Cai *et al.*
Reproductive Biology and Endocrinology (2025) 23:31
https://doi.org/10.1186/s12958-025-01371-6

Reproductive Biology and Endocrinology

RESEARCH

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Preimplantation genetic testing for aneuploidy on previously cryopreserved unbiopsied blastocysts: a cohort study in women with IVF pregnancy loss

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Abstract

Research question Does preimplantation genetic testing for aneuploidy (PGT-A) on cryopreserved unbiopsied blastocysts improve pregnancy outcomes for women with previous IVF-related pregnancy loss?

Methods This retrospective observational study included women who underwent vitrified blastocyst warming procedures, with or without trophectoderm biopsy for PGT-A, between January 2016 and June 2023. Participants had experienced two or more clinical pregnancy losses, with at least one loss following in vitro fertilization (IVF). The primary outcome was the cumulative live birth/ongoing pregnancy rate, analyzed using generalized estimating equations (GEE) with confounding adjustments.

Results The cohort included 146 women, comprising 72 who intended to pursue PGT-A on thawed blastocysts (274 blastocysts) and 74 who proceeded directly to frozen embryo transfer (FET) without prior PGT-A (107 blastocysts). Fourteen women in the PGT-A group had no euploid embryos available for transfer. Among these, two patients had no warmed blastocysts suitable for testing, and twelve had all aneuploid embryoid. The cumulative live birth/ongoing pregnancy rate was significantly lower in the PGT-A group compared to the non-PGT-A group (34.7% [25/72] vs. 52.7% [39/74], adjusted odds ratio [AOR] 0.51, 95% confidence interval [CI]: 0.26–0.99, $P=0.048$). Secondary outcomes, including live birth and pregnancy loss rates after initial FET, were comparable between the two groups. Among tested blastocysts, 58 (82.9%) had at least one euploid embryo, resulting in a euploidy rate of 48.6% (125/257).

Conclusions PGT-A on cryopreserved unbiopsied blastocysts reduces cumulative live birth/ongoing pregnancy rates and could not improve pregnancy outcomes following the initial FET cycle in women with a history of IVF pregnancy loss.

Keywords Preimplantation genetic testing for aneuploidy, Cumulative live birth, Cryopreserved blastocysts, Rewarming, Pregnancy loss, Frozen blastocyst transfer

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Introduction

Chromosomal aneuploidy in human gametes and embryos is a significant contributing factor to pregnancy loss or failure in vitro fertilization (IVF) [1, 2]. Given its biological plausibility, recurrent pregnancy loss (RPL) has been recognized as a common indication for preimplantation genetic testing for aneuploidy (PGT-A), aiming to choose IVF embryos with the highest potential for successful implantation [3, 4], although the evidence supporting improvements in live birth rates remains largely of low certainty [5–7].

Couples with a history of pregnancy loss following in vitro fertilization or intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) treatment and who have previously unbiopsied cryopreserved embryos, may wish to warm these embryos for biopsy and testing. This approach is especially relevant for this group of women due to concerns regarding the potentially heightened prevalence of abnormal embryonic karyotypes among embryos from the same oocyte retrieval cycle [8]. However, to date, there is a lack of robust evidence regarding the benefits of PGT-A on the remaining cryopreserved embryos from this patient population [9, 10]. Additionally, the potential impact of biopsy and two rounds of vitrification on implantation of rewarmed euploid blastocysts should be carefully balanced against the necessity of obtaining PGT-A results [11].

Given the controversy, our study aimed to evaluate the potential benefits of PGT-A on previously vitrified cryopreserved blastocysts as an adjunct to subsequent frozen embryo transfer in women experiencing pregnancy losses following IVF treatment. These findings hold significant implications for patient counseling, offering critical insights into the likelihood of achieving a euploid embryo in future pregnancies.

Materials and methods

Study setting and population

This retrospective observational study included women who underwent vitrified blastocyst warming procedures, with or without trophectoderm biopsy and PGT-A, at a single tertiary fertility center in China between January 2016 and June 2023. In China, women with only one pregnancy loss do not meet the clinical indications for PGT-A. Therefore, this study focused on patients with various infertility factors who experienced two or more clinical pregnancy losses, including at least one loss following prior ART treatment.

