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The impact of low oocyte maturity ratio on blastocyst euploidy rate: a matched retrospective cohort study

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Abstract

Objective To investigate the association between a low oocyte maturity ratio from in vitro fertilization cycle and blastocyst euploidy.

Methods A total of 563 preimplantation genetic testing (PGT) cycles (PGT cycles with chromosomal structural rearrangements were excluded) were performed between January 2021 and November 2022 at our center (average oocyte maturity rate: 86.4% ± 14.6%). Among them, 93 PGT cycles were classified into the low oocyte maturity rate group (group A, < mean − 1 standard deviation [SD]), and 186 PGT cycles were grouped into the average oocyte maturity rate group (group B, mean ± 1 SD). Group B was 2:1 matched with group A. Embryological, blastocyst ploidy, and clinical outcomes were compared between the two groups.

Results The oocyte maturity (metaphase II [MII oocytes]), MII oocyte rate, and two pronuclei (2PN) rates were significantly lower in group A than in group B (5.2 ± 3.0 vs. 8.9 ± 5.0, $P = 0.000$; 61.6% vs. 93.0%, $P = 0.000$; 78.7% vs. 84.8%, $P = 0.002$, respectively). In group A, 106 of 236 blastocysts (44.9%) that underwent PGT for aneuploidy were euploid, which was not significantly different from the rate in group B (336/729, 46.1%, $P = 0.753$). However, euploid blastocysts were obtained only in 55 cycles in group A (55/93, 59.1%), which was lower than the rate in group B (145/186, 78.0%, $P = 0.001$). The clinical pregnancy rate in group B (73.9%) was higher than that in group A (58.0%) ($P = 0.040$).

Conclusion Our results suggest that a low oocyte maturity ratio is not associated with blastocyst euploidy but is associated with fewer cycles with euploid blastocysts for transfer, lower 2PN rates, and lower clinical pregnancy rates.

Keywords Oocyte maturity, PGT, Fertilization, Clinical pregnancy rate, Euploidy rate, Metaphase II oocytes

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Introduction

Oocyte maturation is a key process in human assisted reproductive technology (ART) and is particularly important for intracytoplasmic sperm injection (ICSI) insemination, in which only metaphase II (MII) oocytes are injected with sperm. Thus, all ART doctors try their best to recruit multiple follicular development protocols to harvest numerous mature oocytes. However, despite optimizing ovarian stimulation protocols, approximately 20% of oocytes in the clinic remain immature, stalling at the germinal vesicle (GV) or metaphase I (MI) stages [1, 2]. Although it is possible to induce oocytes maturation using in vitro maturation (IVM), this technique is still not widely used in clinical practice because of its underperformance, including the low rate of blastocyst conversion, implantation and pregnancy compared to conventional in vitro fertilization (IVF) [3, 4].

The maturity of retrieved oocytes is important for fertilization and embryo development. In recent years, several investigators have tried to identify predictive measures for the number of retrieved immature oocytes in controlled ovarian hyperstimulation (COH) programs administered for ART outcomes [1, 5–7]. However, results were inconsistent. A prior study by Halvaei et al. (2012) showed that the rates of fertilization, embryo development, clinical pregnancy, and delivery in a group of patients with less than two immature oocytes were not significantly different from than those of a group of patients with more than two immature oocytes [1]. In contrast, a recent study by Parella et al. (2019) showed significantly reduced normal fertilization (two pronuclei [2PN]), implantation, clinical pregnancy, and live birth rates in the minimal group than in the optimal group [6]. Most recently, a study by Capper et al. (2022) also found that a low oocyte maturity ratio is associated with poor IVF outcomes, including decreased pregnancy and live birth rates but not miscarriage or fertilization rates [7].

Although oocyte maturity is commonly assessed based on IVF outcomes [1, 6, 7], little is known about the relationship between low oocyte maturity ratio and blastocyst ploidy; whether low oocyte maturity ratio leads to a low clinical pregnancy rate by affecting blastocyst euploidy remains unclear. Therefore, we conducted a matched case-control study to determine whether the immature oocyte rate was associated with blastocyst ploidy.

Materials and methods

Study design and participants

In this study, we included all autologous oocyte preimplantation genetic testing (PGT) cycles with at least one blastocyst PGT for aneuploidy (PGT-A) between January 2021 and November 2022, and only the first cycle was analyzed. PGT cycles with chromosomal structural rearrangements (PGT-SR) were excluded because of the potential correlation between chromosomal structural rearrangements and decreased embryo euploidy rate. This study was approved by the Independent Ethics Committee of Boai Hospital of Zhongshan (KY-2023-02-01).

