scientific reports

Check for updates

OPEN

Comparative clinical and reproductive outcomes following non-invasive versus biopsy-based preimplantation genetic testing for aneuploidy: a multicentric cohort study

Divreena Talwar[1], Naval Shah[2]✉, Marcos Meseguer[3]✉, Ritesh Aggarwal[2], Kshitiz Murdia[4], Nitiz Murdia[2], Bhagwat Singh[5], Sourav Rathore[2] & Vipin Chandra[4]

Non-invasive preimplantation genetic testing for aneuploidy (niPGT-A) is emerging as a newer technology alongside conventional trophectoderm biopsy-based PGT-A, aiming to avoid embryo biopsy while enabling genetic assessment through analysis of cell-free DNA. However, its clinical utility in improving reproductive outcomes remains uncertain, particularly in real-world settings. This retrospective multicentric cohort study evaluated reproductive outcomes following niPGT-A and conventional PGT-A in 6,215 patients underwent intracytoplasmic sperm injection across 48 centres of our organisation between January 2023 and July 2024, comprising 15,635 tested blastocysts. Clinical outcomes were analysed in 3,179 frozen embryo transfer (FET) cycles, and multivariable logistic regression was performed to adjust for confounding factors. Across both the overall cohort and euploid FETs, PGT-A was associated with improved reproductive outcomes compared with niPGT-A. Live birth rates were significantly higher following PGT-A in the overall cohort (46.2% vs. 34.5%) and among euploid embryo transfers (46.5% vs. 34.4%), accompanied by significantly higher clinical and ongoing pregnancy rates. These findings remained consistent across single- and double embryo transfers reproductive outcomes. Additionally, after confounders adjustment, PGT-A was still significantly associated with approximately twofold higher odds of clinical pregnancy and live birth. Collectively, these findings suggest that niPGT-A needs further refinement to improve its reliability and clinical efficacy for embryo selection.

Keywords Non-invasive PGT-A, Preimplantation genetic testing for aneuploidy, Preimplantation genetic diagnosis, Euploid PGT-A transfers, Spent culture media, Trophectoderm biopsy

Preimplantation genetic testing (PGT) has become an established component of assisted reproductive technology (ART), with ongoing developments in both its clinical application and underlying methodologies. It is used in patients with or without known chromosomal abnormalities, with the aim of aiding in embryo selection which potentially may reduce time to pregnancy for advanced maternal age (AMA) women[1]. Various biopsy-based approaches, including polar body, cleavage-stage, and trophectoderm (TE) biopsy, have been employed for embryonic chromosomal assessment, demonstrating variable clinical outcomes across different settings. Currently, next-generation sequencing (NGS) following TE biopsy of approximately 5–8 cells represent the most widely adopted approach for preimplantation genetic testing for aneuploidy (PGT-A)[2]. The incorporation

[1]Research & Publications, Indira IVF Hospital Limited, Udaipur, India. [2]Embryology, Indira IVF Hospital Limited, Udaipur, India. [3]IVIRMA Global Research Alliance, IVI Foundation, Instituto de Investigación Sanitaria La Fe (IIS La Fe), Valencia, Spain. [4]Reproductive Medicine, Indira IVF Hospital Limited, Udaipur, India. [5]Management Information System, Indira IVF Hospital Limited, Udaipur, India. ✉email: naval.shah@indiraivf.in; meseguer.escriva@gmail.com

of PGT-A into clinical practice has shifted embryo selection towards chromosomal assessment alongside conventional morphological evaluation, which remains inherently subjective and operator-dependent[3].

Despite its widespread clinical application, TE biopsy remains an invasive procedure requiring specialized equipment and technical expertise, contributing to increased procedural complexity and cost[4,5]. When performed in experienced laboratories, however, TE biopsy has generally not been associated with adverse effects on embryo viability or clinical outcomes. Nonetheless, whether the chromosomal constitution of the TE accurately represents that of the entire embryo remains a subject of ongoing investigation. Several studies have reported concordance between the chromosomal status of the TE and the inner cell mass (ICM)[6–8], whereas others suggest preferential allocation of euploid cells to the ICM[9]. Furthermore, the presence of biological mosaicism adds an additional layer of complexity to the interpretation of genetic results derived from a limited number of biopsied cells[10]. Collectively, these biological and technical considerations have prompted the exploration of alternative strategies for embryo genetic assessment.

Non-invasive preimplantation genetic testing for aneuploidy (niPGT-A) has recently emerged as a potential alternative approach for embryo genetic assessment. This method is based on the analysis of cell-free embryonic DNA (cfDNA) released into the spent culture medium (SCM), enabling prediction of embryo ploidy without direct embryonic manipulation. The presence of embryonic cfDNA in culture media has been demonstrated in several investigations, supporting the feasibility of genetic assessment via cfDNA[11,12]. Subsequently, investigations have explored the concordance between SCM-derived DNA and TE biopsy results and demonstrated varying levels of concordance ranging from 3.5% to 72.2%[13–16]. In contrast, higher concordance rates of up to 85.7%, along with successful clinical outcomes, have been observed in specific patient populations, such as those with recurrent pregnancy loss and reciprocal translocations[17]. Furthermore, Huang et al., 2019[18], using NGS, reported a zero false-negative rate and higher positive predictive value and specificity for niPGT-A compared with TE biopsy.

More recently, a large multicentre prospective study evaluating 1,301 human blastocysts reported a concordance rate of 78.2% between embryonic cfDNA and TE biopsy results. Additional analyses of donated aneuploid blastocysts demonstrated concordance rates of 87.5% with TE biopsy and 84.4% with the ICM, suggesting that cfDNA may reflect chromosomal information originating from both embryonic compartments[19]. These findings have supported the development and increasing clinical adoption of niPGT-A. However, despite encouraging concordance data, evidence regarding the clinical validity of niPGT-A and its impact on reproductive outcomes, including live birth, remains limited and inconsistent, with no robust data comparing clinical outcomes of niPGT-A with TE biopsy-based PGT-A.

Therefore, this multicentric study aimed to evaluate the real-world clinical effectiveness of niPGT-A for embryo selection in assisted reproduction. Using a large cohort of 6,215 patients and 15,635 tested blastocysts, reproductive outcomes from biochemical pregnancy to live birth were compared between niPGT-A and TE biopsy following frozen embryo transfer (FET). Through assessment of clinically meaningful outcomes, this study sought to determine whether niPGT-A achieves reproductive outcomes comparable to TE biopsy-based PGT-A in routine clinical practice.

Materials and methods

Ethics statement

This retrospective multicentre cohort study was approved by the Institutional Review Board and waived from Indira IVF Hospital Institutional Ethics Committee, Udaipur (Approval No.: IIVF-UA00091). All procedures and analyses were conducted in accordance with the ethical standards of the responsible institutional committee and the principles of the Declaration of Helsinki and its subsequent amendments.

Study subjects and design

This retrospective multicentric comparative cohort study was conducted across 48 centres of Indira IVF Hospital Limited, India, between January 2023 and July 2024, including 6,215 intracytoplasmic sperm injection (ICSI) cycles yielding 15,635 tested blastocysts. Among these, 3,560 patients with 9,269 blastocysts underwent niPGT-A, while 2,655 patients with 6,366 blastocysts underwent conventional PGT-A. Genetic testing was offered to patients meeting one or more of the following indications: AMA (female age ≥ 35 years), recurrent pregnancy loss (RPL; two or more consecutive clinical pregnancy losses), or recurrent implantation failure (RIF; failure to achieve implantation following the transfer of two or more good-quality blastocysts as defined by Gardner and Schoolcraft grading system[20] as per Indian ART Act bill[21]. A scheme of the study design is demonstrated in Fig. 1.

Study population

Eligible women were 21–50 years and men 21–55 years undergoing ICSI with autologous sperm. ICSI cycles with both autologous and donated oocytes were included for this investigation if medical indications for genetic testing existed. Both single- and double-vitrified PGT-A and niPGT-A cycles were included. Single-vitrified cycles were defined as embryos that underwent TE biopsy for PGT-A or SCM collection for niPGT-A during the fresh embryo culture post oocyte retrieval and ICSI, followed by a vitrification. Double-vitrified cycles were defined as previously cryopreserved embryos that were subsequently warmed for TE biopsy for PGT-A analysis or 24-hour culture with SCM collection for niPGT-A reporting and were subsequently re-vitrified. Exclusion criteria were abnormal parental karyotypes, conventional IVF, donor sperm, preimplantation genetic testing for monogenic disorders (PGT-M), preimplantation genetic testing for structural rearrangements (PGT-SR), or untreated uterine anomalies (e.g., uterus didelphys, arcuate, unicornuate, bicornuate, septate, or absent uterus).

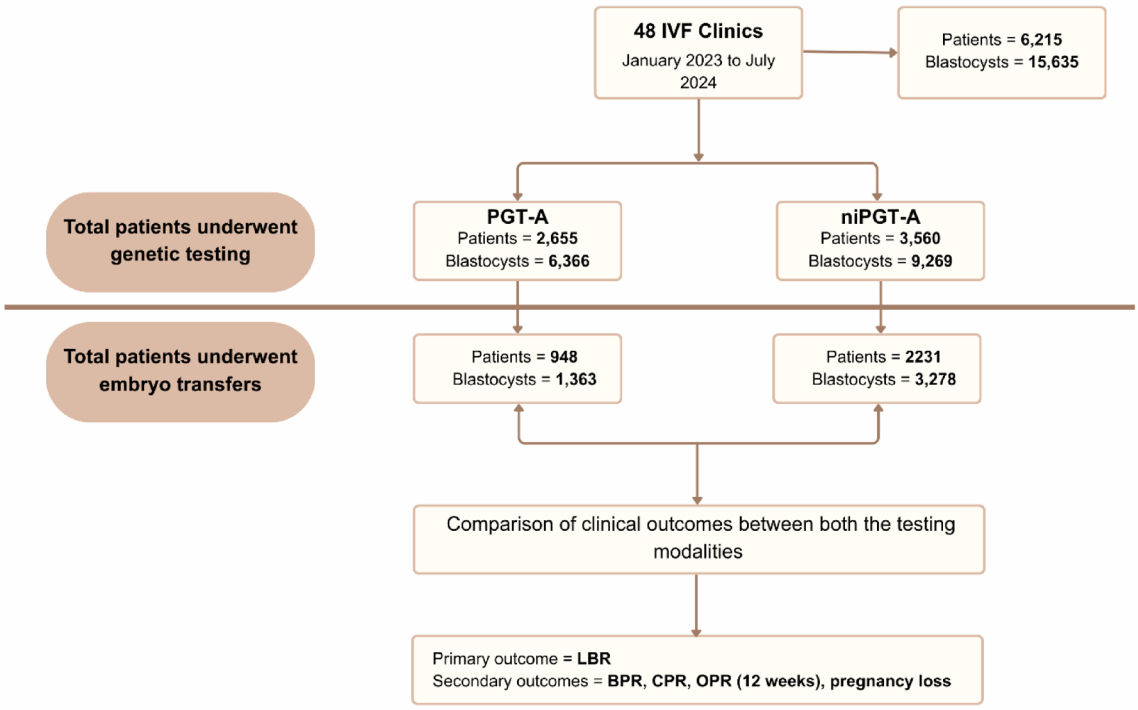


Fig. 1. Scheme of study design.