All patients underwent a comprehensive RPL workup, which included an evaluation of the uterine cavity and blood tests to assess for parental karyotypes, and to detect the presence of hypothyroidism, hyperprolactinemia, and antiphospholipid syndrome. Women who had experienced prior pregnancy losses attributed to aneuploidy, or whose embryonic karyotypes were untested or confirmed as euploid, were eligible in the study. All participants had at least one previous cryopreserved blastocyst available. The exclusion criteria for the study were as follows: chromosomal abnormalities in either member of the couple; donor cycles; women whose embryos underwent PGT for monogenic disorders (PGT-M) or structural rearrangements (PGT-SR); and couples with cleavage-stage embryos that were warmed and extended to blastocyst stage for biopsy. Women with factors related to immunologic disorders including thyroid dysfunction, antiphospholipid syndrome, and uterine malformation were also excluded.

Women who underwent PGT-A on warmed embryos were categorized as the PGT-A group. Those who underwent subsequent frozen embryo transfer (FET) of morphologically graded embryos without biopsy and aneuploidy testing during the same time period were classified as the non-PGT-A group.Follow-up of subsequent re-thawing cycles was conducted until December 2024. The first live birth resulting from the thawing of blastocysts in the frozen cycle cohort was documented for each patient. Patient and clinical data were collected from the Northwest Women's and Children's Hospital in Xi'an, China. The study was approved by the Institutional Review Board (IRB) (No. 2023003) and conforms to the provisions of the Declaration of Helsinki.

Embryo transfer procedures

The technical procedures for embryo scoring, vitrification, warming, and transfer at our center have been previously documented [12]. All blastocysts were graded at first cryopreservation using modified Gardner and Cornell's criteria [13]. Blastocysts with Gardner's A or B grades for inner cell mass (ICM) and trophectoderm (TE) were defined as good quality blastocysts and any grade lower than this was categorised as fair quality. Throughout the study period, we consistently thawed all vitrified blastocysts and conducted biopsies for patients undergoing aneuploidy testing on cryopreserved embryos.

Embryos were thawed on the morning of the transfer day, only the surviving blastocysts that were fully re-expanded and had sufficient quality, with no signs of degeneration, were suitable to undergo a biopsy. Expanded blastocysts were biopsied around 3 pm using a noncontact laser to remove five to ten trophoblast ectoderm cells. Following biopsy, all blastocysts underwent revitrification. The biopsied specimens were subjected to analysis using next-generation sequencing (NGS). The process of library preparation and sequencing followed previously established methods [14]. Briefly, two amplification steps, including preamplification and exponential

amplification, were carried out according to the manufacturer's instructions (ChromInst, Xukang Medical Science & Technology Co., Ltd). Library quantification for sample pooling was performed using quantitative real-time polymerase chain reaction, followed by sequencing on an Illumina NGS platform.

FET cycles were carried out using different protocols based on the women's ovulatory status [15]. Natural cycles (NC) were employed for ovulatory women, while hormone replacement treatment (HRT) cycles, with or without gonadotropin-releasing hormone (GnRH) agonist suppression, were utilized for anovulatory women. In a natural cycle, blastocyst transfer was performed on the fifth day after ovulation, whereas in a HRT cycle with or without GnRH agonist suppression, it was conducted on the sixth day of progesterone supplementation.

Within the PGT-A group, a single euploid blastocyst was rewarmed and transferred during each cycle [16]. When multiple euploid blastocysts were available, the blastocyst with the highest morphological score was prioritized for rethawing. In the non-PGT-A group, embryos were selected for transfer based solely on their morphologic features, following the scoring procedure outlined above. While a maximum of two embryos could be transferred, preference was given to a single blastocyst transfer The embryo transfer procedure was guided by transabdominal ultrasound after confirming embryo survival post-thawing. Luteal phase support was maintained until the 10th week of pregnancy.