Patient treatment

Standard COH protocols were used in this study. Briefly, patients underwent either a gonadotropin-releasing hormone (GnRH) agonist or GnRH antagonist protocol for ovulation induction. Ovarian stimulation was performed using recombinant follicle-stimulating hormone (rFSH) (Gonal-F, Merck-Serono, Geneva, Switzerland) in combination with a GnRH agonist (Triptorelin Acetate, Ipsen Pharma Biotech, France) or antagonist (Cetrorelix Acetate, Merck-Serono, Geneva, Switzerland). The initial doses were based on antral follicle counts, female age, body mass index (BMI), anti-Müllerian hormone (AMH) level, and FSH level. Subsequent doses were adjusted according to the follicle growth and serum estradiol levels. Human chorionic gonadotropin (HCG) and/or GnRH agonists were administered when at least three leading follicles reached a mean diameter of ≥18 mm. Transvaginal oocyte retrieval was scheduled approximately 36 h later.

Determination of oocyte maturity

Oocytes were incubated for 4–6 h prior to ICSI (about 40–42 h after hCG injection). During this time, cumulus cells were removed by exposure to a medium containing 40 IU/Ml hyaluronidase (Cumulase; Halozyme Therapeutics, Inc., San Diego, CA). Following denudation, oocytes at prophase I displayed GV, whereas oocytes at MI displayed broken GV without the extrusion of the first polar body. Whenever the first polar body was present, the oocytes were considered MII and could be injected. Cycles were classified into different groups according to the proportion of oocytes that had their first polar body extruded (MII) at the time of cumulus removal. Initial, we determined the mean and standard deviation (SD) for the oocyte maturity ratio, and categorized maturity as high (>1 SD above the mean), average (mean ± 1 SD), below average (1–2 SD below the mean), and low (>2 SD below the mean), as in a previous study [7]. However, because the cases of high and low maturity groups were relatively few, the below average and low groups were re-grouped as the low oocyte maturity group (group A), while the average and high maturity groups were re-grouped into high oocyte maturity (group B, 2:1 matched with group A, and matched priority to the high group cases). A flow diagram of this study is shown in Fig. 1.

