverse outcomes: association or causation? Hum Reprod Update. 2023; 29(5):694-696.
20.Bortoletto P, Romanski PA, Magaoay BI, Rosenwaks Z and Spandorfer SD. Time from oocyte retrieval to frozen embryo transfer in the natural cycle does not impact reproductive or neonatal outcomes. Fertil Steril. 2021; 115(5):1232-1238.
21.Wang R, Pan W, Yu L, Zhang X, Pan W, Hu C, Wen L, Jin L and Liao S. AI-Based Optimal Treatment Strategy Selection for Female Infertility for First and Subsequent IVF-ET Cycles. Journal of medical systems. 2023; 47(1):87.
22.Volodarsky-Perel A, Eldar-Geva T, Holzer HE, Schonberger O, Reichman O and Gal M. Cryopreserved embryo transfer: adjacent or non-adjacent to failed fresh long GnRH-agonist protocol IVF cycle. Reprod Biomed Online. 2017; 34(3):267-273.
23.Song JY, Dong FY, Li L, Zhang XX, Wang AJ, Zhang Y, Gao DD, Xiao JM and Sun ZG. Immediate versus delayed frozen embryo transfer in women following a failed IVF-ET attempt: a multicenter randomized controlled trial. Reprod Biol Endocrinol. 2021; 19(1):131.
24.Santos-Ribeiro S, Polyzos NP, Lan VT, Siffain J, Mackens S, Van Landuyt L, Tournaye H and Blockeel C. The effect of an immediate frozen embryo transfer following a freeze-all protocol: a retrospective analysis from two centres. Hum Reprod. 2016; 31(11):2541-2548.
25.Lattes K, Checa MA, Vassena R, Brassesco M and Vernaeve V. There is no evidence that the time from egg retrieval to embryo transfer affects live birth rates in a freeze-all strategy. Hum Reprod. 2017; 32(2):368-374.

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