Outcomes

The primary outcome was defined as the cumulative rate of live births or ongoing pregnancies achieved within 18 months following warming of unbiopsied cryopreserved blastocysts. Secondary outcomes included the cumulative incidences of biochemical pregnancy, clinical pregnancy, and pregnancy loss. Additionally, pregnancy outcomes following the first FET and aneuploidy rates were assessed. Live birth was defined as the delivery of a fetus exhibiting signs of life. Ongoing pregnancy was defined as a viable intrauterine pregnancy after 12 weeks of gestation. Biochemical pregnancy was defined as serum beta human chorionic gonadotrophin (hCG) > 20 IU/L after 12 days of transfer. Uterine clinical pregnancy referred to at least one uterine gestational sac observed at ultrasonography. Pregnancy loss was defined as spontaneous demise of a pregnancy before 24 weeks of gestation.

Statistical analysis

Continuous variables are expressed as median and interquartile range and categorical values are presented as percentages. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using generalized estimating equations (GEE) with a robust variance estimate, accounting for the non-independence of repeated cycles from individual patients. The outcomes of all women who intended to undergo PGT-A on cryopreserved blastocysts were analyzed, including those whose embryos did not survive post-thawing or who had no euploid embryos available for transfer. The results were adjusted for potential confounders, such as maternal age at oocyte retrieval and embryo transfer, number of previous pregnancy losses, presence of aneuploidy in products of conception, embryo quality, and the number of blastocysts transferred. A p-value < 0.05 was considered statistically significant.

Results

A total of 146 couples were included in the analysis, with 72 undergoing PGT-A of the remaining cryopreserved embryos, while 74 were offered conventional FET management without testing for aneuploidy (Non PGT-A) (Fig. 1). The mean age of women at the time of oocyte retrieval was 35 years. The majority of women in both groups had experienced two previous pregnancy losses (61.1% in the PGT-A group and 58.1% in the non-PGT-A group), with no significant difference observed between the two groups. Compared with the non PGT-A group, the PGT-A group had a higher rates of history of aneuploid loss (68.1% vs 39.2%, $P < 0.01$). There were no significant differences between the two groups in other characteristics between the two groups (Table 1).

PGT results

In the PGT-A cohort, 93.8% (257/274) of vitrified-warmed blastocysts survived the warming process, re-expanded, and were suitable for biopsy. Two women in the PGT-A group had no blastocysts available for genetic testing after warming. Among those with blastocysts tested, 82.9% women (58/70) had at least one euploid embryo for transfer, corresponding to an overall euploidy rate of 48.6% (125/257). A total of 12 patients had no euploid embryos available after testing. Among these, 11 women had embryos that were all aneuploid, while one patient had two aneuploid embryos and one mosaic embryo. Mosaic embryos were diagnosed in 5.8% of embryos and there were no mosaic embryo transfers.

Clinical outcomes

In the PGT-A group, among women with transferable euploid embryos, one patient had not undergone embryo transfer due to divorce (Fig. 1). By the end of the follow-up period, 71 FET cycles had been performed in the PGT-A group, compared to 90 FET cycles in the non-PGT-A group (Table 2). The average number of