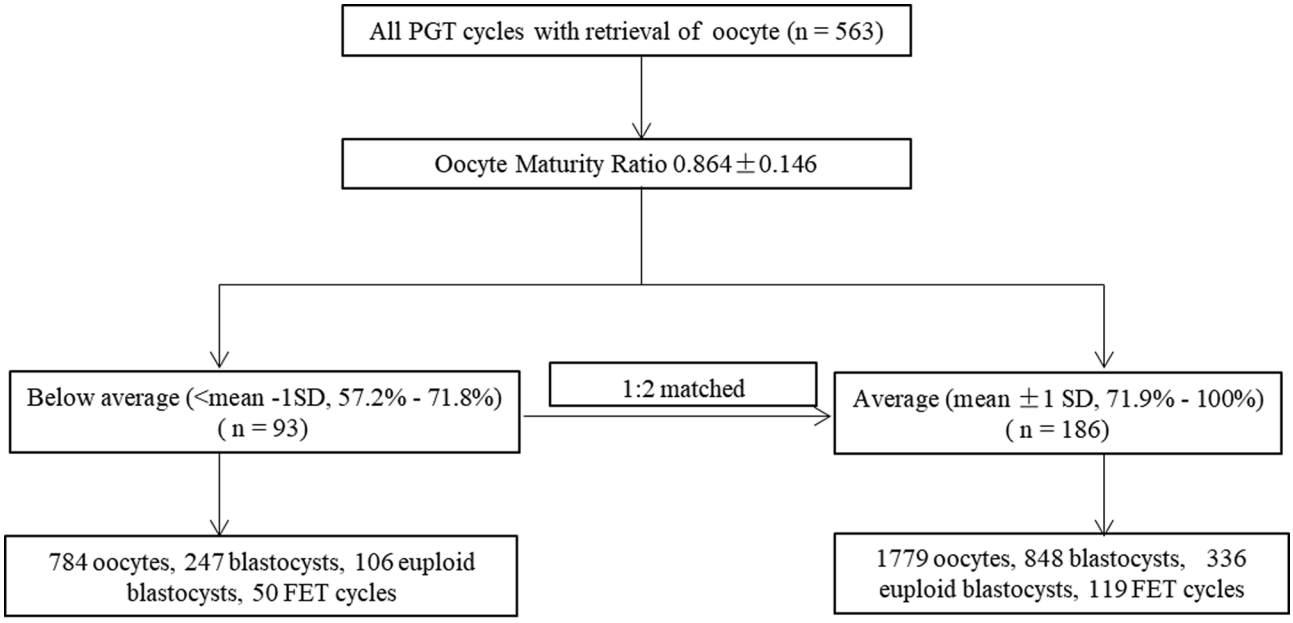

Fig. 1 A flow diagram of this study

Embryo culture and blastocyst biopsy

Each of the MII oocytes was microinjected in the culture media (G-MOPS, Vitrolife Sweden AB, Vastra Frolunda, Sweden). The injected oocytes were washed twice and then individually placed in fresh droplets of G1 (Vitrolife Sweden AB, Vastra Frolunda, Sweden) covered with mineral oil. Fertilization was assessed 16–18 h after ICSI (about 55–58 h after hCG injection). Zygotes were cultured individually in G1 medium under 6% CO2 and 5% O2 at 37 °C in a humidified benchtop incubator (K-MINC-1000, COOK, Brisbane, Australia). On day 3, embryos were transferred to G2 (Vitrolife) medium and cultured individually under the same conditions until day 5 or 6.

Biopsy was performed on day 5 or 6 according to the blastocyst grade on that day [8]. All blastocysts were subjected to trophectoderm (TE) cell biopsy using a laser, and 5–10 TE cells were biopsied. After biopsy, blastocysts were cryopreserved by vitrification according to the manufacturer's protocol (ARSCI Inc., Canada) and stored in liquid nitrogen.

NGS protocol for the TE biopsy

The multiple annealing and loop-based amplification cycle (MALBAC)-based single-cell whole genome amplification (WGA) kit (catalogue number KT110700324; Yikon Genomics Ltd, Suzhou, China) was applied to amplify DNA from TE cells, in accordance with the manufacturer's instructions. To analyze the ploidy status of the blastocysts, the amplified DNA of the trophectoderm samples was sequenced using a NextSeq 550 sequencer (catalogue number SY-415-1002; Illumina, Inc., San Diego, CA, USA) with a single-ended read length of 55 bp. Approximately two million raw reads were generated for each trophectodermal sample. Genome-wide copy number variations (CNV) was analyzed to determine the ploidy status of each embryo, as previously described [9]. Embryos with less than 20% aneuploidy in the TE sample were classified as euploid, those over 80% were classified as aneuploid, and those between 20% and 80% were described as mosaic [10].

Frozen embryo transfer

The endometrium was prepared for transfer in one of two ways: natural cycle (NC) or hormone replacement therapy (HRT). Patients with regular ovulation were treated with NC. Briefly, transvaginal ultrasonography was performed to monitor follicular growth and endometrial thickness. If the thickness of the endometrium reached ≥ 8 mm, the cycle was considered suitable for frozen embryo transfer (FET). If the patient did not ovulate regularly, the HRT cycle was selected. Administration of oral estrogen (4–6 mg/day) was initiated on the 3rd day of the menstrual cycle. When the endometrial thickness reached ≥ 8 mm, injections of progesterone (60 mg/day) were administered before FET [11].

Matched case-control study

In total, 93 PGT cycles were included in the low oocyte maturity rate group. The matched group consisted of 186 cycles, with an average oocyte maturity rate. Matching was accomplished using the following criteria: [1] age (± 1 year); [2] infertility type (primary infertility or secondary infertility); [3] reason for PGT-A; [4] type of protocol