Oocyte stimulation, oocyte retrieval and ICSI

Controlled ovarian stimulation was performed using recombinant follicle-stimulating hormone (rFSH) (Intas Pharmaceutical, India) in combination with a gonadotropin-releasing hormone (GnRH) antagonist protocol (BDR Pharmaceutical, India). Follicular development was monitored by transvaginal ultrasonography, and final oocyte maturation was triggered using either 250 µg recombinant human chorionic gonadotropin (rhCG) (Bharat Serums and Vaccines, India) or a GnRH agonist when at least three follicles reached a diameter of 18–20 mm. Oocyte retrieval was performed 36 h after trigger under transvaginal ultrasound and retrieved oocytes were cultured in a single-step culture medium (Vitromed, Germany) at 37 °C under 6% CO_2 and 6% O_2. Cumulus–oocyte complexes were denuded approximately 38 h post-trigger using hyaluronidase. ICSI was performed approximately 40 h after trigger using HEPES-buffered handling medium (Vitromed, Germany) on an ICSI workstation equipped with micromanipulators (Shivani Scientific, India). Sperm preparation was performed using either density gradient centrifugation (PureSperm, Prime Healthcare, India) or the swim-up method (Vitromed, Germany) depending on semen parameters. All embryological procedures, including oocyte retrieval, denudation, and ICSI, were conducted in temperature-controlled IVF chambers equipped with HEPA and activated carbon filtration systems (Shivani Scientific, India).

Embryo culture and sample collection

After ICSI, embryos were cultured in one-step medium (Vitromed, Germany) under controlled laboratory conditions (37 °C and 6% CO_2). Fertilization was confirmed by the presence of two pronuclei 17–18 h post-injection. Embryos were then cultured to the blastocyst stage (day 5 or day 6), with morphology assessed using the Gardner and Schoolcraft grading system[20].

For PGT-A, Day 5 or Day 6 blastocysts were graded according to the Gardner and Schoolcraft grading system[20] and good-quality (AA, AB, BA) and fair-quality (BB) blastocysts underwent TE biopsy. TE biopsy was performed using standard micromanipulation techniques. Briefly, 5–8 TE cells were isolated using laser-assisted cutting and flicking methods, sequentially washed and tubed along with 2 µL of PBS into RNase- and DNase-free PCR tubes already containing 2 µL of lysis buffer. The blastocyst post tubing was vitrified, and samples were labelled with unique identifiers, maintained under contamination-controlled conditions, stored at − 20 °C before transportation to the genetic laboratory for chromosomal analysis.

For niPGT-A, embryos were cultured until day 3 and subsequently washed before transfer into fresh culture dishes containing 22 µL droplets of one-step medium. Embryos were maintained in individual culture until day 5 or day 6, after which 20 µL of SCM was collected for genetic analysis. Samples were handled under sterile conditions, transferred into RNase- and DNase-free PCR tubes, stored at −20 °C, before transportation to the genetic laboratory.

In both testing approaches, embryos were vitrified immediately following sample collection.

Preimplantation genetic testing counselling

Prior to PGT, all patients received standardized genetic counselling regarding the purpose, potential benefits, and limitations based on currently existing literature. During counselling, patients were informed about both testing modalities – TE biopsy-based PGT-A and niPGT-A – including their methodological differences, cost, available validation data, and limitations reported in the literature. Following counselling, the choice of testing modality was made by the patients in consultation with their treating clinician. Based on patient preference, they underwent niPGT-A or PGT-A.

Preimplantation genetic testing

Both TE biopsy and niPGT-A samples were processed in a National Accreditation Board for Testing and Calibration Laboratories (NABL)-accredited genetic laboratory.

For TE biopsy-based PGT-A, chromosomal assessment was performed using NGS following whole-genome amplification (WGA) with multiple annealing and looping-based amplification cycles (MALBAC) technology. Sequencing was carried out on the Ion GeneStudio™ S5 Plus System (Thermo Fisher Scientific, USA), and sequencing data were analysed using ChromGo (Yikon Genomics, China) for chromosomal copy number analysis. Based on copy number variation profiles, embryos were classified as euploid (≤ 30% aneuploid cells), low-level mosaic (31–50% aneuploid cells), high-level mosaic (51–70% aneuploid cells), aneuploid (> 70% aneuploid cells), complex aneuploid (embryos demonstrating multiple chromosomal abnormalities) and chaotic (embryos exhibiting extensive genomic instability involving multiple chromosomes). In the absence of euploid embryos, patients were counselled regarding the potential transfer of low-level mosaic embryos in accordance with current ESHRE survey results and good practice recommendations on managing chromosomal mosaicism[22]. Transfer of high-level mosaic embryos was not recommended; however, in selected cases patients elected to proceed following detailed genetic counselling and documented informed consent.

The samples dispatched to the genetic laboratory for niPGT-A were analysed using NGS with a commercially available and validated Yikon Genomics kit following the manufacturer's protocol. The downstream workflow, including WGA using MALBAC, sequencing on the Ion GeneStudio™ S5 Plus System, and chromosomal copy number analysis using ChromGo, and embryo classification thresholds, was same to that used for TE biopsy-based PGT-A. Additionally, embryos showing multiple chromosomal abnormalities were reported as complex mosaic, while samples with insufficient DNA concentration were classified as non-informative. In the absence of euploid embryos, patients were offered the option of transferring low-level mosaic embryos as that of PGT-A following appropriate counselling and informed consent. Transfer of high-level mosaic, non-informative, aneuploid or complex mosaic embryos were not recommended. According to our standard operating procedures (SOPs), high-level mosaic embryos were recommended to be discarded following patient consent, whereas patients with non-informative embryos were counselled regarding repeat genetic testing using TE biopsy. However, in exceptional circumstances, some patients elected to proceed with transfer of high-level mosaic or non-informative embryos with the aim of reducing time to pregnancy. These patients were subjected to extensive genetic counselling regarding potential risks and limitations with written consent.

All genetic testing procedures were conducted in accordance with laboratory quality assurance standards, internal validation protocols, and established clinical laboratory practices.

Endometrial preparation, embryo thawing and embryo transfer

Endometrial preparation for FET cycles were performed using oral oestradiol valerate (Abbott, USA), with endometrial development monitored by transvaginal ultrasonography. Progesterone supplementation was initiated using 8% vaginal progesterone gel (Akums Drugs, India) administered twice daily in combination with oral dydrogesterone (10 mg twice daily). Embryo warming was performed following the availability of genetic testing results using a commercially available vitrification and warming kit (Kitazato Corporation, Shizuoka, Japan) according to the manufacturer's protocol. Embryo transfer was scheduled once an adequate endometrial thickness of ≥ 7 mm was achieved.

A maximum of two embryos were permitted for transfer in accordance with the provisions of the ART Act of India[21]. Patients were counselled regarding the risks associated with multiple pregnancy prior to embryo transfer. The decision to perform single embryo transfer (SET) or double embryo transfer (DET) was made based on embryo availability and patient preference following clinical counselling including euploid embryos. Embryo transfer was performed under transabdominal ultrasound guidance using a soft transfer catheter. Luteal phase support with progesterone supplementation was continued until confirmation of pregnancy and, in the event of a positive test, was maintained through the first trimester.

Data collection

Data for this study were obtained from the institution's electronic medical record (EMR) system, anonymized, and assigned unique tracking numbers to ensure confidentiality. When follow-up data (clinical outcomes) were unavailable, patients were contacted by telephone up to three times. Despite these efforts, follow-up data were not obtained for 23 patients (1.0%) in the niPGT-A group and 22 patients (2.3%) in the PGT-A group at 12 weeks, and for 119 patients (5.3%) in the niPGT-A group and 130 patients (13.3%) in the PGT-A group at the live birth endpoint. To ensure data accuracy and integrity, all entries were independently reviewed, verified and audited by the institution's clinical research associate team.

Standardisation and quality control

Prior to implementation of the PGT-A and niPGT-A programmes, standardised laboratory protocols and SOPs were developed and uniformly implemented across all participating centres. For PGT-A, designated embryologists underwent structured training through the organisation's embryology training academy, followed

by competency assessment and validation by certified embryology trainer before independently performing TE biopsy at their respective centres.

For niPGT-A, SOPs for SCM collection, handling, storage, and transport were developed in collaboration with the accredited genetic laboratory. The validated protocol and SOPs were disseminated to all designated embryologists through structured theoretical and hands-on training. To ensure uniform implementation, a validated embryologist from the accredited genetic laboratory visited each participating centre to provide on-site training, followed by laboratory validation using five embryos per centre. Following successful competency assessment of embryologists and laboratory validation, the niPGT-A protocol was implemented uniformly across all participating centres. To minimise inter-centre variability and ensure consistency in cfDNA sampling and analysis, all SCM samples were collected using the same validated protocol, stored under standardised conditions, and transported through a uniform logistics pathway to an accredited genetic laboratory for chromosomal analysis. Oversight of programme implementation, protocol adherence, and ongoing quality assurance was provided by the Head of Embryology – Genetics.

