



Original article

Oocyte-intrinsic aging drives whole-chromosome aneuploidy independent of ovarian reserve beyond maternal age as risk factor

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ABSTRACT

This retrospective cross-sectional study investigated whether maternal age functions as an independent risk factor for chromosomal aneuploidy in day-5 blastocysts through oocyte-intrinsic aging mechanisms rather than quantitative ovarian reserve reduction. A total of 490 infertile couples (2472 day-5 blastocysts) underwent preimplantation genetic testing for aneuploidy (PGT-A) at An Sinh Hospital (Vietnam, 2023). Patients were stratified into three maternal age groups: < 35 years (n = 244, 1261 embryos), 35–40 years (n = 217, 1085 embryos), and ≥ 40 years (n = 29, 126 embryos). Trophectoderm biopsies were analyzed using next-generation sequencing with BlueFuse Multi software. Multivariate logistic regression evaluated maternal age, oocyte quantity, and blastocyst development as independent predictors of aneuploidy. Results demonstrated significant age-related decline in euploid rates from 36.24% in women < 35 years to 20.63% in women ≥ 40 years ($p < 0.0001$), with corresponding increase in aneuploidy from 20.86% to 41.27% ($p < 0.0001$). Maternal age remained independently predictive after adjusting for oocyte quantity (OR 1.052/year; 95% CI 1.031–1.073; $p < 0.001$), demonstrating that age operates through oocyte-intrinsic aging mechanisms rather than reserve-related effects. Day-5 blastocyst count emerged as a prognostic biomarker (OR 0.965; $p = 0.002$) compared to initial oocyte quantity. Mosaic embryo rates remained age-independent (approximately 40–43%; $p = 0.383$), indicating post-zygotic rather than meiotic origins. These findings imply that this indication-enriched PGT-A cohort (advanced maternal age, prior IVF failure, male-factor infertility, and a history of genetic disease) has higher aneuploidy rates than non-selected IVF patients and support age-group patient counseling, use of day-5 blastocyst development as a practical biomarker of oocyte and embryonic potential, and systematic consideration of PGT-A in women ≥ 35 years, particularly in those ≥ 40 years.

1. Introduction

Chromosomal abnormalities represent the leading cause of implantation failure, early miscarriage, and congenital anomalies in assisted reproductive technology [1]. The establishment of chromosomally normal embryo selection through preimplantation genetic testing remains essential for improving in vitro fertilization (IVF) success rates and ensuring genetic health of offspring [2]. Among numerous factors influencing embryonic genetic integrity, maternal age has emerged as the primary determinant of oocyte quality, meiotic competence, and the propensity for chromosomal segregation errors [3].

Maternal age-related increase in aneuploidy is well-established in

natural conceptions, with risk accelerating markedly after age 35 years [1]. This phenomenon becomes increasingly critical during in vitro fertilization cycles, fundamentally influencing treatment planning, prognosis, and reproductive outcomes [4]. The biological mechanisms underlying age-related aneuploidy encompass multiple molecular cascades including progressive degradation of cohesin complex proteins maintaining sister chromatid cohesion, spindle checkpoint assembly complex dysfunction, reduced crossover frequency between homologous chromosomes, telomere shortening, and cumulative mitochondrial dysfunction with oxidative stress accumulation [3,5]. These molecular processes collectively impair the fidelity of meiotic chromosome segregation, particularly during the extended dictyotene arrest unique to

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female meiosis that may persist for 10–50 years [6,7].

Preimplantation genetic testing for aneuploidy (PGT-A) utilizing next-generation sequencing (NGS) has revolutionized embryo selection by enabling precise detection of chromosomal abnormalities, including whole chromosome aneuploidies and segmental imbalances at resolution previously unattainable with fluorescence in situ hybridization (FISH) or comparative genomic hybridization (CGH) technologies [8]. NGS permits simultaneous analysis of all 24 chromosomes (22 autosomes plus sex chromosomes) from a single trophoctoderm biopsy specimen with both high sensitivity and specificity, facilitating accurate classification of euploid, aneuploid, and mosaic embryos [9].

However, comprehensive data regarding the relationship between maternal age and day-5 blastocyst chromosomal status in Vietnamese populations remain scarce. Only two prior Vietnamese studies [10,11] have examined this relationship using NGS-based approaches, with data remaining insufficient for comprehensive clinical counseling and treatment optimization. The present study was undertaken to [1] quantify the relationship between maternal age and chromosomal aneuploidy in day-5 blastocysts using NGS-based PGT-A in a large Vietnamese cohort; [2] evaluate the independence of maternal age as a risk predictor when simultaneously accounting for ovarian stimulation parameters and embryo development kinetics; [3] provide granular statistical modeling stratified by clinically relevant age categories; and [4] furnish evidence-based clinical guidance for individualized ART counseling and treatment planning in the Vietnamese healthcare context.