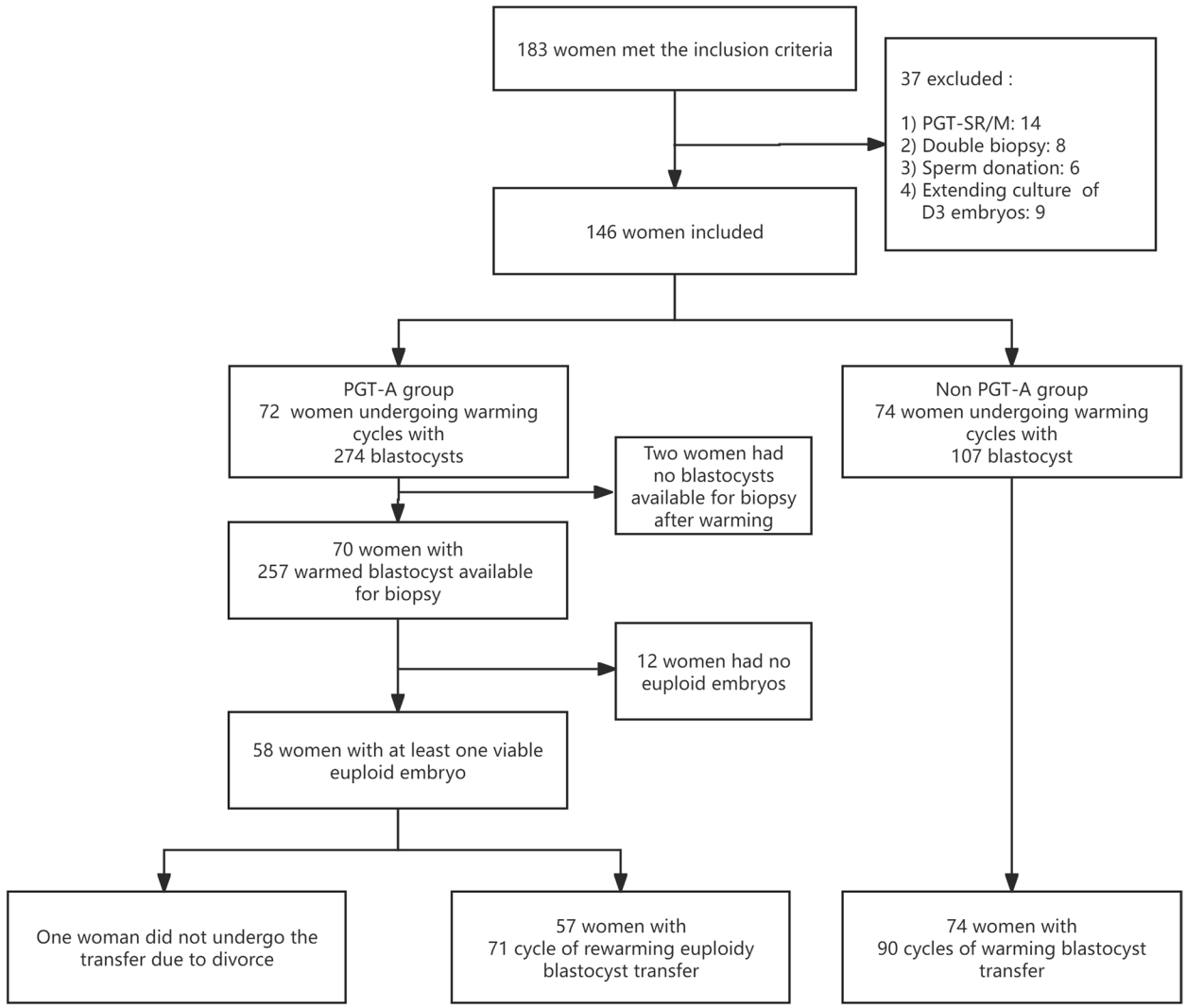

Fig. 1 Flowchart of study cohort selection

blastocysts transferred was significantly higher in the non-PGT-A group with 1.2 embryos compared to 1.0 embryos transferred in the PGT-A group ($P<0.01$). In the PGT-A group, only one cycle involved double blastocyst transfer, compared to 17.8% of cycles in the non-PGT-A group. The proportions of Day 5 blastocysts and good-quality embryos transferred were comparable between the two groups.

The cumulative live birth/ongoing pregnancy rate was significantly lower in the PGT-A group compared to the non-PGT-A group (34.7% [25/72] vs. 52.7% [39/74]; adjusted odds ratio [AOR]: 0.51, 95% confidence interval [CI]: 0.26–0.99, $P=0.048$). The sustained implantation rate resulting in live birth per thawed blastocyst was 25/274 (9.1%) in the PGT-A group, compared to 39/107 (36.4%) in the non-PGT-A group ($P<0.001$) (Fig. 1). However, no significant differences were observed between the PGT-A and non-PGT-A groups in terms of the cumulative incidence of clinical pregnancy (AOR: 0.61, 95% CI: 0.30–1.22) or total pregnancy loss (AOR: 1.00, 95% CI: 0.46–2.15) (Table 3). Additionally, reproductive outcomes following the initial cycle of FET, including live birth rates and pregnancy loss rates, were comparable between the two groups (Table 3). When restricted to women undergoing single blastocyst transfers, there was also no significant difference in pregnancy outcomes following the first FET between the two groups (Table 4). Furthermore, we conducted a subgroup analysis stratified by embryonic status in women with previous pregnancy losses (Fig. S1). In both subgroups, women undergoing PGT-A exhibited lower outcomes, which aligns with the results of the primary analysis. However, significant differences were observed only among women without a history of aneuploidy loss.