All participating centres operated under a unified organisational quality management system and adhere to standardised clinical and laboratory protocols throughout the study period. Regular internal audits, competency assessments and continuous performance monitoring were conducted to ensure compliance with organisation SOP and consistency of practice across the network.

Outcome measures

The primary outcome of the study is live birth rate (LBR). Secondary outcomes included biochemical pregnancy rate (BPR), clinical pregnancy rate (CPR), ongoing pregnancy rate (OPR) at 12 weeks and categorisation of pregnancy losses into early biochemical loss, first-trimester loss, and second-trimester loss. All outcomes were defined according to standard clinical criteria. Biochemical pregnancy was determined by the presence of serum β-hCG (> 100IU/L) in maternal blood 14 days after embryo transfer. Clinical pregnancy was confirmed by the visualization of a viable gestational sac on transvaginal ultrasound at or beyond 6 weeks of gestation. Ongoing pregnancy was defined as a viable pregnancy continuing beyond 12 weeks of gestation, confirmed by ultrasonography. Live birth was defined as the delivery of one or more infants beyond 23 weeks of gestation. Biochemical loss was defined as a positive β-hCG test without subsequent visualization of a gestational sac at 6 weeks of gestation. First-trimester loss referred to the loss of a clinically confirmed pregnancy before 12 weeks of gestation, while second-trimester loss was defined as pregnancy loss between 12 and 20 weeks of gestation.

Statistical analysis

All analyses were performed using SPSS Statistics, Version 28.0 (IBM Corp., Armonk, NY, USA). Graphical outputs were generated in R software (R Foundation for Statistical Computing, Vienna, Austria). Baseline demographic and clinical characteristics were summarized using descriptive statistics. Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR), as appropriate, and categorical variables were reported as counts and percentages. Between-group comparisons of continuous variables were performed using the student's t test or Mann–Whitney U test, depending on distribution, while categorical variables were compared using the Pearson χ^2 test.

Clinical outcomes were analysed per embryo transfer in both the overall cohort and the euploid-only population. Additionally, subgroup analyses were conducted stratified by SET and DET and single- and double-vitrified embryos. Furthermore, separate subgroup analyses comparing single- and double-vitrified embryos were also conducted within the niPGT-A and PGT-A cohorts to evaluate the impact of vitrification on clinical outcomes in both the testing modality. A multivariable binary logistic regression model was constructed to assess the association between testing modality (niPGT-A vs. PGT-A) and clinical pregnancy and live birth after adjusting for maternal and paternal age and endometrial thickness and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were reported. This regression model incorporated aforementioned covariates, while embryo transfer strategy (SET vs. DET) and vitrification status (single- vs. double-vitrified embryos, including within-group comparisons for the niPGT-A and PGT-A cohorts) were evaluated separately through predefined stratified analyses. Additionally, BMI and embryo morphology were not included in the model because these characteristics were broadly comparable between the two testing modalities. Predicted probabilities of live birth and clinical pregnancy by testing group were calculated at the mean maternal and paternal age and endometrial thickness. Cases of lost to follow-up were documented separately and excluded from inferential testing. A two-tailed p value < 0.05 was considered statistically significant.

Results

Participant characteristics

Baseline demographic and clinical characteristics are summarized in supplementary table 1A. Women in the niPGT-A group were slightly older (36.2 ± 4.3 vs. 35.7 ± 4.5 years), had lower AFC (12.8 ± 7.2 vs. 14.3 ± 7.3) and AMH (2.6 ± 2.4 vs. 2.8 ± 2.4 ng/mL) compared with the PGT-A group. Additionally, BMI demonstrated no statistical significance between both the testing modalities. Furthermore, the morphology of transferred blastocysts was compared between the two testing modalities. The distribution of good-quality (90.3% vs. 90.8%) and fair-quality (9.7% vs. 9.2%) blastocysts did not differ significantly between the niPGT-A and PGT-A cohorts ($p = 0.645$) and is demonstrated in supplementary table 1B.

Embryo ploidy distribution

In the niPGT-A group, 3,560 patients (9,269 blastocysts) were analysed (supplementary Fig. 1 A). Embryo distribution included 43.4% euploid ($n = 4,019$), 19.6% low-level mosaic ($n = 1,817$), 7.5% high-level mosaic ($n = 697$), 14.1% aneuploid ($n = 1,303$), 1.6% complex aneuploid ($n = 156$), and 13.8% non-informative ($n = 1,277$).

Embryo transfers were performed in 2,231 patients, including euploid, mosaic, and non-informative embryos (supplementary table 2 A).

In the PGT-A group, 2,655 patients (6,366 blastocysts) were analysed (supplementary Fig. 1B). Of these, 43.8% were euploid ($n = 2,789$), 5.4% low-level mosaic ($n = 341$), 2.2% high-level mosaic ($n = 144$), 31.5% aneuploid ($n = 2,008$), 10.6% complex aneuploid ($n = 675$), 2.0% chaotic ($n = 122$), and 4.5% non-informative ($n = 287$). Embryo transfers were performed in 948 patients, limited to euploid and mosaic embryos (supplementary table 2B).

The euploid distribution was analysed as depicted in supplementary Tables 3 and euploidy distribution did not differ significantly between both the testing modalities.

Distribution by maternal age

The distribution of ploidy categories by maternal age is shown in Fig. 2. In the niPGT-A group, women < 30 years had 42% euploid and 13% aneuploid embryos. With increasing age, euploidy declined to 38% in women 45–50 years, while aneuploidy rose to 33%. Low- and high-level mosaicism were consistent across ages (24–31%), and complex mosaicism was detected in 1–2%. Non-informative results (failed amplification) remained stable at 10–15% (Fig. 2A).

In the PGT-A group, women < 30 years had 56.5% euploid and 25.1% aneuploid embryos. Euploidy declined to 24.4% in women ≥ 45 years, while aneuploidy increased to 38.5%. Low-level mosaicism ranged from 2.6% to 6.5% and demonstrated decline trend with increase in age. However, inverse relationship was demonstrated in high-level mosaic embryos, and it was augmented with increase in age. Complex aneuploidy was more frequent with age, reaching 19.9% at 40–44 years and 16.7% at ≥ 45 years. Chaotic embryos were rare but increased with age (1.1% <30 vs. 6.4% ≥45). Non-informative embryos comprised ≤ 6% across groups (Fig. 2B).

Clinical outcomes in euploid transfers

Clinical outcomes of euploid transfers are summarized in Table 1A. A total of 1,609 niPGT-A and 908 PGT-A transfers were analysed. The BPR was higher in the PGT-A group compared with niPGT-A (69.9% vs. 59.0%, $p < 0.001$). The CPR also significantly favoured PGT-A (66.2% vs. 47.6%, $p < 0.001$). Ongoing pregnancy at 12 weeks was achieved in 60.8% of PGT-A transfers compared with 41.1% of niPGT-A transfers ($p < 0.001$). The LBR was 46.5% after PGT-A compared with 34.4% after niPGT-A ($p < 0.001$). Pregnancy loss rates were higher after niPGT-A. Biochemical loss occurred in 19.2% of niPGT-A pregnancies compared with 5.3% after PGT-A ($p < 0.001$). First-trimester loss was 11.2% after niPGT-A compared with 4.6% after PGT-A ($p = 0.002$). Second-trimester loss did not differ significantly (5.2% vs. 4.2%, $p > 0.999$).

Euploid SET and DET outcomes

Clinical outcomes stratified by SET and DET in the euploid population are shown in Table 1B. In the SET cohort, 926 niPGT-A and 511 PGT-A transfers were analysed. The BPR was higher with PGT-A compared with niPGT-A (64.0% vs. 50.0%, $p < 0.001$). Clinical pregnancy also significantly favoured PGT-A (59.5% vs. 40.1%, $p < 0.001$). Ongoing pregnancy at 12 weeks occurred in 52.3% of PGT-A transfers compared with 35.3% of niPGT-A transfers ($p < 0.001$). The LBR was 38.7% after PGT-A compared with 29.9% after niPGT-A ($p < 0.001$). Biochemical loss was greater in niPGT-A compared with PGT-A (19.8% vs. 7.0%, $p < 0.001$). First-trimester and second-trimester loss rates were not statistically significant between groups (9.2% vs. 7.9%, $p = 0.065$; 4.6% vs. 3.4%, $p = 0.980$).

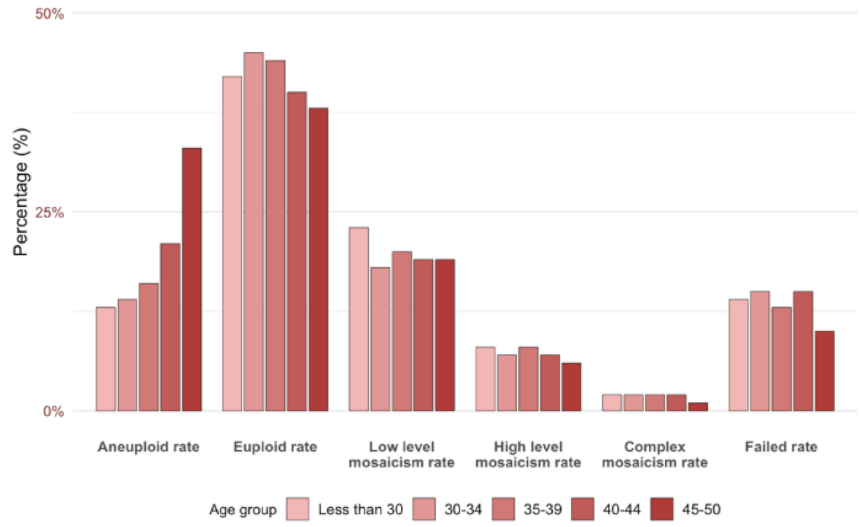
In the DET cohort, 683 niPGT-A and 397 PGT-A transfers were analysed. The BPR was significantly higher with PGT-A (77.6%) compared with niPGT-A (71.2%, $p = 0.022$). Clinical pregnancy also significantly improved following PGT-A (74.8% vs. 57.8%, $p < 0.001$). Ongoing pregnancy at 12 weeks was achieved in 71.8% of PGT-A transfers versus 49.0% of niPGT-A transfers ($p < 0.001$). The LBR was 56.4% after PGT-A compared with 40.4% after niPGT-A ($p < 0.001$). Biochemical loss was greater in niPGT-A compared with PGT-A (18.2% vs. 3.6%, $p < 0.001$). First-trimester loss was also higher after niPGT-A compared with PGT-A (13.2% vs. 1.3%, $p < 0.001$). Second-trimester loss rates were similar (5.8% vs. 5.1%, $p > 0.999$).