2. Materials and methods

2.1. Ethical statement

Before conducting the research, the research subjects were informed about the purpose of the research, as well as their rights and responsibilities as research participants. Private information related to the research subjects was kept confidential. Participation in the research was completely voluntary, and research subjects were not required to disclose demographic characteristics such as gender. Before participating, all subjects carefully reviewed and signed the informed consent documents, including the research information sheet and the consent form. This research received approval from the Biomedical Research Ethics Council and adhered to the ethical principles and guidelines of the research institution.

2.2. Study design and selection criteria

This retrospective cross-sectional analysis included 490 consecutive patients who underwent *in vitro* fertilization (IVF) with trophoctoderm (TE) biopsy and preimplantation genetic testing for aneuploidy (PGT-A) of day-5 blastocysts at the Unit of Assisted Reproduction, An Sinh Hospital, Ho Chi Minh City, Vietnam, between January 1 and December 31, 2023.

Patients were eligible if they met at least one of the following indications for PGT-A including (1) maternal age ≥ 35 years; (2) ≥ 2 previous failed IVF cycles; (3) male factor infertility; or (4) personal or family history of genetic disease. Male factor infertility was defined according to institutional semen analysis criteria based on WHO reference ranges and included oligozoospermia, asthenozoospermia, teratozoospermia, or combined defects. Patients were excluded if embryos failed to reach blastocyst stage by day 5, clinical data were incomplete, or samples failed quality control measures during genetic testing.

2.3. Patient stratification

The cohort was stratified into three maternal age groups: < 35 years ($n = 244$), $35\text{--}40$ years ($n = 217$), and ≥ 40 years ($n = 29$). This categorization aligns with established reproductive age benchmarks wherein advanced maternal age is defined as ≥ 35 years, the threshold at which

age-related reproductive risks and chromosomal aneuploidy increase significantly.

2.4. Controlled ovarian hyperstimulation and oocyte retrieval

All patients underwent standard protocols for controlled ovarian hyperstimulation using either gonadotropin-releasing hormone (GnRH) agonist or antagonist approaches, administered in conjunction with recombinant follicle-stimulating hormone (FSH) or human menopausal gonadotropin (hMG). Final oocyte maturation was triggered using either recombinant human chorionic gonadotropin (rhCG) or GnRH agonist, depending on institutional protocol and individual patient characteristics. Oocyte retrieval was performed 35–36 h following trigger injection via transvaginal ultrasound-guided aspiration.

2.5. Fertilization and embryo culture

Retrieved oocytes were assessed for meiosis II (MII) completion and processed for fertilization via conventional IVF or intracytoplasmic sperm injection (ICSI) based on semen analysis parameters. Fertilization was confirmed at 16–18 h post-insemination by observation of two distinct pronuclei. Embryos were cultured in sequential media (G-CLEAVETM and G-BLASTTM, Vitrolife) under controlled atmosphere conditions (5% CO₂, 5% O₂, and 90% N₂) in a time-lapse monitoring incubator (EmbryoScope, Vitrolife) permitting non-invasive morphological assessment at regular intervals. All semen samples are evaluated using standardized procedures at the same andrology laboratory prior to the initiation of treatment cycles, and the decision to use IVF or ICSI is based on the facility's predefined protocols to reduce variability related to sperm handling.

2.6. Blastocyst development assessment

Blastocyst development was evaluated on days 3, 5, and 6 according to established morphological grading criteria (Gardner classification). Day-5 blastocysts meeting quality parameters, specifically expansion grades ≥ 3 with inner cell mass grades A or B and trophoctoderm grades A or B, were selected for trophoctoderm biopsy. Embryos not meeting these criteria were cultured to day 6 for reassessment or discarded if morphological parameters remained suboptimal.

2.7. Trophoctoderm biopsy and DNA processing

Trophoctoderm biopsies (5–10 cells) were performed on selected day-5 blastocysts using a laser-assisted mechanical dissection technique (Saturn 5 Laser, Research Instruments). Biopsied TE specimens were transferred into sterile 0.2 mL tubes and immediately submitted to the genetics laboratory for processing. DNA extraction and whole genome amplification (WGA) were performed using the SurePlex protocol (BlueGnome), which employs a non-PCR amplification method to generate sufficient template DNA. Following WGA, samples underwent NGS library preparation utilizing the VeriSeq PGS kit [12].