Table 1 Baseline characteristics of the study participants

Item	Group A (*n* = 93)	Group B (*n* = 186)	*P*
Age (years)	37.1 ± 4.8	37.2 ± 4.7	0.843
AMH	2.9 ± 4.2	2.9 ± 2.4	0.941
BMI (kg/m^2)	22.3 ± 3.1	22.8 ± 2.9	0.151
Infertility type			1.000
Primary infertility	14 (15.1%)	28 (15.1%)	
Secondary infertility	79 (84.9%)	158 (84.9%)	
Reason for PGT-A			1.000
Advanced age (≥ 38)	59 (63.4%)	118 (63.4%)	
Recurrent miscarriage	18 (19.4%)	36 (19.4%)	
Repeated implantation failure	7 (7.5%)	14 (7.5%)	
PGT-M	9 (9.7%)	18 (9.7%)	
Stimulation protocols			1.000
Antagonist protocol	58 (62.4%)	116 (62.4%)	
Agonist protocol	35 (37.6%)	70 (37.6%)	
Number of abortions	0.8 ± 0.9	0.8 ± 0.7	1.000
Duration of stimulation (days)	10.0 ± 2.1	10.1 ± 1.6	0.896
Total gonadotrophins dose	2771.9 ± 816.1	2705.2 ± 705.1	0.481
Antral Follicle Counting	9.3 ± 4.8	10.5 ± 5.9	0.079

AMH, Anti-Müllerian hormone; BMI, body mass index; PGT-A, Preimplantation genetic testing for aneuploidy; PGT-M, preimplantation genetic testing for monogenetic

used for COH; [5] the doses of HCG; [6] number of abortions (classified as 1, 2, or >2 times); and [7] the oocyte retrieval physician. Exact matching was required for criteria 1–6; for criterion 7, we attempted to perform matching as closely as possible. To reduce potential bias, researchers were blinded to the reproductive and PGT-A outcomes during the matching process. If multiple patients met the criteria, a single patient was chosen randomly.

Statistical analysis

Data are presented as the mean±standard deviation. Statistical comparisons between the two experimental groups were evaluated via a two-tailed Student t-test, if the data distribution passed the normality test, while the Mann–Whitney U test was used for comparison if the data distribution failed the normality test. A chi-squared test or Fisher's exact test was used to compare the fertilization rate, 2PN rate, etc. Statistical significance was set at $P<0.05$.

Results

Baseline characteristics of the study participants

PGT cycles (excluding PGT-SR cycles) with at least one retrieved oocyte performed between January 2021 and November 2022 at our center were reviewed for eligibility. Cycles without blastocyst euploidy for analysis were excluded. Finally, 563 cycles were included in the study. The average oocyte maturity rate was 86.4% ± 14.6%.

Table 2 Embryological outcomes in the two groups

Item	Group A (*n* = 93)	Group B (*n* = 186)	*P*
Mean oocytes retrieval	8.4 ± 4.7	9.6 ± 5.4	0.084
Mean no. of MII oocytes	5.2 ± 3.0	8.9 ± 5.0	0.000
MII oocytes rate	61.6% (483/784)	93.0% (1655/1779)	0.000
2PN rate	78.7% (380/483)	84.8% (1403/1655)	0.002
Cleavage rate	98.2% (373/380)	98.9% (1388/1403)	0.226
Blastocyst rate	66.2% (247/373)	61.1% (848/1388)	0.069
Blastocyst suitable for biopsy rate	95.5% (236/247)	86.0% (729/848)	0.000
Euploid rate	44.9% (106/236)	46.1% (336/729)	0.753
Mosaic rate	7.2% (17/236)	7.4% (54/729)	0.917
Cycles with euploid blastocyst rate	59.1% (55/93)	78.0% (145/186)	0.001

2PN, 2 Pronuclear

Among these, 93 PGT cycles were grouped into the low oocyte maturity rate group (group A, ≤ 71.8%), with an incidence rate of 16.5%. A total of 186 PGT cycles were 2:1 matched and grouped as the high (71.9–100%) oocyte maturity rate group (group B). The baseline characteristics of the study population are shown in Table 1. No significant differences were observed in age (37.1±4.8 vs. 37.2±4.7, $P=0.843$), BMI (22.3±3.1 vs. 22.8±2.9, $P=0.151$), AMH (2.9±4.2 vs. 2.9±2.4, $P=0.941$), primary or secondary infertility rate ($P=1.000$), reason for PGT-A ($P=1.000$), stimulation protocols ($P=1.000$), number of abortions ($P=1.000$), duration of stimulation ($P=0.896$), total gonadotrophins dose ($P=0.481$), or antral follicle counting ($P=0.079$) between the two groups (Table 1).