Table 1 Demographic characteristics of women in the PGT-A and non-PGT-A groups

	PGT-A (N = 72)	Non PGT-A (N = 74)	*P* value
Female age at retrieval (y, mean ± SD)	34.9 ± 5.0	34.8 ± 3.2	0.83
Female age categories, y			0.06
< 35	32 (44.4%)	35 (47.3%)	
35–37	16 (22.2%)	26 (35.1%)	
≥ 38	24 (33.3%)	13 (17.6%)	
Male age at retrieval (y, mean ± SD)	36.4 ± 6.0	35.7 ± 3.0	0.33
Body mass index (kg/m^2, median, IQR)	22.5 (20.7–25.1)	22.8 (20.7–24.7)	0.85
Infertility duration (y, median, IQR)	2 (1–3)	2 (1–3)	0.63
Gravidity			1.00
2	36 (50.0%)	37 (50.0%)	
≥ 3	36 (50.0%)	37 (50.0%)	
Parity			0.09
0	58 (80.6%)	67 (90.5%)	
≥ 1	14 (19.4%)	7 (9.5%)	
Previous pregnancy losses			0.71
2	44 (61.1%)	43 (58.1%)	
≥ 3	28 (38.9%)	31 (41.9%)	
At least one instance of chromosomal abnormality detected by pregnancy or fetal tissue	49 (68.1%)	29 (39.2%)	< 0.01
Smoking or passive smoking	11 (15.3%)	15 (20.3%)	0.43
Cause of subfertility[a]			0.39
Tubal factor	27 (37.5%)	35 (47.3%)	
Ovulation disorder	10 (13.9%)	4 (5.4%)	
Endometriosis gradel/II	4 (5.6%)	5 (6.8%)	
Male factor	22 (30.6%)	17 (23.0%)	
Unexplained	7 (9.7%)	11 (14.9%)	
Other	2 (2.8%)	2 (2.7%)	
Insemination method			0.32
IVF	59 (81.9%)	65 (87.8%)	
ICSI	13 (18.1%)	9 (12.2%)	
AFC, median, IQR	14 (9–18)	12 (8–18)	0.26
AMH (ng/mL, median, IQR)	3.2 (1.8–4.5)	2.5 (1.6–5.1)	0.39
Stimulation protocol			0.65
Agonist	28 (38.9%)	26 (35.1%)	
Depot agonist	22 (30.6%)	20 (27.0%)	
Antagonist	22 (30.6%)	28 (37.8%)	
Total blastocysts cryopreserved (median, IQR)	4 (3–5)	3 (2–4)	0.06
Good quality blastocysts cyopreserved (median, IQR)[b]	1 (0–2)	1 (0–2)	0.20

Data are mean ± SD or median (IQR) or n (%)

PGT-A Preimplantation genetic testing for aneuploidy, *AFC* Antral follicle count, *AMH* Anti-Müllerian Hormone, *IVF* in-vitro fertilization, *ICSI* Intracytoplasmic sperm injection

[a] Shown with a maximum of one event per woman

[b] Blastocysts with Gardner's A or B grades for ICM and TM

Discussion

According to on our data, PGT-A on cryopreserved unbiopsied blastocysts reduces cumulative live birth/ongoing pregnancy rates across subsequent FET cycles in women with a history of IVF pregnancy loss. In addition, no improvement in pregnancy outcomes was observed in the initial FET cycle following the application of PGT-A.

Although PGT-A is designed to test for aneuploidy, a feature particularly relevant for women with RPL, available evidence indicates that its value in predicting live

Table 2 Parameters of laboratory and transfer cycles between the PGT-A and non-PGT-A groups