2.8. Next-generation sequencing analysis

Sequencing was conducted on the MiSeq platform (Illumina) with 2×150 base pair reads generating approximately 500,000 reads per sample. Bioinformatic analysis employed BlueFuse Multi software (Illumina) with comparison to the GRCh37 (hg19) reference genome. Copy number variations (CNVs) were detected and classified based on whole chromosome and segmental imbalances. Genomic quality control filters were applied to ensure data integrity prior to embryo classification.

2.9. Classification of chromosomal status

Embryos were classified according to Illumina manufacturer recommendations and validated consensus criteria into three categories:

Embryos were classified according to Illumina manufacturer recommendations and validated consensus criteria [8] into three categories. Euploid embryos were defined as chromosomally normal with a balanced chromosome complement and normal copy number across all 24 chromosomes (\log_2 ratio ≈ 0). Aneuploid embryos were classified as chromosomally abnormal, characterized by complete loss or gain of one or more whole chromosomes, such as monosomy or trisomy (\log_2 ratio < -0.4 or > 0.4), or by substantial segmental imbalances of ≥ 10 Mb. Mosaic embryos were defined as those with chromosomal imbalance present in 20–80% of cells within the biopsied sample, indicated by \log_2 ratio values between -0.4 and $+0.4$, or between $+0.4$ and $+0.8$, depending on the proportion threshold.

2.10. Statistical analysis

Descriptive statistics are presented as mean \pm standard deviation (SD) for continuous variables and frequencies with percentages for categorical variables. Between-group comparisons employed Chi-square tests for categorical variables and analysis of variance (ANOVA) with Bonferroni correction for continuous variables. Post-hoc pairwise comparisons were performed when significant overall group differences were identified.

Univariate logistic regression evaluated associations between individual predictor variables (maternal age, number of retrieved oocytes, number of day-5 blastocysts) and embryonic aneuploidy risk. Multivariate logistic regression modeling incorporated all three variables simultaneously as independent predictors of aneuploidy status (binary outcome: euploid vs. “abnormal” [aneuploid + mosaic aneuploid + mosaic]) for the primary clinically focused analysis; euploid vs. aneuploid for a predefined secondary analysis addressing meiotic errors). Results are presented as odds ratios (OR) with 95% confidence intervals (CI). Statistical significance was defined as two-sided $p < 0.05$.

All analyses were performed using R version 4.2.1 (R Foundation for Statistical Computing) and Microsoft Excel 2021. Missing data were handled via list-wise deletion and represented $< 2\%$ of the original dataset.

3. Results

3.1. Patient characteristics and demographic distribution

Baseline demographic and clinical characteristics are presented in Table 1. Mean maternal age in the three groups was 30.75 ± 2.54 years (< 35 years), 37.18 ± 1.68 years (35–40 years), and 43.07 ± 2.62 years (≥ 40 years), respectively ($p < 0.001$). The age distribution reflected a predominance of younger patients with progressively fewer patients in the oldest age stratum, typical of IVF populations in clinical practice.

Anti-Müllerian hormone (AMH) levels demonstrated a statistically

Table 1
Demographic and hormonal characteristics by maternal age group.

Characteristic	Maternal age groups			p-value
	< 35 years	35–40 years	≥ 40 years	
N (patients)	244	217	29	-
Mean age, years \pm SD	30.75 ± 2.54	37.18 ± 1.68	43.07 ± 2.62	< 0.001
AMH, ng/mL \pm SD	3.47 ± 1.86	2.85 ± 1.71	2.01 ± 1.12	< 0.001
Oocytes retrieved \pm SD	19.06 ± 9.87	20.06 ± 10.98	18.31 ± 15.56	0.268
MII oocytes, %	86.1%	82.7%	79.5%	0.076

AMH, Anti-Müllerian hormone; SD, standard deviation; MII, metaphase II

significant decline with advancing maternal age: 3.47 ± 1.86 ng/mL (< 35 years) versus 2.85 ± 1.71 ng/mL (35–40 years) versus 2.01 ± 1.12 ng/mL (≥ 40 years), $p < 0.001$ (Kruskal-Wallis test). The significant difference in AMH between the youngest and oldest groups was 1.46 ng/mL (42% reduction).