Embryological outcomes

No significant differences were observed in the mean oocytes retrieval (8.4±4.7 vs. 9.6±5.4, $P=0.084$), cleavage (98.2% vs. 98.9%, $P=0.226$), or blastocyst (66.2% vs. 61.1%, $P=0.069$) rates between the two groups (Table 2). The mean number of MII oocytes (oocyte maturity), the MII oocyte rate, and 2PN rate were significantly lower in group A than in group B (5.2±3.0 vs. 8.9±5.0, $P=0.000$; 61.6% vs. 93.0%, $P=0.000$; 78.7% vs. 84.8%, $P=0.002$; respectively) (Table 2). The percentage of blastocysts suitable for biopsy in group A (236/247, 95.5%) was higher than that in group B (729/848, 86.0%) ($P<0.01$) (Table 2).

PGT-A outcomes

In group A, 106 of 236 blastocysts subjected to PGT-A were euploid. In group B, 336 of 729 blastocysts subjected to PGT-A were euploid. Thus, there were no significant differences in the euploid blastocyst rate (44.9% vs. 46.1%, $P=0.753$) or mosaic rate (17/236, 7.2% vs. 54/729, 7.4%, $P=0.917$) between the two groups. However, euploid blastocysts were obtained in 55 cycles out of 93 in group A (59.1%), which was lower than that in group B (145/186, 78.0%, $P=0.001$).

Clinical outcomes

The number of first FET cycles transferred to a single blastocyst was 50 and 119 in Groups A and B, respectively. Table 3 presents the main clinical outcomes of the two groups. No ectopic pregnancies were observed in either group. Some of the cycles are still ongoing pregnancies; therefore, we did not compare the live birth rate. No significant difference was observed in endometrial thickness (9.2±1.3 VS. 9.5±1.7, P=0.260), biomedical pregnancy rate (32/50, 64.0% vs. 90/119, 75.6%, P=0.123), or miscarriage rate (2/50, 4.0% vs. 2/119, 1.7%, P=0.365) between the two groups. However, the clinical pregnancy rate in group B (88/119, 73.9%) was higher than that in group A (29/50, 58.0%) (P=0.040).

Discussion

Oocyte maturity is an essential step in fertilization and embryonic development. Some studies have suggested that a low oocyte maturity ratio is an important factor related to poor IVF outcomes, including decreased implantation, pregnancy, and live birth rates [6, 7]. Many studies have tried to identify biomarkers for or predictive methods to assess oocyte maturity, including such ones as the concentration of leptin in follicular fluid and use of state-of-the-art machine learning algorithm [12–14]. Besides, some studies used artificial intelligence and blastocyst morphology to predict human embryo ploidy status [15, 16]. However, there have been no studies on the relationship between oocyte maturity ratio and blastocyst ploidy.

In the present study, multiple matching criteria were used to minimize the effect of clinical heterogeneity observed between patients. Hence, maternal age, infertility type, reason for PGT-A, type of protocol used for COH, number of abortions, and doses of HCG, all of which are known to affect blastocyst ploidy and oocyte maturity were matched. We further attempted to match oocyte retrieval physicians as closely as possible, as the surgeon's ability to aspirate relatively small antral follicles during oocyte retrieval also affects the proportion of MII oocytes [17, 18]. Our results show that higher 2PN rate was accompanied by a decrease in the proportion of MII oocytes. This result corresponded with those of previous studies [6, 7]. Similarly, the results of the study by Parella et al. (2019) showed that the rate of three-pronuclear (3PN) increased with a diminishing proportion of MII [6].

Table 3 Clinical outcomes of the two groups

Item	Group A (n=93)	Group B (n=186)	P
FET cycles	50	119	
Endometrium thickness (mm)	9.2 ± 1.3	9.5 ± 1.7	0.260
Biomedical pregnancy rate (%)	32/50 (64.0)	90/119 (75.6)	0.123
Clinical pregnancy rate (%)	29/50 (58.0)	88/119 (73.9)	0.040
Miscarriage rate (%)	2/50 (4.0)	2/119 (1.7)	0.365

FET, Frozen embryo transfer

The main objective of this study was to investigate whether a low oocyte maturity ratio in the ICSI cycle can predict the blastocyst euploidy rate. Unfortunately, there was no relationship between low oocyte maturity ratio and the euploidy rate or mosaic rate in this study. This outcome can be attributed to three reasons. First, there were relatively few low oocyte maturity cases, with only 93 cycles and 236 blastocysts for PGT-A. Second, blastocysts from 3PN embryos would lead to a higher proportion of aneuploidy [19]. However, these blastocysts were discarded before PGT-A. Therefore, a low oocyte maturity ratio may not increase the aneuploidy rate caused by 3PN. Third, cases of low oocyte maturity rate (>2 SD below the mean) were relatively rare in our center. Therefore, we defined the low oocyte maturity rate group as those>1 SD below the mean (<71.8%). However, comparison of cases of low oocyte maturity rate (>2 SD below the mean) with the high oocyte maturity ratio group (>1 SD above the mean) may provide more conclusive results.