Clinical outcomes of niPGT-A versus PGT-A stratified by vitrification status in euploid embryo transfers

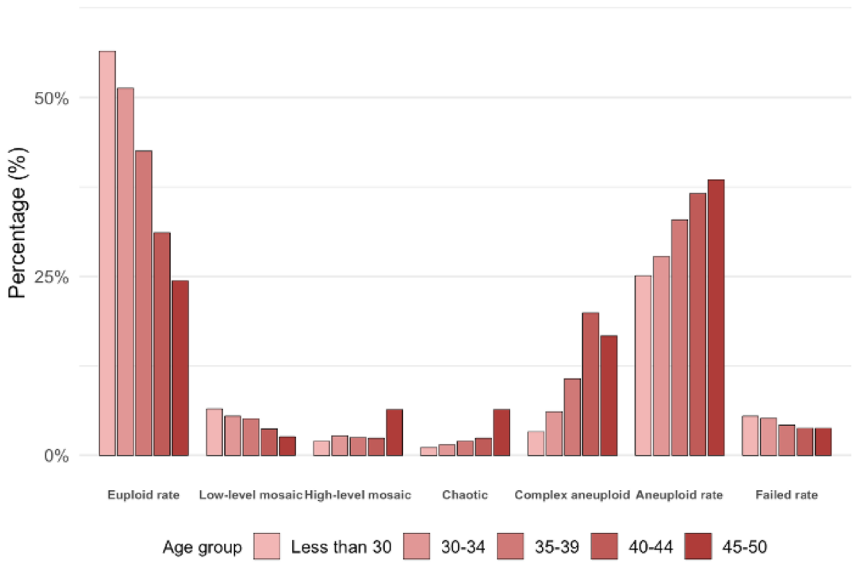
A total of 1,671 single-vitrified transfers (niPGT-A: $n = 1,239$; PGT-A: $n = 432$) and 864 double-vitrified transfers (niPGT-A: $n = 370$; PGT-A: $n = 476$) were analysed and is demonstrated in Table 1C.

In single-vitrified embryo transfers, the BPR was significantly lower in the niPGT-A group compared with PGT-A (57.6% vs. 73.4%, $p < 0.001$). The biochemical loss rate was significantly higher following niPGT-A (20.7% vs. 3.8%, $p < 0.001$). CPR was also significantly lower in the niPGT-A group compared with PGT-A (45.7% vs. 70.6%, $p < 0.001$). Similarly, first-trimester loss occurred significantly more frequently after niPGT-A (10.8% vs. 3.3%, $p = 0.015$). Ongoing pregnancy at 12 weeks was achieved in 39.5% of niPGT-A transfers compared with 65.7% of PGT-A transfers ($p < 0.001$). The LBR was 32.3% following niPGT-A compared with 48.6% after PGT-A ($p < 0.001$). Second-trimester loss was also higher in the niPGT-A group (12.7% vs. 4.6%, $p < 0.001$).

In double-vitrified embryo transfers, the BPR was not statistically significant between niPGT-A and PGT-A (63.5% vs. 66.8%, $p = 0.344$). However, biochemical loss remained significantly higher in the niPGT-A group (14.9% vs. 6.9%, $p = 0.006$). CPR was significantly lower following niPGT-A compared with PGT-A (54.1% vs. 62.2%, $p = 0.020$). First-trimester loss was also significantly more frequent in the niPGT-A group (12.5% vs. 6.1%, $p = 0.023$). Ongoing pregnancy at 12 weeks was achieved in 46.8% of niPGT-A transfers compared with 56.3% following PGT-A ($p = 0.002$). LBR were not statistically significant between the groups (41.4% vs. 44.5%, $p = 0.974$), as were second-trimester loss rates (3.5% vs. 3.7%, $p = 0.890$).



(A)



(B)

Fig. 2. **(A)** Distribution of niPGT-A embryo ploidy categories by maternal age group. *Legend*: Percentage distribution of embryo ploidy status assessed by niPGT-A across maternal age groups (< 30, 30–34, 35–39, 40–44, and 45–50 years). Categories include euploid, aneuploid, low-level mosaic, high-level mosaic, complex aneuploid, and failed/non-informative embryos. **(B)** Distribution of PGT-A embryo ploidy categories by maternal age group. *Legend*: Percentage distribution of embryo ploidy status assessed by PGT-A across maternal age groups (< 30, 30–34, 35–39, 40–44, and 45–50 years). Categories include euploid, aneuploid, low-level mosaic, high-level mosaic, complex aneuploid, chaotic, and failed/non-informative embryos.

(A) Comparison of reproductive outcomes following niPGT-A and PGT-A			
Outcomes	**niPGT-A (n = 1609)**	**PGT-A (n = 908)**	**p value**
Biochemical pregnancy rate[a]	59.0%	69.9%	< 0.001
Biochemical loss rate[b]	19.2%	5.3%	< 0.001
Clinical pregnancy rate[c]	47.6%	66.2%	< 0.001
First-trimester loss rate[d]	11.2%	4.6%	0.002
Ongoing pregnancy rate (12 weeks)[e]	41.1%	60.8%	< 0.001
Second-trimester loss rate[f]	5.2%	4.2%	> 0.999
Live birth rate[g]	34.4%	46.5%	< 0.001

(B) Comparison of reproductive outcomes following niPGT-A and PGT-A stratified by SET and DET						
Outcomes	**niPGT-A (n = 926)**	**PGT-A (n = 511)**	**p value**	**niPGT-A (n = 683)**	**PGT-A (n = 397)**	**p value**
	SET			**DET**		
Biochemical pregnancy rate[a]	50.0%	64.0%	< 0.001	71.2%	77.6%	0.022
Biochemical loss rate[b]	19.8%	7.0%	< 0.001	18.2%	3.6%	< 0.001
Clinical pregnancy rate[c]	40.1%	59.5%	< 0.001	57.8%	74.8%	< 0.001
First-trimester loss rate[d]	9.2%	7.9%	0.065	13.2%	1.3%	< 0.001
Ongoing pregnancy rate (12 wks)[e]	35.3%	52.3%	< 0.001	49.0%	71.8%	< 0.001
Second-trimester loss rate[f]	4.6%	3.4%	0.980	5.8%	5.1%	> 0.999
Live birth rate[g]	29.9%	38.7%	< 0.001	40.4%	56.4%	< 0.001

(C) Single-vitrified versus double-vitrified embryo transfer						
	Single-vitrified embryo transfers (n = 1671)			**Double-vitrified embryo transfers (n = 864)**		
Outcomes	**niPGT-A (n = 1239)**	**PGT-A (n = 432)**	**p value**	**niPGT-A (n = 370)**	**PGT-A (n = 476)**	**p value**
Biochemical pregnancy rate[a]	57.6%	73.4%	< 0.001	63.5%	66.8%	0.344
Biochemical loss rate[b]	20.7%	3.8%	< 0.001	14.9%	6.9%	0.006
Clinical pregnancy rate[c]	45.7%	70.6%	< 0.001	54.1%	62.2%	0.020
First-trimester loss rate[d]	10.8%	3.3%	0.015	12.5%	6.1%	0.023
Ongoing pregnancy rate (12 wks)[e]	39.5%	65.7%	< 0.001	46.8%	56.3%	0.002
Second-trimester loss rate[f]	12.7%	4.6%	< 0.001	3.5%	3.7%	0.890
Live birth rate[g]	32.3%	48.6%	< 0.001	41.4%	44.5%	0.974

(D) Comparison of clinical outcomes between single-vitrified and double-vitrified within the niPGT-A and PGT-A cohorts						
	niPGT-A (n = 1609)			**PGT-A (n = 908)**		
Outcomes	**Single-vitrified niPGT-A (n = 1239)**	**Double-vitrified niPGT-A (n = 370)**	**p value**	**Single-vitrified PGT-A (n = 432)**	**Double-vitrified PGT-A (n = 476)**	**p value**
Biochemical pregnancy rate[a]	57.6	63.5	0.047	73.4	66.8	0.036
Biochemical loss rate[b]	20.7	14.9	0.193	3.8	6.9	0.163
Clinical pregnancy rate[c]	45.7	54.1	0.005	70.6	62.2	0.008
First trimester loss rate[d]	10.7	12.5	0.192	3.3	6.1	0.427
Ongoing pregnancy rate (12 Wks)[e]	39.5	46.8	0.028	65.7	56.3	0.009
Second trimester loss rate[f]	5.8	3.5	0.292	4.6	3.7	0.065
Live birth rate[g]	32.3	41.4	0.004	48.6	44.5	0.010

(E) Logistic regression predictors of clinical pregnancy (n = 2517, niPGT-A = 1609, PGT-A = 908)						
Predictors	**Estimate**	**Standard Error**	**z value**	**OR**	**95% CI**	**p value**
Intercept	1.299	0.497	2.612	3.67	(1.38, 9.72)	0.009
Group: PGT-A (Ref. niPGT-A)	0.761	0.086	8.767	2.14	(1.80, 2.53)	< 0.001
Maternal Age	-0.043	0.011	-3.707	0.95	(0.93, 0.98)	< 0.001
Paternal Age	-0.012	0.010	-1.169	0.98	(0.96, 1.00)	0.242
Endometrial Thickness	0.071	0.037	1.938	1.08	(0.99, 1.15)	0.052