	PGT-A	Non PGT-A	*P*- value
No. of Patients	72	74	
Women with surviving blastocysts for testing or transfer after warming	70	74	
Tested blastocysts/total number of surviving blastocysts	257/274 (93.8)	–	
Ploidy status of embryos tested			
Euploid (%)	125/257 (48.6)	–	
Aneuploid (%)	117/257 (45.5)	–	
Mosaic (%)	15/257 (5.8)	–	
Women with at least one euploid embryo	58	–	
Cycles of FET	71	90	
Female age at transfer (y, mean ± SD)	34.5 ± 4.4	33.8 ± 3.3	0.21
Endometrial thickness (mm, median, IQR)	9.2 (8.5–10.4)	9.2 (8.5–10.6)	0.65
Endometrial preparation, n (%)			0.52
Natural cycle	27 (38.0)	37 (41.1)	
HRT	27 (38.0)	38 (42.2)	
GnRH-a + HRT	17 (23.9)	15 (16.7)	
No. of blastocyst transferred	1.0 ± 0.1	1.2 ± 0.4	< 0.01
1, n (%)	70 (98.6)	74 (82.2)	< 0.01
2, n (%)	1 (1.4)	16 (17.8)	
Cycles with good-quality blastocysts transferred* (%)	38/71 (46.9)	42/90 (46.7)	0.97
Cycles with D5 blastocysts transferred (%)	61/71 (85.9)	76/90 (84.4)	0.76

Data are mean ± SD or median (IQR) or n (%)

PGT-A Preimplantation genetic testing for aneuploidy, *FET* Frozen embryo transfer, *NC* Natural cycle, *HRT* Hormone replacement treatment

* Blastocysts with Gardner's A or B grades for ICM and TM

Table 3 Clinical outcomes between the PGT-A and non-PGT-A groups

	PGT-A group	Non- PGT-A group	Multivariate OR (95% CI)	Adjusted *P*-value
Cumulative outcomes	N = 72	N = 74		
Live birth/ongoing pregnancy	25 (34.7%)	39 (52.7%)	0.51 (0.26, 0.99)	0.048
Biochemical pregnancy	46 (63.9%)	57 (77.0%)	0.53 (0.26, 1.10)	0.089
Clinical pregnancy	43 (59.7%)	53 (71.6%)	0.61 (0.30, 1.22)	0.159
Total pregnancy loss[a]	19 (26.4%)	18 (24.3%)	1.00 (0.46, 2.15)	0.994
Outcomes after the first cycle of FET	N = 57[b]	N = 74		
Live birth	21 (36.8%)	33 (44.6%)	0.96 (0.44, 2.07)	0.912
Biochemical pregnancy	37 (64.9%)	49 (66.2%)	1.12 (0.53, 2.37)	0.767
Clinical pregnancy	35 (61.4%)	47 (63.5%)	1.04 (0.49, 2.17)	0.925
Total pregnancy loss[a]	15 (26.3%)	16 (21.6%)	1.31 (0.55, 3.12)	0.537

Adjusted for the age of women at oocyte retrieval and embryo transfer, the number of pregnancy losses, embryonic status in previous losses, embryo quality, and the number of blastocysts transferred

ET Embryo transfer, *CI* confidence interval, *PGT-A* Preimplantation genetic testing for aneuploidy

[a] Excluding ectopic pregnancies

[b] One patient with euploidy blastocysts did not undergo embryo transfer due to divorce

birth outcomes remains of low quality [4]. A retrospective analysis of the SART-CORS database, which compared couples with RPL undergoing FET with or without PGT-A, demonstrated a significantly higher live birth rate across all age categories in the PGT-A group after adjustment for confounding factors, while no differences were observed in pregnancy loss rates [3]. Nonetheless, it should be noted that the SART-CORS data were presented on a per-embryo-transfer basis and did not account for women in the PGT-A group who initiated

Table 4 Clinical outcomes after the initial FET limited to the transfer of a single blastocyst

	PGT-A group	Non PGT-A group	Multivariate OR (95% CI)	Adjusted *P*-value
No. of cycles	*N* = 57	*N* = 63		
Biochemical pregnancy	37 (64.9%)	39 (61.9%)	0.88 (0.42, 1.87)	0.741
Clinical pregnancy	35 (61.4%)	38 (60.3%)	0.96 (0.44, 2.09)	0.916
Total pregnancy loss[a]	15 (26.3%)	13 (20.6%)	0.74 (0.31, 1.76)	0.502
Live birth	21 (36.8%)	26 (41.3%)	1.10 (0.50, 2.39)	0.815