3.2. Oocyte retrieval and maturation

Mean oocytes retrieved were comparable across the three age groups: 19.06 ± 9.87 (< 35 years), 20.06 ± 10.98 (35–40 years), and 18.31 ± 15.56 (≥ 40 years), $p = 0.268$ (Table 1). However, the proportion of mature (MII) oocytes showed a trend toward decline with advancing age of 86.1% (< 35 years), 82.7% (35–40 years), and 79.5% (≥ 40 years), though this did not reach statistical significance ($p = 0.076$). Mean MII oocyte counts were also comparable: 16.74 ± 9.25 (< 35 years), 17.48 ± 9.79 (35–40 years), and 17.52 ± 19.64 (≥ 40 years) ($p = 0.915$).

3.3. Fertilization rate and age-related decline

Fertilization rates declined progressively and significantly with maternal age, $62.03\% \pm 17.31\%$ (< 35 years) versus $57.70\% \pm 17.62\%$ (35–40 years) versus $54.00\% \pm 20.44\%$ (≥ 40 years) ($p = 0.0057$) (Table 2). The absolute difference in fertilization rate between the youngest and oldest groups was 8.03 %age points. Linear regression analysis revealed that each additional year of maternal age was associated with an estimated 0.54 %age point decline in fertilization rate ($p = 0.01$) (Fig. 1), equating to a cumulative 5.4 %age point reduction across a 10-year span.

3.4. Blastocyst formation kinetics across development days

The number of embryos progressing to different developmental stages demonstrated age-related decline, with differential patterns for day-3, day-5, and day-6 developmental competency (Supplementary Table 1). For day-3 Embryos, mean day-3 embryo counts were low across all groups (0.12, 0.24, 0.21, respectively) with statistically significant between-group variation ($p = 0.0284$). For day-5 blastocysts (primary analysis), mean day-5 blastocyst counts declined significantly with advancing maternal age 3.75 ± 2.83 (< 35 years), 2.84 ± 2.31 (35–40 years), and 2.62 ± 2.18 (≥ 40 years) ($p = 0.0154$). This represents a 30% decline in day-5 blastocyst availability when comparing women ≥ 40 years to those < 35 years. The proportion of retrieved oocytes reaching day-5 blastocyst stage declined from 61.8% (< 35 years) to 56.2% (35–40 years) to 49.7% (≥ 40 years) ($p = 0.015$). For day-6 blastocysts (secondary analysis), mean day-6 blastocyst counts were more stable across age groups: 1.64 ± 1.72 (< 35 years), 1.84 ± 1.81 (35–40 years), and 1.34 ± 1.56 (≥ 40 years) ($p = 0.106$), indicating no statistically significant age-related decline.

3.5. Chromosomal analysis by maternal age

A total of 2472 day-5 blastocysts underwent complete chromosomal analysis by NGS-based PGT-A (Fig. 2). The embryo distribution across

Table 2
Fertilization and embryo development parameters by maternal age group.

Parameter	Maternal age groups			p-value
	< 35 years	35–40 years	≥ 40 years	
Mean fertilization rate, % \pm SD	62.03 ± 17.31	57.70 ± 17.62	54.00 ± 20.44	0.0057
Mean MII oocytes \pm SD	16.74 ± 9.25	17.48 ± 9.79	17.52 ± 19.64	0.915
Day-5 blastocyst rate, %	61.8%	56.2%	49.7%	0.015

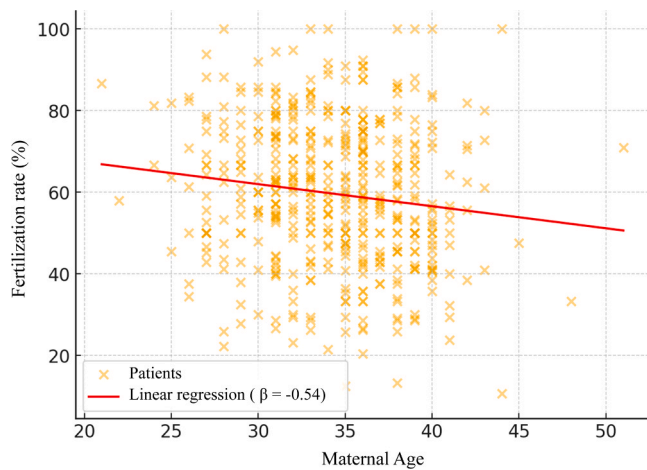


Fig. 1. Fertilization rates demonstrated age-dependent decline across maternal age groups.

maternal age groups was: 1261 embryos (<35 years, 51.0%), 1085 embryos (35–40 years, 43.9%), and 126 embryos (≥40 years, 5.1%), reflecting the age distribution of the patient cohort. Because only 29 patients and 126 embryos were included in the ≥ 40 years group, the estimates for this oldest age group have wider confidence intervals and should be interpreted cautiously, although they are consistent with the general trend related to age.