(F) Predicted adjusted probability of clinical pregnancy						
Group	**Maternal Age**	**Paternal Age**	**ET**	**Predicted Probability**	**SE**	**95% CI**
niPGT-A	36.0	38.5	8.8	47.7%	0.013	(45.3%, 50.2%)
PGT-A	36.0	38.5	8.8	66.2%	0.016	(63.0%, 69.2%)

(G) Logistic regression predictors of live birth (n = 2304, niPGT-A = 1522, PGT-A = 782)						
Predictors	**Estimate**	**Standard Error**	**z value**	**Odds Ratio**	**95% CI**	**p value**
Intercept	1.177	0.526	2.234	3.24	(1.15, 9.11)	0.025
Group: PGT-A (Ref. niPGT-A)	0.704	0.090	7.817	2.02	(1.69, 2.41)	< 0.001
Maternal Age	-0.036	0.012	-2.972	0.96	(0.94, 0.98)	0.002
Paternal Age	-0.019	0.011	-1.769	0.98	(0.96, 1.00)	0.076
Endometrial Thickness	0.037	0.039	0.949	1.03	(0.96, 1.12)	0.342

(H) Predicted adjusted probability of live birth						
Group	**Maternal Age**	**Paternal Age**	**ET**	**Predicted Probability**	**SE**	**95% CI**
niPGT-A	36.0	38.5	8.8	36.4%	0.012	(33.9%, 38.8%)
PGT-A	36.0	38.5	8.8	53.6%	0.018	(50.1%, 57.2%)

Table 1. Reproductive outcomes comparison following niPGT-A and PGT-A in euploid population. (E) Footnotes: Null deviance = 3470.6, Residual deviance = 3354.8, AIC = 3364.8 VIF = 1, indicating no collinearity issue (F) Footnotes: ET = endometrial thickness, SE = standard error, CI = confidence interval (G) Footnotes: Null deviance = 3139.4, Residual deviance = 3046.0, AIC = 3056.0, VIF = 1, Indicating no collinearity issue (H) Footnotes: ET = endometrial thickness, SE = standard error, CI = confidence interval Legend: Statistical analysis was performed using Pearson Chi Square test and logistic regression [a]The biochemical pregnancy rate was calculated as the percentage of embryo transfers that resulted in a biochemical pregnancy (defined as a b-human chorionic gonadotropin (b-hCG) level > 100 IU/L detected via maternal blood testing). [b]The biochemical loss rate was defined as positive b-hCG test without subsequent visualization of a gestational sac visualised at 6 weeks of ultrasonographic scan. [c]The clinical pregnancy rate was calculated as number of viable gestational sac visualised at 6 weeks of ultrasonographic scan. [d]The first trimester loss rate was defined as the proportion of clinically confirmed pregnancies that resulted in pregnancy loss before 12 weeks of gestation. [e]The ongoing pregnancy rate was calculated as the percentage of embryo transfers resulting in a viable pregnancy continuing beyond 12 weeks of gestation, confirmed by ultrasonography. [f]The second-trimester loss rate was defined as the proportion of clinically confirmed pregnancies that resulted in pregnancy loss between 12 and 20 weeks of gestation. [g]The live birth rate was calculated as the percentage of embryo transfers resulting in the delivery of one or more live infants beyond 23 weeks of gestation.

Clinical outcomes of euploid embryo transfers following single versus double vitrification within the PGT-A and niPGT-A groups

Clinical outcomes were further compared between single-vitrified and double-vitrified euploid embryo transfers within the niPGT-A and PGT-A cohorts (Table 1D). The niPGT-A cohort comprised 1,609 embryo transfers, including 1,239 single-vitrified and 370 double-vitrified transfers. The PGT-A cohort comprised 908 embryo transfers, including 432 single-vitrified and 476 double-vitrified transfers.

Within the niPGT-A cohort, double-vitrified embryo transfers were associated with significantly improved reproductive outcomes compared with single-vitrified embryo transfers. Higher biochemical pregnancy (63.5% vs. 57.6%, $p = 0.047$), clinical pregnancy (54.1% vs. 45.7%, $p = 0.005$), ongoing pregnancy at 12 weeks (46.8% vs. 39.5%, $p = 0.028$), and live birth rates (41.4% vs. 32.3%, $p = 0.004$) were observed following double vitrification. However, biochemical, first-trimester, and second-trimester pregnancy loss rates were not statistically different between the two groups.

In contrast, within the PGT-A cohort, single-vitrified embryo transfers demonstrated improved reproductive outcomes compared with double-vitrified transfers, with significantly higher biochemical pregnancy (73.4% vs. 66.8%, $p = 0.036$), clinical pregnancy (70.6% vs. 62.2%, $p = 0.008$), ongoing pregnancy at 12 weeks (65.7% vs. 56.3%, $p = 0.009$), and live birth rates (48.6% vs. 44.5%, $p = 0.010$). Similar to the niPGT-A cohort, no significant differences were observed in biochemical, first-trimester, or second-trimester pregnancy loss rates.

Regression analysis and prediction of clinical pregnancy and live birth in euploid embryo transfers

A total of 2,517 women were included for regression analysis of clinical pregnancy (niPGT-A = 1609; PGT-A = 908) and 2,304 women for live birth (niPGT-A = 1522, PGT-A = 782). Logistic regression was adjusted for co-variates (maternal age, paternal age, and endometrial thickness) demonstrated that PGT-A transfers were associated with significantly higher odds of clinical pregnancy compared with niPGT-A (OR, 2.14; 95% CI, 1.80–2.53; $p < 0.001$) (Table 1E). After the adjustment of the covariates, the adjusted predicted probability of clinical pregnancy was 47.7% (95% CI, 45.3%–50.2%) in the niPGT-A group and 66.2% (95% CI, 63.0%–69.2%) in the PGT-A group (Table 1F). Similar trends were observed in live birth, the logistic regression after adjustment of the covariates depicted that PGT-A transfers were significantly associated with higher odds of live birth (OR, 2.02; 95% CI, 1.69–2.41; $p < 0.001$) (Table 1G). With covariates adjustment the predicted probability of live birth was 36.4% (95% CI, 33.9%–38.8%) in niPGT-A and 53.6% (95%CI, 50.1%–57.2%) in PGT-A group (Table 1H).

Overall clinical outcomes

Clinical outcomes of niPGT-A and PGT-A are summarized in table 2A. A total of 2,231 patients underwent embryo transfers following niPGT-A and 948 following PGT-A. The BPR was significantly higher in the PGT-A group compared with niPGT-A (69.2% vs. 59.0%, $p<0.001$). The CPR also significantly improved following PGT-A (65.5% vs. 47.8%, $p<0.001$). Ongoing pregnancy at 12 weeks occurred in 60.2% of PGT-A transfers versus 41.6% of niPGT-A transfers ($p<0.001$). A similar trend was observed for LBR (46.2% vs. 34.5%, $p<0.001$). Pregnancy loss rates were higher after niPGT-A. The biochemical loss rate was 18.9% for niPGT-A compared with 5.3% for PGT-A ($p<0.001$). First-trimester loss occurred in 10.9% of niPGT-A clinical pregnancies versus 4.5% after PGT-A ($p<0.001$). Second-trimester loss was slightly higher following niPGT-A (5.7% vs. 4.0%, $p<0.001$).

(A) Comparison of reproductive outcomes following niPGT-A and PGT-A			
Outcomes	**niPGT-A (n = 2231)**	**PGT-A (n = 948)**	**p value**
Biochemical pregnancy rate[a]	59.0%	69.2%	< 0.001
Biochemical loss rate[b]	18.9%	5.3%	< 0.001
Clinical pregnancy rate[c]	47.8%	65.5%	< 0.001
First-trimester loss rate[d]	10.9%	4.5%	< 0.001
Ongoing pregnancy rate (12 weeks)[e]	41.6%	60.2%	< 0.001
Second-trimester loss rate[f]	5.7%	4.0%	< 0.001
Live birth rate[g]	34.5%	46.2%	< 0.001

(B) Comparison of reproductive outcomes following niPGT-A and PGT-A stratified by SET and DET						
Outcomes	**niPGT-A (n = 1184)**	**PGT-A (n = 533)**	**p value**	**niPGT-A (n = 1047)**	**PGT-A (n = 415)**	**p value**
	SET			**DET**		
Biochemical pregnancy rate[a]	49.9%	63.2%	< 0.001	69.2%	76.9%	< 0.001
Biochemical loss rate[b]	18.8%	6.8%	< 0.001	19.0%	3.8%	< 0.001
Clinical pregnancy rate[c]	40.5%	58.9%	< 0.001	56.1%	74.0%	< 0.001
First-trimester loss rate[d]	8.8%	7.6%	0.067	12.8%	1.3%	< 0.001
Ongoing pregnancy rate (12 wks)[e]	35.9%	51.8%	< 0.001	47.9%	71.1%	< 0.001
Second-trimester loss rate[f]	4.6%	3.2%	0.080	6.6%	4.9%	0.098
Live birth rate[g]	30.7%	38.5%	< 0.001	38.8%	56.1%	< 0.001

(C) Single-vitrified versus double-vitrified PGT embryo transfers						
	Single-vitrified PGT embryo transfers (n = 2144)			**Double-vitrified PGT embryo transfers (n = 1035)**		
Outcomes	**niPGT-A (n = 1687)**	**PGT-A (n = 457)**	**p value**	**niPGT-A (n = 544)**	**PGT-A (n = 491)**	**p value**
Biochemical pregnancy rate[a]	57.6%	72.4%	< 0.001	63.2%	66.2%	0.329
Biochemical loss rate[b]	20.2%	3.92%	< 0.001	15.1%	6.2%	0.002
Clinical pregnancy rate[c]	45.9%	69.6%	< 0.001	53.7%	61.7%	0.010
First-trimester loss rate[d]	10.2%	3.1%	0.007	13.0%	5.9%	0.002
Ongoing pregnancy rate (12 wks)[e]	40.0%	64.8%	< 0.001	46.3%	56.0%	< 0.001
Second-trimester loss rate[f]	6.3%	4.4%	0.420	4.1%	3.6%	0.311
Live birth rate[g]	32.7%	48.4%	< 0.001	40.3%	44.2%	0.569