Adjusted for the woman's age at oocyte retrieval and embryo transfer, number of pregnancy losses, embryonic status in previous losses, and embryo quality

FET Frozen embryo transfer, *CI* Confidence interval, *PGT-A* Preimplantation genetic testing for aneuploidy

[a] One woman was diagnosed with an ectopic pregnancy

a cycle but failed to achieve a euploid embryo for transfer, potentially introducing selection bias. Theoretically, as revealed in the literature, PGT-A does not improve the cumulative live birth rate, since its primary purpose is to optimize the embryo selection process rather than directly enhance embryo quality [5, 17]. Murugappan et al. investigating the use of PGT-A in cases of unexplained RPL reported comparable live birth rates (63% vs. 68%) and pregnancy loss rates (18% vs. 25%) between patients treated with PGT-A and those who are expectantly managed [18]. Different from the above study, all women included in our study were infertile, had IVF indications, and were undergoing FET cycles.

The efficacy of PGT-A in women with RPL has been a longstanding subject of debate, with the additional complexity introduced by double vitrification further complicating the understanding of its implication. Our data indicated that PGT-A did not increase but decreased the cumulative live birth/ongoing pregnancy outcomes. The sustained implantation rate leading to live birth per thawed blastocyst was 9.1% for the PGT-A group, whereas the corresponding rate for the non-PGT-A group was 36.4%. These results are consistent with the published theoretical model, which demonstrated the superiority of non-PGT embryo transfers over PGT blastocyst transfers in terms of cumulative live birth rates [19]. A key contributing factor could be the adverse impact of biopsy combined with two rounds of vitrification on embryonic viability. In our previous study, we demonstrated that double vitrification-warming combined with a single biopsy significantly reduces live birth rates and increases pregnancy loss compared to a single round of warming and biopsy [14]. Supporting these findings, a recent systematic review and meta-analysis by Bickendorf et al. underscored the detrimental effects of combining a single biopsy with double vitrification on clinical outcomes following euploid blastocyst transfer. Specifically, the meta-analysis revealed significant reductions in clinical pregnancy rates (six studies, n = 13,284; RR = 0.84, 95% CI: 0.76–0.92) and live birth/ongoing pregnancy rates (seven studies, n = 16,800; RR = 0.79, 95% CI: 0.69–0.91), alongside a notable increase in miscarriage rates (five studies, n = 15,781; RR = 1.48, 95% CI: 1.31–1.67) [10]. Another possible explanation for our findings is that we did not perform mosaic embryo transfers in our study. There is growing evidence demonstrating that live births can result from the transfer of mosaic embryos, albeit with lower implantation rates and increased miscarriage rates, depending on percent and type of mosaicism [20, 21]. Their exclusion may have further contributed to the observed outcomes, potentially underestimating the success rates associated with embryo transfer.

Contrary to expectations, our results show that PGT-A did not improve pregnancy rates or reduce pregnancy loss rates in the initial FET cycle. The comparable outcomes observed between groups after the initial FET cycle could be explained by the higher prevalence of double blastocyst transfers in the Non PGT-A group, which may have compensated for the reduced success rates associated with embryonic aneuploidy. However, a sub-analysis restricted to single blastocyst transfers demonstrated that this factor did not significantly influence outcomes.

In the current cohort of PGT-A, 45.5% of all tested blastocysts were classified as aneuploid. This aneuploidy rate demonstrated a strong correlation with advancing maternal age. However, no significant differences were noted in the aneuploidy rates between individuals with a history of aneuploid pregnancy losses and those without. Although the role of aneuploidy in pregnancy loss is well established, its significance in RPL remains less clear. Some earlier studies reported higher aneuploidy rates in patients with RPL [22, 23]; however, more recent studies have reported comparable rates [24, 25]. The aneuploidy rate observed in our study was found to be similar to the fetal chromosomal anomalies reported in sporadic miscarriages [26, 27]. The systematic review of 19 studies investigating the cytogenetic findings of pregnancy tissue after miscarriage revealed that the pooled prevalence of

fetal chromosomal anomalies in sporadic miscarriages was 45% [26]. This was further corroborated by another study, which demonstrated that the aneuploidy rates in products of conception from patients with RPL were not significantly higher than those observed in sporadic miscarriages [1]. This suggests that factors other than embryonic genetic anomalies may contribute to RPL. Therefore, definitive evidence regarding the benefits of PGT-A in this patient population is still needed.