For euploid embryos (chromosomally normal), the proportion of chromosomally normal euploid embryos showed marked and statistically significant decline with advancing maternal age (Fig. 2) including < 35 years, 457/1261 (36.24%); 35–40 years, 283/1085 (26.08%); ≥ 40 years, 26/126 (20.63%); $\chi^2 = 46.6, p < 0.0001$. This represents a 15.61 %age point absolute decline and a 43% relative reduction in euploid embryo proportion when comparing women < 35 years to those ≥ 40 years. Clinically, patients < 35 years require approximately 3 embryos to identify 1 euploid embryo, whereas patients ≥ 40 years require approximately 5 embryos.

For aneuploid embryos (chromosomally abnormal), chromosomal abnormalities increased substantially and significantly with advancing maternal age (Fig. 2): < 35 years, 263/1261 (20.86%); 35–40 years, 361/1085 (33.27%); ≥ 40 years, 52/126 (41.27%); $\chi^2 = 46.6, p < 0.0001$. The absolute increase in aneuploid frequency was 20.41 %

age points, representing a doubling from the youngest to oldest age group and approximately a 5% increment per year of maternal age.

For mosaic embryos, the proportion of mosaic embryos remained remarkably stable across all maternal age groups (Fig. 2): < 35 years, 541/1261 (42.90%); 35–40 years, 441/1085 (40.65%); ≥ 40 years, 48/126 (38.10%); $\chi^2 = 1.91, p = 0.383$. The mean mosaic rate across all groups was 40.5% (range: 38.1–42.9%), demonstrating age-independence of mosaicism.

3.6. Classification of chromosomal abnormalities

For detailed chromosomal abnormality classification, whole-chromosome aneuploidies (trisomy and monosomy) comprised the majority of chromosomal abnormalities (536 cases, 21.7% of total embryos) (Supplementary Figure 1), with frequency increasing significantly with maternal age including 14.0% (<35 years), 28.8% (35–40 years), and 36.5% (≥40 years). Segmental aneuploidies remained relatively stable: 5.4% (<35 years), 3.1% (35–40 years), and 4.0% (≥40 years). Sex chromosome abnormalities occurred at similarly low frequencies across all age groups (<1.3%).

3.7. Univariate logistic regression analysis

Univariate logistic regression was used to assess the relationship between maternal age and the risk of embryonic aneuploidy in a sample of 2472 embryos (Supplementary Figure 2). The analysis revealed that for each additional year of maternal age, the odds of aneuploidy increased by 5.7% (odds ratio [OR] = 1.057; 95% confidence interval [CI]: 1.036–1.078; $p < 0.001$). Over a 5-year period, such as from age 35–40, this translated to a cumulative increase in the odds of aneuploidy by approximately 31%.

Multivariate logistic regression was then performed to evaluate the simultaneous effects of maternal age, number of retrieved oocytes, and number of day-5 blastocysts as independent predictors of embryonic aneuploidy status across the same 2472 embryos (Table 3). Maternal age remained the strongest independent predictor, with an OR of 1.052 per year (95% CI: 1.031–1.073) ($p < 0.001$), indicating a 5.2% increased odds of aneuploidy per year, a minimal change from the univariate analysis that underscores maternal age as a robust risk factor. Additionally, the number of retrieved oocytes showed a modest positive association with aneuploidy risk (OR = 1.010; 95% CI: 1.003–1.018; $p = 0.008$). In contrast, the number of day-5 blastocysts had a