(D) Comparison of clinical outcomes according to vitrification status within the niPGT-A and PGT-A cohorts						
	niPGT-A (n = 2231)			**PGT-A (n = 948)**		
Outcomes	**Single-vitrified niPGT-A (n = 1687)**	**Double-vitrified niPGT-A (n = 544)**	**p value**	**Single-vitrified PGT-A (n = 457)**	**Double-vitrified PGT-A (n = 491)**	**p value**
Biochemical pregnancy rate[a]	57.6%	63.2%	0.021	72.4%	66.2%	0.041
Biochemical loss rate[b]	20.3%	15.1%	0.184	3.9%	6.8%	0.228
Clinical pregnancy rate[c]	45.9%	53.7%	0.002	69.6%	61.7%	0.011
First trimester loss rate[d]	10.2%	13.1%	0.254	3.1%	5.9%	0.371
Ongoing pregnancy rate (12 Wks)[e]	40.0%	46.3%	0.010	64.8%	56.0%	0.011
Second trimester loss rate[f]	6.3%	4.1%	0.270	4.4%	3.6%	0.094
Live birth rate[g]	32.7%	40.3%	0.004	48.4%	44.2%	0.014

(E) Logistic regression predictors of clinical pregnancy (n = 3179, niPGT-A = 2231, PGT-A = 948)						
Predictors	**Estimate**	**Standard Error**	**z value**	**Odds Ratio**	**95% CI**	**p value**
Intercept	1.819	0.434	4.184	6.17	(2.63, 14.47)	< 0.001
Group: PGT-A (Ref. niPGT-A)	0.710	0.081	8.756	2.03	(1.73, 2.38)	< 0.001
Maternal Age	-0.038	0.010	-3.723	0.96	(0.94, 0.98)	< 0.001
Paternal Age	-0.022	0.009	-2.471	0.97	(0.96, 0.99)	0.013
Endometrial Thickness	0.042	0.031	1.321	1.04	(0.98, 1.11)	0.186

(F) Predicted adjusted probability of clinical pregnancy						
Group	**Maternal Age**	**Paternal Age**	**ET**	**Predicted Probability**	**SE**	**95% CI**
niPGT-A	36.1	38.6	8.8	48.0%	0.011	(45.9%, 50.1%)
PGT-A	36.1	38.6	8.8	65.3%	0.016	(62.2%, 68.3%)

(G) Logistic regression predictors of live birth (n = 2930, niPGT-A = 2112, PGT-A = 818)						
Predictors	**Estimate**	**Standard Error**	**z value**	**Odds Ratio**	**95% CI**	**p value**
Intercept	1.554	0.461	3.36	4.73	(1.91, 11.68)	< 0.001
Group: PGT-A (Ref. niPGT-A)	0.670	0.084	7.95	1.95	(1.65, 2.31)	< 0.001
Maternal Age	-0.033	0.011	-3.06	0.97	(0.95, 0.99)	0.002
Paternal Age	-0.027	0.010	-2.83	0.97	(0.95, 0.99)	0.004
Endometrial Thickness	0.020	0.034	0.59	1.02	(0.96, 1.09)	0.550

(H) Predicted adjusted probability of live birth						
Group	**Maternal Age**	**Paternal Age**	**ET**	**Predicted Probability**	**SE**	**95% CI**
niPGT-A	36.1	38.7	8.9	36.5%	0.011	(34.4%, 38.6%)
PGT-A	36.1	38.7	8.9	52.9%	0.018	(49.5%, 56.4%)

Table 2. Reproductive outcomes comparison following niPGT-A and PGT-A in the overall population. (E) Footnotes: Null deviance=4394.8, Residual deviance=4260.9, AIC=4270.9, VIF=1, Indicating no collinearity issue (G) Footnotes: Null deviance=3971.2, Residual deviance=3857.6, AIC=3867.6, VIF=1, Indicating no collinearity issue (H) Footnotes: ET = endometrial thickness, SE = standard error, CI = confidence interval Legend: Statistical analysis was performed using Pearson Chi Square test and logistic regression [a]The biochemical pregnancy rate was calculated as the percentage of embryo transfers that resulted in a biochemical pregnancy (defined as a b-human chorionic gonadotropin (b-hCG) level > 100 IU/L detected via maternal blood testing). [b]The biochemical loss rate was defined as positive b-hCG test without subsequent visualization of a gestational sac visualised at 6 weeks of ultrasonographic scan. [c]The clinical pregnancy rate was calculated as number of viable gestational sac visualised at 6 weeks of ultrasonographic scan. [d]The first trimester loss rate was defined as the proportion of clinically confirmed pregnancies that resulted in pregnancy loss before 12 weeks of gestation. [e]The ongoing pregnancy rate was calculated as the percentage of embryo transfers resulting in a viable pregnancy continuing beyond 12 weeks of gestation, confirmed by ultrasonography. [f]The second-trimester loss rate was defined as the proportion of clinically confirmed pregnancies that resulted in pregnancy loss between 12 and 20 weeks of gestation. [g]The live birth rate was calculated as the percentage of embryo transfers resulting in the delivery of one or more live infants beyond 23 weeks of gestation.

Outcomes stratified by SET and DET in overall population

Clinical outcomes stratified by SET and DET are presented in table 2B. The SET cohort included 1,184 niPGT-A and 533 PGT-A transfers, and the DET cohort included 1,047 niPGT-A and 415 PGT-A transfers.In the SET group, the BPR was significantly higher after PGT-A compared with niPGT-A (63.2% vs. 49.9%, $p<0.001$). The CPR was too significantly higher in the PGT-A group (58.9% vs. 40.5%, $p<0.001$). Ongoing pregnancy at 12 weeks was achieved in 51.8% of PGT-A transfers versus 35.9% of niPGT-A transfers ($p<0.001$). The LBR was 38.5% after PGT-A and 30.7% after niPGT-A ($p<0.001$). Biochemical loss was significantly more frequent with niPGT-A (18.8%) compared with PGT-A (6.8%, $p<0.001$). First-trimester and second-trimester losses did not differ significantly, although both were numerically higher in the niPGT-A group (8.8% vs. 7.6%, $p=0.067$; 4.6% vs. 3.2%, $p=0.080$).In the DET group, the BPR was significantly higher after PGT-A compared with niPGT-A (76.9% vs. 69.2%, $p<0.001$). Clinical pregnancy was achieved in 74.0% of PGT-A transfers compared with 56.1% of niPGT-A transfers ($p<0.001$). Ongoing pregnancy at 12 weeks occurred in 71.1% of PGT-A transfers compared with 47.9% of niPGT-A transfers ($p<0.001$). The LBR was 56.1% in the PGT-A group versus 38.8% in the niPGT-A group ($p<0.001$). Biochemical loss was significantly more frequent with niPGT-A (19.0%) compared with PGT-A (3.8%, $p<0.001$). First-trimester loss was also significantly higher in niPGT-A (12.8%) compared with PGT-A (1.3%, $p<0.001$). Second-trimester loss rates were not statistically different (6.6% vs. 4.9%, $p=0.098$).

Clinical outcomes of niPGT-A versus PGT-A stratified by vitrification status in overall embryo transfers

Clinical outcomes were stratified according to single-vitrified and double-vitrified overall embryo transfers, comprising 2144 single-vitrified and 1035 double-vitrified cycles, as presented in table 2C. Among single-vitrified transfers, 1687 embryos underwent niPGT-A and 457 underwent PGT-A, whereas in double-vitrified transfers 544 embryos were analysed using niPGT-A and 491 using PGT-A.In single-vitrified cycles, niPGT-A demonstrated a significantly lower BPR compared with PGT-A (57.6% vs 72.4%, $p<0.001$). The biochemical loss rate was markedly significantly higher in the niPGT-A group (20.2%) compared with the PGT-A group (3.92%, $p<0.001$). Similarly, the CPR was significantly higher following PGT-A (69.6%) than niPGT-A (45.9%, $p<0.001$). The first-trimester loss rate was significantly higher in the niPGT-A group (10.2%) compared with PGT-A (3.1%, $p=0.007$). Consequently, the ongoing pregnancy rate at 12 weeks was significantly improved in PGT-A cycles (64.8%) compared with niPGT-A (40.0%, $p<0.001$). The LBR was also significantly higher in the PGT-A group (48.4%) than in the niPGT-A group (32.7%, $p<0.001$). Second-trimester loss rates were not statistically significant between groups (6.3% vs 4.4%, $p=0.420$).In double-vitrified embryo transfer cycles, the BPR was not statistically significant between niPGT-A and PGT-A (63.2% vs 66.2%, $p=0.329$). However, the biochemical loss rate remained significantly higher in the niPGT-A group (15.1% vs 6.2%, $p=0.002$). The CPR was significantly higher following PGT-A (61.7%) compared with niPGT-A (53.7%, $p=0.010$). Similarly, the first-trimester loss rate was significantly higher in the niPGT-A group (13.0% vs 5.9%, $p=0.002$). The ongoing pregnancy rate at 12 weeks significantly improved PGT-A (56.0%) compared with niPGT-A (46.3%, $p<0.001$). However, the LBR was not statistically significant between niPGT-A and PGT-A (40.3% vs 44.2%, $p=0.311$), with similar second-trimester loss rates (4.1% vs 3.6%, $p=0.311$) respectively.

Clinical outcomes following single versus double vitrification within the PGT-A and niPGT-A groups in overall population

The effect of vitrification status on reproductive outcomes was further evaluated within each testing modality using the overall transfer cohort (table 2D). The niPGT-A cohort comprised 2,231 embryo transfers, including 1,687 single-vitrified and 544 double-vitrified transfers, while the PGT-A cohort comprised 948 embryo transfers, including 457 single-vitrified and 491 double-vitrified transfers. Within the niPGT-A cohort, double-vitrified embryo transfers were associated with significantly higher BPR (63.2% vs. 57.6%, p = 0.021), CPR (53.7% vs. 45.9%, p = 0.002), OPR at 12 weeks (46.3% vs. 40.0%, p = 0.010), and LBR (40.3% vs. 32.7%, p = 0.004) compared with single-vitrified transfers. Conversely, within the PGT-A cohort, single-vitrified embryo transfers demonstrated significantly higher BPR (72.4% vs. 66.2%, p = 0.041), CPR (69.6% vs. 61.7%, p = 0.011), OPR at 12 weeks (64.8% vs. 56.0%, p = 0.011), and LBR (48.4% vs. 44.2%, p = 0.014) than double-vitrified transfers. Biochemical, first-trimester, and second-trimester pregnancy loss rates were not statistically significant between single- and double-vitrified embryo transfers in both testing modalities.