In this study, we found that the cycles with euploid blastocysts in group A were lower than those in group B. This result is reasonable as the MII oocyte rate and 2PN rates were both lower in the low oocyte maturity rate group, and there were fewer blastocysts for PGT-A in each cycle. Finally, there were more cycles without euploid blastocysts for transfer in group (A) Furthermore, the clinical pregnancy rate was significantly higher in group (B) This result corresponds with those of previous studies [6, 7]. It is known that the antral follicle provides a complex microenvironment for oocyte differentiation that includes three distinct types of somatic cells (theca, granulosa, and cumulus) that support the oocyte during its growth and maturation [20]. Further, the interaction between the surrounding cumulus cells and oocytes has a major contribution to oocyte meiotic and developmental competence [21, 22], including nuclear and cytoplasmic maturation [23]. In fact, oocyte competence is dependent on nuclear and cytoplasmic maturity. Nuclear maturity was validated by extrusion of the first polar body, which was used to clinically evaluate oocyte maturity. However, cytoplasmic maturity is also important, including the accumulation of polyadenylated maternal RNA and proteins necessary for gamete fusion during fertilization and mitotic divisions of the embryo under the control of oocyte mRNA until the embryonic genome is gradually activated [24, 25], but there is currently no available method to evaluate subtle cytosolic and membrane changes in the clinic [26, 27]. Nevertheless, cytoplasmic maturity is known to be linked to the activation of specific genetic and epigenetic signaling pathways

that influence embryonic development and subsequent implantation [28]. We expected the low oocyte maturity group to be accompanied by low cytoplasmic maturity, which may have potentially exerted some negative effects on the cohort of healthy mature oocytes. This factor may also influence the embryo development ability, leading to lower implantation and clinical pregnancy rates.

This study has some limitations. First, as this is a retrospective study, selection bias cannot be excluded. Second, this study had a relatively small sample size; to better understand the relationship between low oocyte maturity and ploidy of blastocysts, a larger sample is required. Third, cases of low oocyte maturity rate (>2 SD below the mean) were relatively rare, and inclusion of cases with extremely low oocyte maturities would better reflect the relationship between low oocyte maturity and the ploidy of blastocysts.

In conclusion, this matched retrospective cohort study suggests that the low number of mature oocytes does not impact the blastocyst euploidy rate. But it may be associated with low fertilization and, clinical pregnancy rates, along with fewer cycles with euploid blastocysts. At last, these results may lead to less chance with cumulative live birth.

Abbreviations

PGT	Preimplantation genetic testing
SD	Standard deviation
MII	Metaphase II
2PN	Two pronuclei
ART	Assisted reproductive technology
ICSI	Intracytoplasmic sperm injection
MI	Metaphase I
GV	Germinal vesicle
IVM	In vitro maturation
IVF	In vitro fertilization
COH	Controlled ovarian hyperstimulation
PGT-A	PGT for aneuploidy
PGT-SR	PGT cycles with chromosomal structural rearrangements
GnRH	Gonadotropin-releasing hormone
rFSH	Recombinant follicle-stimulating hormone
BMI	Body mass index
AMH	Anti-Müllerian hormone
HCG	Human chorionic gonadotropin
TE	Trophectoderm
WGA	Whole genome amplification
CNV	Copy number variations
NC	Natural cycle
HRT	Hormone replacement therapy
FET	Frozen embryo transfer
3PN	Three-pronuclear

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Author contributions
ZHO and XFL- conception and design of the study. ZHO, JD, NQL, XWF, XJW, and JLL- data collection, statistical analysis, construction of figures and tables. ZHO and XFL-drafted the article and revised the article. All- reviewed the manuscript and approved the version to be published.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The studies involving human participants were reviewed and approved by the ethics committee of Boai Hospital of Zhongshan (KY-2023-02-01). The authors have consented for publication.

Conflict of interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Competing interests
The authors declare no competing interests.

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