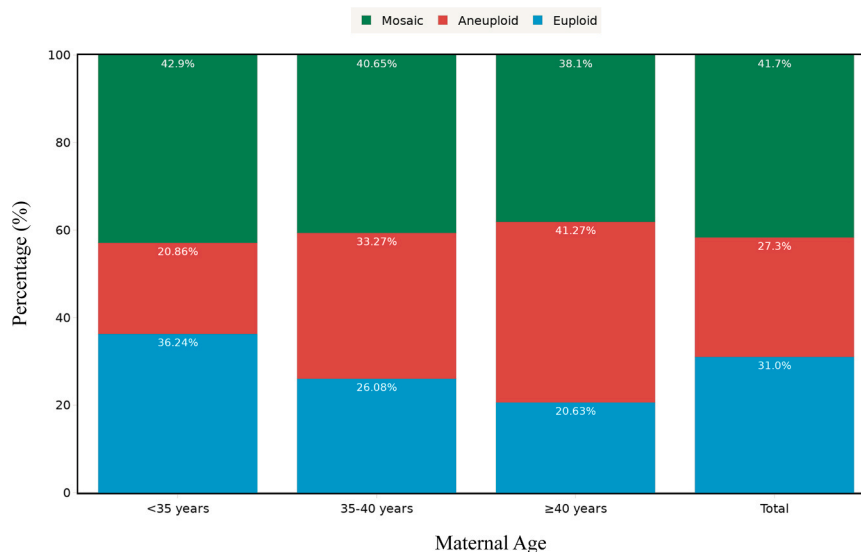


Fig. 2. PGT-A results by maternal age group demonstrate the primary finding of progressive decline in euploid embryo rates, decreasing from 36.24% in women < 35 years to 20.63% in women ≥ 40 years.

Table 3
Independent predictors of aneuploidy identified by multivariate logistic regression.

Predictor	Odds Ratio	95% CI	p-value	N Embryos
Maternal age (per 1-year increase)	1.052	1.031–1.073	< 0.001	2472
Number of retrieved oocytes (per 1 oocyte)	1.010	1.003–1.018	0.008	2472
Number of day-5 blastocysts (per 1 blastocyst)	0.965	0.944–0.987	0.002	2472

significant protective effect, with each additional blastocyst associated with a 3.5% reduction in aneuploidy odds (OR = 0.965; 95% CI: 0.944–0.987; $p = 0.002$).

4. Discussion

This cohort's demographic characteristics reflect patterns consistent with clinical IVF populations globally, with statistically significant ($p < 0.001$) stratification of maternal age across three groups (Table 1). The predominance of younger patients (244 in <35 years) progressively declining to 29 patients in the ≥ 40 years category demonstrates typical fertility treatment-seeking patterns [13]. Serum AMH levels declined dramatically ($p < 0.001$) across age strata (Table 1), representing a 42% reduction reflecting well-established age-related ovarian reserve depletion [14]. This finding aligns with documented AMH decline rates of approximately 0.31 ng/mL/year in women aged 33–43 years. In particular, mean oocyte retrieval remained comparable across age groups (19.06 ± 9.87 – 18.31 ± 15.56) ($p = 0.268$) despite substantially reduced AMH in older patients, a paradox reflecting survivor bias wherein poor-responder cycles undergo cancellation prior to retrieval, systematically excluding the most severely affected individuals from analysis [15]. The elevated standard deviation in the ≥ 40 years group (± 15.56 versus ± 9.25) indicates greater heterogeneity in oocyte retrieval outcomes, encoding the underlying differential cycle completion based on ovarian response. In addition, all patients in this study group met at least one clinical indication for PGT-A (advanced maternal age, previous IVF failure, male-related infertility, or personal/familial genetic disease), resulting in a higher proportion of at-risk couples compared to the general IVF population and potentially contributing to the relatively high absolute aneuploidy rate observed.

The observed trend toward declining meiosis II (MII) oocyte proportions with advancing maternal age (Table 1) approaches statistical significance and reflects age-related impairment of oocyte cytoplasmic maturation. While the p -value reflects limited statistical power in the oldest age category ($n = 29$), the magnitude of decline (6.6 %age points) is biologically meaningful and aligns with prior investigations demonstrating higher oocyte immaturity rates in women aged ≥ 41 years [16]. This pattern suggests impaired cytoplasmic maturation mediated through multiple mechanisms: mitochondrial dysfunction with reduced ATP synthesis [17], oxidative stress pathway activation, proteostatic disruption with altered protein synthesis regulation [18], and compromised cumulus-oocyte cooperativity and growth factor signaling [19]. Mean MII oocyte counts demonstrated equivalence across age groups (Table 1), though this apparent homogeneity masks age-related selection bias wherein older women achieving retrieval represent preferentially good-responders, artificially elevating their MII oocyte means.