Strengths and limitations

This study evaluates common treatment strategies for women who experienced pregnancy loss following IVF and had cryopreserved, unbiopsied blastocysts. Unlike previous research that primarily focused on outcomes following the transfer of euploid embryos, our analysis provides a comprehensive examination of cumulative live birth rates associated with two distinct treatment approaches, offering broader insight into available options.

However, several limitations should be acknowledged. First, due to the relatively small sample size, a post hoc power analysis was conducted, revealing a study power of approximately 0.61 for the cumulative live birth rate. In contrast, the power for secondary outcomes was suboptimal. For example, the cumulative incidence of clinical pregnancy (AOR: 0.61, 95% CI: 0.30–1.22) exhibited a power of only 0.33. The limited sample size increases the risk of type II errors, potentially leading to false-negative findings. To further validate these results, future prospective studies with larger cohorts are necessary. Nonetheless, the insights gained from this study are valuable for counseling IVF patients with a history of two or more pregnancy losses, particularly regarding the likelihood of achieving a live birth and other related pregnancy outcomes. Second, the retrospective design of this study introduces potential biases. Women in the PGT-A group exhibited a higher incidence of previous aneuploid losses compared to those in the non-PGT-A group. This finding aligns with current clinical practice, as there is no established recommendation for the use of PGT-A in couples with unexplained RPL without identified chromosomal abnormalities [5, 28]. Additionally, both single and double blastocyst transfers were included, with a higher proportion of women in the non-PGT-A group undergoing double blastocyst transfer. To mitigate these biases, extensive efforts were made, including the use of multivariable regression models and subgroup analyses stratified by embryonic status of prior losses, with a focus on single blastocyst transfers. While these approaches helped control for various confounding factors, we acknowledge that some residual confounding may still persist. Therefore, prospective studies with larger and more homogeneous cohorts are needed to confirm these findings and further elucidate the outcomes. Finally, it is important to note that not all blastocysts were utilized before achieving a live birth during the study period, which may have influenced the results.

Conclusion

PGT-A on cryopreserved unbiopsied blastocysts may not offer benefits for women in this specific cohort and could potentially be detrimental when assessing cumulative live birth rates. Well-designed prospective trials comparing FET with PGT-A versus non-PGT-A on unbiopsied cryopreserved blastocysts in this specific patient population are warranted.

Abbreviations

PGT-A	Preimplantation genetic testing for aneuploidy
RPL	Recurrent pregnancy loss
FET	Frozen embryo transfer
PGT-M	Preimplantation genetic testing for monogenic disorders
PGT-SR	Preimplantation genetic testing for structural rearrangements
NC	Natural cycles
HRT	Hormone replacement treatment
IVF	In vitro fertilization
ICSI	Intracytoplasmic sperm injection
ET	Embryo transfer
ICM	Inner cell mass
TE	Trophectoderm
NGS	Next-generation sequencing
GnRH	Gonadotropin-releasing hormone
hCG	Human chorionic gonadotrophin
GEE	Generalized estimating equations

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12958-025-01371-6.

Supplementary Material 1.

Acknowledgements

The authors wish to thank all of the staff from Northwest Women's and Children's Hospital for their assistance with the data collection, and we thank all of the patients in this study.

Clinical trial number

Not applicable.

Consent to participate

Not applicable.

Authors' contributions

H.C. and J.S. participated in the design of the study. H.B., X.X., W.L., D.W contributed to data acquisition, analyses and data interpretation. H.C and M.K participated in writing the manuscript. D.Z., J.S., H.B., X,W and W.L revised the manuscript. All authors read and approved the final manuscript.

Funding

This study was funded by Science and Technology Department of Shaanxi Province, China (S2022-YF-YBSF-0517) and Natural Science Basic Research Program of Shaanxi (Program No.2024JC-YBMS-677).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study protocol was approved by the Northwest Women's and Children's Hospital Internal Review Board, which granted a waiver regarding the requirement for written informed consent (approval number 2023003).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 27 January 2025 Accepted: 21 February 2025
Published online: 03 March 2025

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