Regression analysis and prediction of clinical pregnancy and live birth in overall population

For logistic regression analysis of clinical pregnancy 3,179 women were included (niPGT-A=2231 and PGT-A=948) and for live birth 2,930 women were included (niPGT-A=2112 and PGT-A=818). After adjustments for the covariates, PGT-A transfers were associated with significantly higher odds of achieving clinical pregnancy (OR=2.03; 95% CI=1.73–2.38; p<0.001) with the predicted probability of 48% (95% CI = 45.9%–50.1%) in niPGT-A group and 65.3% (95% CI=62.2%–68.3%) in PGT-A group (table 2E & 2F). Similarly, the live birth was significantly associated with higher odds in PGT-A group (OR=1.95; 95% CI=1.65–2.31; p<0.001) with the predicted probability of 36.5% (95% CI = 34.4%–38.6%) in niPGT-A group and 52.9% (95% CI=49.5%–56.4%) in PGT-A group (table 2G & 2H).

Exploratory outcomes in failed amplification

Exploratory analysis of patients underwent non-informative niPGT-A with SET/DET was performed on 38 patients and is demonstrated in Supplementary Table 4. The BPR was 65.7%, and the CPR was 50.0%. Ongoing pregnancy at 12 weeks was achieved in 44.7%, and the LBR was 44.7%. Biochemical loss occurred in 24.0% of pregnancies, first-trimester loss in 10.5%, and second-trimester loss in 0%.

Discussion

The discovery of cfDNA analysis has catalysed a paradigm shift in reproductive genetics and prenatal testing. Within ART, niPGT-A offers the potential to reduce the technical complexity, economic burden, and ethical concerns associated with TE biopsy. The initial report introducing niPGT-A as a technique by Xu et al. 2016[17] reported high analytical accuracy, with sensitivity and specificity of 88% and 84%, respectively. According to their clinical protocols, SCM collection was performed on day 5 post day 3 embryo thawing in sample size of 42 embryos from 17 patients and reported five successful live births. However, subsequent studies evaluating clinical outcomes based on these protocols have remained limited and heterogeneous largely due to small sample sizes, inconsistent use of live birth as a primary endpoint, and the lack of clear stratification between single- and double-vitrified PGT embryo transfers in both niPGT-A and TE biopsy-based PGT-A cycles.

In this large multicentric real-world cohort investigation, we compared reproductive outcomes following niPGT-A and conventional TE biopsy-based PGT-A. Although euploidy rates and maternal age-related trends were broadly comparable between the two modalities, PGT-A was consistently associated with improved reproductive outcomes. Transfers of PGT-A–tested embryos were associated with improved biochemical pregnancy, clinical pregnancy, ongoing pregnancy, and live birth rates across both overall and euploid-only analyses. Importantly, multivariable logistic regression demonstrated that PGT-A conferred approximately twofold higher odds of achieving both clinical pregnancy and live birth compared with niPGT-A, even after adjustment for relevant confounders. Collectively, these findings highlight a distinction between analytical performance and real-world clinical effectiveness, suggesting that niPGT-A needs further refinement to improve its reliability and clinical efficacy.

When stratified by single- versus double-vitrified genetic testing ICSI cycles, PGT-A demonstrated significantly higher live birth rates than niPGT-A in single-vitrified embryo transfers, whereas no statistically significant difference was observed in double-vitrified embryo transfers. Furthermore, comparison of single- and double-vitrified embryo transfers within each testing modality revealed contrasting patterns. Within the PGT-A cohort, single-vitrified embryo transfers were consistently significantly associated with improved reproductive outcomes compared with double-vitrified transfers, whereas within the niPGT-A cohort, double-vitrified embryo transfers demonstrated significantly higher biochemical pregnancy, clinical pregnancy, ongoing pregnancy, and live birth rates than single-vitrified transfers. These findings align with the prior report describing reduced reproductive outcomes in PGT-A cycles following re-biopsy or freeze–thaw cycles[23].

Conversely, the improved outcomes observed following double vitrification within the niPGT-A cohort may suggest that repeated vitrification and warming influences the quantity and/or quality of embryonic cfDNA released into the SCM. This hypothesis is supported by the recent investigation by Wen et al. (2026), who reported that niPGT-A performed on frozen–thawed embryos demonstrated higher cfDNA yield, improved sensitivity, positive predictive value, and greater concordance with embryo chromosomal status compared with fresh embryos[24]. This investigation findings aligns with the published report and suggest that vitrification and subsequent warming may influence cfDNA availability and thereby enhance the analytical performance of SCM-based genetic testing. Alternatively, vitrification may alter factors affecting signal quality and interpretation, including potential sources of biological noise such as maternal cell contamination (MCC). Although the

biological mechanisms underlying these observations remain incompletely understood, they warrant further mechanistic investigation.

From a clinical perspective, these findings may open new avenues for the application of niPGT-A in patients who elect to undergo genetic testing on previously cryopreserved embryos following unsuccessful treatment cycles. In this setting, niPGT-A may provide a non-invasive alternative to biopsy-based PGT-A by avoiding additional TE biopsy and embryo micromanipulation, while potentially offering a more accessible option for appropriately selected patients. However, given the exploratory nature of these subgroup findings, prospective validation is required before these observations can be translated into routine clinical practice. If confirmed, these findings may provide novel insights into the biological determinants of niPGT-A performance and inform future strategies for its optimisation and clinical implementation.

Furthermore, a higher miscarriage rate was observed in the niPGT-A group, both in the overall cohort and among embryos classified as euploid. These findings underscore the importance of evaluating embryo selection technologies using patient-relevant outcomes rather than analytical concordance alone. In this context, the present study contributes large-scale real-world evidence indicating that the real-world effectiveness of niPGT-A when compared with conventional biopsy-based PGT-A.

Additionally, embryos with failed or inconclusive niPGT-A results demonstrated live birth rates comparable to those with reported niPGT-A findings. Interestingly, failed or inconclusive niPGT-A results were observed in good-quality blastocysts (AA, AB, and BA) as defined by Gardner and Schoolcraft grading system[20]. In these cases, embryo selection was primarily guided by morphology as requested by patients, reflecting a pragmatic clinical approach to avoid delaying embryo transfer and time to pregnancy. These findings warrant further investigation into the biological basis of niPGT-A failure in good-quality blastocysts and the mechanisms underlying successful reporting of these embryos.

The significant reason for the discrepancy between reproductive outcomes of both the modalities, despite comparable euploidy distribution likely arises because of quantity, quality and origin of cfDNA, presence of MCC, i.e. DNA presented in SCM originated from residual cumulus cells, or DNA amplification failures[14,15,25–27]. This cfDNA may originate from apoptotic cells, cellular debris, or compromised blastomeres[28], and may therefore disproportionately represent abnormal or non-viable cell populations. In addition, MCC and technical challenges related to DNA amplification may further compromise signal specificity. In aggregate, these factors may result in a biologically plausible yet clinically misleading representation of embryonic ploidy.

Most existing reports have focused primarily on assessing analytical concordance between niPGT-A and PGT-A rather than their translation into clinical outcomes. Subsequent to the Xu et al., (2016) report[17], Feichtinger et al., (2017) report[13], analysed 22 blastocysts and reported a diagnostic concordance of 72.2% between cfDNA in the SCM and polar body PGT-A results. Later, Rubio et al., (2020)[19] extended this evidence in a larger cohort of 1,301 human blastocysts, demonstrating a concordance rate of 78.2%. Ho et al., (2018)[14] subsequently reported a 65% concordance between cfDNA and TE biopsy samples, while Yeung et al., (2019)[16] observed a 62% and 82.4% concordance for autosomal and sex chromosomes respectively. Although these investigations collectively established proof-of-principle for niPGT-A, they remained limited by small sample sizes, heterogeneous clinical protocols for niPGT-A implementation, and clinical validations. Importantly, none of these studies determined the reproductive potential of all the embryos underwent niPGT-A testing. However, few reports have documented healthy live births following niPGT-A[16,17,29]; but these cases involved the transfer of embryos confirmed as euploid by TE biopsy rather than niPGT-A alone making it arduous to demonstrate the clinical impact of embryos detected by niPGT-A. To our knowledge, the present study represents one of the biggest datasets and it is the first large-scale efforts to assess this clinical translation, bridging the gap between analytical validation and reproductive efficacy.

The present findings contribute to the ongoing evaluation of emerging embryo selection technologies and their potential role in fertility care. Although niPGT-A has gained clinical interest as a less invasive alternative to biopsy-based genetic testing, its clinical value ultimately depends on its ability to improve patient outcomes. Notably, a recent prospective study by Wu et al., (2025) reported low specificity (50.7%) for euploid embryo detection by niPGT-A compared with biopsy-based PGT-A, highlighting the potential for false-positive classifications and discrepancies in reproductive outcomes, and concluded that the technology, at present, not ready for routine clinical implementation[30]. Similarly, this investigation too highlights the value of evaluating niPGT-A using robust clinical endpoints, including live birth, to better understand their real-world clinical effectiveness before widespread integration into routine practice. Taken together, the observed findings of this investigation align with the Wu et al., (2025)[30] work and suggests that niPGT-A as a technology is yet to evolve.

The major strength of this study lies in its large multicentric real world cohort data, offering a robust comparison. Unlike previous investigations, this study has evaluated reproductive outcomes, potentially providing clinical relevance of niPGT-A integration into clinical practices. These findings evaluated both testing modalities in medically indicated patients offering clinical insights into which approach is compelling for these patients who are more likely to undergo genetic testing. Additionally, the parallel assessment of overall and euploid outcomes, along with stratification by embryo transfer type (SET vs. DET) and vitrification status, including both between- and within-testing modality comparisons, enhances the clinical interpretability and applicability of the findings.