A significant and progressive decline in fertilization rates with advancing maternal age was observed in women < 35 years and women ≥ 40 years ($p = 0.0057$, Table 2), representing an 8.03 %age point absolute decline. Linear regression analysis demonstrated 0.54 %age point decline per advancing year ($p = 0.01$) (Fig. 1), equating to 5.4 %age point cumulative reduction across a 10-year span. These findings establish maternal age as a significant independent predictor of fertilization competence, with magnitude aligning with published

international data [20,21]. Age-related fertilization rate decline reflects multiple impaired oocyte cytoplasmic competence parameters: zona pellucida structural alterations and reduced sperm-binding capacity, cortical granule positioning and exocytosis dysfunction, compromised calcium signaling machinery, and altered pronuclear formation kinetics [22,23]. Premature cortical granule exocytosis in aged oocytes releases ovastacin aberrantly, which cleaves and cross-links ZP2 prior to fertilization, creating a zona pellucida structurally resembling post-fertilization state and preventing sperm binding [23]. Additionally, age-related calcium store depletion and reduced calcium release kinetics impair oocyte activation, with oxidative stress-mediated damage to IP3 receptors and calcium transporters further compromising this critical machinery [24].

Blastocyst formation kinetics revealed significant age-related decline ($p = 0.0154$) in day-5 blastocyst formation (Table 2, Supplementary Table 1), representing 30% reduction in day-5 blastocyst availability in women ≥ 40 years. The proportion of oocytes reaching day-5 stage declined from 61.8% to 49.7% ($p = 0.015$). In contrast, day-6 blastocyst counts remained stable (1.64 ± 1.72 – 1.34 ± 1.56) ($p = 0.106$) (Supplementary Table 1), indicating developmental delay kinetics rather than developmental incompetence. This divergent pattern reflects impaired embryonic oxidative phosphorylation and ATP synthesis capacity with advancing maternal age [25]. Early embryonic development depends critically on mitochondrial ATP synthesis as glycolysis remains suppressed during preimplantation stages. Studies measuring mitochondrial oxygen consumption rates demonstrate age-related decline in embryonic OCR at morula stage, with reduced OCR directly correlating to prolonged developmental intervals necessary for achieving blastocyst expansion [25]. Day-5 achievement emerges as a biomarker of superior embryo quality and developmental competence [26], with clinical implications supporting prioritization of day-5 blastocyst biopsy and transfer when available.

The present analysis of 2472 day-5 blastocysts demonstrated marked decline ($p < 0.0001$) in euploid embryo proportions with advancing maternal age in women < 35 years and women ≥ 40 years (Fig. 2), representing absolute 15.61 %age point and relative 43% reduction. This translates clinically to requiring approximately 3 embryos for one euploid in younger women versus 5 embryos in women ≥ 40 years. Univariate logistic regression established that each advancing year conveyed 5.7% increased ($p < 0.001$) aneuploidy odds (OR 1.057, 95% CI 1.036–1.078) (Supplementary Figure 2). In particular, multivariate analysis including retrieved oocyte number and day-5 blastocyst count demonstrated maternal age remained the dominant independent predictor (OR 1.052, 95% CI 1.031–1.073) ($p < 0.001$) (Table 3), with minimal change from univariate reflecting that age operates as independent risk factor rather than proxy for oocyte quantity. This establishes maternal age-related aneuploidy as reflecting fundamental oocyte-intrinsic aging rather than secondary effects of reduced quantity.

Whole-chromosome aneuploidies comprised 21.7% of all embryos and demonstrated striking age-dependency (Supplementary Figure 1), representing nearly three-fold increase. Specific autosomal trisomies show distinct patterns: trisomy 21 increases rapidly from < 1% to approximately 5% in women 40–45 years, while trisomies 13 and 18 increase approximately two-fold [27]. Preferential T21 increase reflects chromosome 21-specific vulnerability to premature sister chromatid separation and age-related cohesin loss related to chromosomal size and centromeric structure [5]. The primary mechanistic driver of whole-chromosome aneuploidy is progressive cohesin complex protein loss established exclusively during fetal oogenesis with no replenishment throughout reproductive life, producing age-dependent deterioration reaching critical thresholds between ages 35–40 years [28,29]. Cohesin loss leads to premature sister chromatid separation, representing the most common meiotic segregation error in aged oocytes [30]. Additionally, spindle checkpoint assembly complex desensitization occurs through age-related ROS accumulation causing preferential SAC component degradation and altered protein homeostasis [31], while

altered recombination patterns show 2–3-fold increased achiasmate chromosome pairs with age [5].