We have realized that this investigation is limited by its retrospective, non-randomised design, and despite adjustment for measured confounders using multivariable regression, residual confounding and selection bias cannot be completely excluded. Furthermore, this report lacks the diagnostic concordance of niPGT-A compared with PGT-A. However, the commercially validated niPGT-A platform employed has demonstrated high analytical accuracy by various investigations[17,18,31,32]. Additionally, regulatory restrictions limited genetic testing to medically indicated cases, potentially limiting the generalizability of these findings to lower-

risk populations. Finally, unidentified biological and technical factors associated with niPGT-A that remain incompletely understood within the current literature cannot be excluded.

Future investigations should focus on prospective clinical validation focusing on patient clinical outcomes including live birth rate. Furthermore, improved understanding of the biological characteristics of embryonic cfDNA, and refinement of analytical algorithms capable of capturing biologically meaningful indicators of embryonic competence will enhance its clinical efficacy.

Conclusion

This large multicentric real-world cohort study evaluated reproductive outcomes following niPGT-A and conventional biopsy-based PGT-A in routine clinical practice. Although euploidy distributions and maternal age-related trends were broadly similar between the two testing modalities, reproductive outcomes from biochemical pregnancy through live birth were significantly improved following biopsy-based PGT-A as compared with niPGT-A. These findings remained consistent across embryo transfer strategies and after adjustment for relevant covariates. However, because of this retrospective nature of this investigation, prospective validation is required to confirm these findings and further define the clinical role of niPGT-A and biopsy-based PGT-A in embryo selection.

Data availability

The datasets generated and analysed during the current study are available from the corresponding authors on a reasonable request.

Received: 26 June 2026; Accepted: 27 August 2026
Published online: 12 September 2026

References

1. Eliner, Y. et al. The impact of preimplantation genetic testing for aneuploidy on time to live birth in in vitro fertilization. *Fertil. Steril.* **124**, 281–289 (2025).
2. Coonen, E. et al. ESHRE PGT Consortium good practice recommendations for the detection of structural and numerical chromosomal aberrations. *Hum. Reprod. Open* (2020). (2020).
3. Yan, J. et al. Live Birth with or without Preimplantation Genetic Testing for Aneuploidy. *N. Engl. J. Med.* **385**, 2047–2058 (2021).
4. Capalbo, A. et al. Consistent and reproducible outcomes of blastocyst biopsy and aneuploidy screening across different biopsy practitioners: a multicentre study involving 2586 embryo biopsies. *Hum. Reprod.* **31**, 199 (2015).
5. Neal, S. A. et al. High relative deoxyribonucleic acid content of trophectoderm biopsy adversely affects pregnancy outcomes. *Fertil. Steril.* **107**, 731–736e1 (2017).
6. Vera-Rodriguez, M. & Rubio, C. Assessing the true incidence of mosaicism in preimplantation embryos. *Fertil. Steril.* **107**, 1107–1112 (2017).
7. Fragouli, E. et al. Analysis of implantation and ongoing pregnancy rates following the transfer of mosaic diploid-aneuploid blastocysts. *Hum. Genet.* **136**, 805–819 (2017).
8. Chuang, T. H. et al. Concordance between different trophectoderm biopsy sites and the inner cell mass of chromosomal composition measured with a next-generation sequencing platform. *Mol. Hum. Reprod.* **24**, 593–601 (2018).
9. Liu, J. et al. DNA microarray reveals that high proportions of human blastocysts from women of advanced maternal age are aneuploid and mosaic. *Biol Reprod* **87** (6), 148, e1-9 (2012).
10. Gleicher, N. et al. A single trophectoderm biopsy at blastocyst stage is mathematically unable to determine embryo ploidy accurately enough for clinical use. *Reprod Biol. Endocrinol* **15**, 33, e1-8 (2017).
11. Stigliani, S., Anserini, P., Venturini, P. L. & Scaruffi, P. Mitochondrial DNA content in embryo culture medium is significantly associated with human embryo fragmentation. *Hum. Reprod.* **28**, 2652–2660 (2013).
12. Shamonki, M. I., Jin, H., Haimowitz, Z. & Liu, L. Proof of concept: preimplantation genetic screening without embryo biopsy through analysis of cell-free DNA in spent embryo culture media. *Fertil. Steril.* **106**, 1312–1318 (2016).
13. Feichtinger, M. et al. Non-invasive preimplantation genetic screening using array comparative genomic hybridization on spent culture media: a proof-of-concept pilot study. *Reprod. Biomed. Online.* **34**, 583–589 (2017).
14. Ho, J. R. et al. Pushing the limits of detection: investigation of cell-free DNA for aneuploidy screening in embryos. *Fertil. Steril.* **110**, 467–475e2 (2018).
15. Vera-Rodriguez, M. et al. Origin and composition of cell-free DNA in spent medium from human embryo culture during preimplantation development. *Hum. Reprod.* **33**, 745–756 (2018).
16. Yeung, Q. S. Y. et al. A prospective study of non-invasive preimplantation genetic testing for aneuploidies (NiPGT-A) using next-generation sequencing (NGS) on spent culture media (SCM). *J. Assist. Reprod. Genet.* **36**(8), 1609–1621 (2019).
17. Xu, J. et al. Noninvasive chromosome screening of human embryos by genome sequencing of embryo culture medium for in vitro fertilization. *Proc. Natl. Acad. Sci. U S A.* **113**, 11907–11912 (2016).
18. Huang, L. et al. Noninvasive preimplantation genetic testing for aneuploidy in spent medium may be more reliable than trophectoderm biopsy. *Proc. Natl. Acad. Sci. U S A.* **116**, 14105–14112 (2019).
19. Rubio, C. et al. Multicenter prospective study of concordance between embryonic cell-free DNA and trophectoderm biopsies from 1301 human blastocysts. *Am. J. Obstet. Gynecol.* **223**, 751e1–751e13 (2020).
20. Balaban, B. & Gardner, D. K. Morphological Assessment of Blastocyst Stage Embryos: Types of Grading Systems and Their Reported Outcomes. *Hum. Gametes Preimplantation Embryos: Assess. Diagnosis*. 31–43. https://doi.org/10.1007/978-1-4614-6651-2_4 (2013).
21. The Assisted Reproductive Technology. (Regulation) Act, 2021 and the Surrogacy (Regulation) Act, 2021 | Indian Council of Medical Research | Government of India. https://www.icmr.gov.in/the-assisted-reproductive-technology-regulation-act-2021-and-the-surrogacy-regulation-act-2021
22. De Rycke, M. et al. ESHRE survey results and good practice recommendations on managing chromosomal mosaicism. *Hum. Reprod. Open* (2022). (2022).
23. Dave, D. D. et al. P-295 Consequences of embryos transfer following fresh biopsy (single vitrification) and frozen-thaw embryo biopsy (double vitrification) after preimplantation genetic testing for aneuploidy: A retrospective cohort analysis. *Human Reproduction* **40**, (i344) (2025).
24. Wen, X. et al. Comparative study of noninvasive chromosomal screening using blastocyst culture media: frozen–thawed embryos outperform fresh embryos. *Front. Endocrinol. (Lausanne).* **17**, 1851046 (2026).

25. Hammond, E. R., Shelling, A. N. & Cree, L. M. Nuclear and mitochondrial DNA in blastocoele fluid and embryo culture medium: evidence and potential clinical use. *Hum. Reprod.* **31**, 1653–1661 (2016).
26. Hammond, E. R. et al. Characterizing nuclear and mitochondrial DNA in spent embryo culture media: genetic contamination identified. *Fertil. Steril.* **107**, 220–228e5 (2017).
27. Capalbo, A. et al. Diagnostic efficacy of blastocoel fluid and spent media as sources of DNA for preimplantation genetic testing in standard clinical conditions. *Fertil. Steril.* **110**, 870–879e5 (2018).
28. Handayani, N. et al. The origin and possible mechanism of embryonic cell-free DNA release in spent embryo culture media: a review. *J. Assist. Reprod. Genet.* **40**, 1231 (2023).
29. Fang, R. et al. Chromosome screening using culture medium of embryos fertilised in vitro: a pilot clinical study. *J Transl Med* **17**, 73, e1-8 (2019).
30. Wu, T. F. et al. Extended blastocyst culture improves DNA yield in non-invasive preimplantation genetic testing for aneuploidy but diagnostic specificity remains limited. *Scientific Reports 2025 15:1* 15, 34906- (2025).
31. Huang, L. et al. Non-invasive Preimplantation Genetic Testing of Embryonic Genome in Spent Culture Medium. https://doi.org/10.1101/2024.11.10.24317049
32. Fang, R. et al. Chromosome screening using culture medium of embryos fertilised in vitro: a pilot clinical study. *J. Transl Med.* **17**, 73 (2019).

Author contributions
D.T. contributed to conceptualization, methodology, formal analysis, investigation, and writing of the original draft. N.S. contributed to conceptualization, methodology, investigation, resources, and review and editing of the manuscript. M.M. contributed to formal analysis, methodology, investigation, and review and editing of the manuscript. R.A. contributed to methodology, resources, and supervision. K.M. contributed to project administration and resources. N.M. contributed to project administration and resources. B.S. contributed to data curation. S.R. contributed to data curation and software. V.C. contributed to project administration, resources, supervision, and review and editing of the manuscript. All authors reviewed and approved the final manuscript.

Funding
No funding was received for conducting this research.

Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
This retrospective study was approved by the Institutional Ethics Committee of Indira IVF Hospital Limited, Udaipur. The approval number for this study is IIVF-UA00091.

Consent to participate
The requirement for obtaining informed consent from participants was waived by the Indira IVF Hospital Institutional Ethics Committee, Udaipur due to the retrospective nature of the study and the use of de-identified/anonymised data.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-026-69506-2.

Correspondence and requests for materials should be addressed to N.S. or M.M.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2026