In contrast, segmental aneuploidies (chromosomal segments ≥ 10 Mb) remained remarkably age-independent (5.4–4.0%), with no significant age trend (Supplementary Figure 1). This age-independence despite pronounced whole-chromosome age-dependency indicates mechanistically distinct etiologies: segmental abnormalities arise through non-allelic homologous recombination between genomic repetitive sequences, paternal meiotic errors, post-zygotic rearrangements, and chromothripsis-like phenomena [3], mechanisms unaffected by female reproductive aging. Approximately 77% of segmental deletions demonstrate paternal origin (OR 3.4–5.1 for paternal vs. maternal), further supporting mechanistic distinctness from age-related maternal oocyte defects.

Sex chromosome aneuploidies similarly showed age-independence (Supplementary Figure 1), reflecting multifactorial etiologies from both parental meiotic systems and post-zygotic errors rather than maternal aging-specific mechanisms. Mosaic embryo proportions remained age-independent at approximately 40% across all groups ($p = 0.383$) (Fig. 2), indicating post-zygotic mitotic origins distinct from meiotic aneuploidy. These divergent age-dependencies provide essential mechanistic context establishing specific clinical applicability of PGT-A for detecting and avoiding maternal age-related whole-chromosome aneuploidies rather than segmental or sex chromosome abnormalities [32,33].

Although the primary focus of this analysis was maternal age and oocyte intrinsic mechanisms, male factors also appeared in some couples and were one of the indications for PGT-A in this cohort. Detailed semen parameters and paternal age were not included as independent variables in the multivariate models, which is a limitation. However, modern evidence suggests that paternal age has little or no significant impact on the rate of total aneuploidy in blastocysts, while maternal age has a strong exponential impact, particularly for autosomal trisomies [27]. Therefore, although male-factor infertility and sperm quality remain clinically important for fertilization and embryonic development, omitting them in regression models is unlikely to reverse the main conclusion that maternal age-related oocyte intrinsic defects are the primary drivers of total aneuploidy.

Retrieved oocyte number demonstrated modest positive association with aneuploidy risk (OR 1.010, $p = 0.008$) (Table 3), likely reflecting confounding by indication and survivor bias wherein higher retrievals occur in younger women with more aggressive stimulation yet older women with lower baseline reserve are simultaneously enrolled. The minimal effect size (1.01 per oocyte) suggests limited independent clinical significance. In striking contrast, day-5 blastocyst count demonstrated significant protective effect (OR 0.965, $p = 0.002$) (Table 3), with each additional day-5 embryo reducing aneuploidy odds by 3.5% substantially exceeding oocyte quantity effects. This protective day-5 effect reflects selection for superior oocyte quality rather than direct protection: women producing multiple day-5 blastocysts possess oocytes with intact meiotic checkpoints, better-preserved mitochondrial function, robust cohesin complexes, and superior developmental competence [26]. These quality metrics simultaneously support rapid development kinetics and reduced segregation errors. The distinction is clinically vital: a 42-year-old woman retrieving 20 oocytes retains identical baseline aneuploidy risk per oocyte as one retrieving 10 oocytes, though absolute burden increases proportionally. Identifying oocytes developing into day-5 blastocysts provides meaningful quality stratification. Predictive modeling studies incorporating multiple variables [34] achieve AUCs of 0.80–0.83 for discriminating high-risk ($\geq 50\%$ aneuploidy) populations, with maternal age emerging as strongest independent predictor. Day-5 achievement, a simplified developmental pace measure, provides prognostic effects independent of age and oocyte number, supporting developmental competence as clinically relevant quality metric, with morphokinetic parameters predicting aneuploidy at 75–80% accuracy [26].

This study findings have several practical implications for counseling and treatment planning. For women < 35 years, the lower baseline aneuploidy rate and the need for approximately three embryos to identify a normal blastocyst suggest that the number of oocytes exceeding a certain threshold may be less important than selecting for the quality of day 5 blastocysts. For women 35–40 years, the average aneuploidy rate (26–33%) and the strong independent influence of maternal age justify individualized discussion of PGT-A to avoid transferring chromosomally aneuploid embryos, with particular attention to the number of day 5 blastocysts as an indicator of potential oocyte quality. For women ≥ 40 years, where approximately 41% of blastocysts are aneuploid, practical counseling should include the possibility of needing multiple cycles and/or embryo accumulation strategies to obtain a normal blastocyst, and PGT-A may be particularly helpful in minimizing unsuccessful embryo transfers. In summary, these data support age-group counseling and emphasize that achieving blastocysts by day-5 is a more informative quality indicator than the number of oocytes retrieved.

5. Limitations and mechanistic perspectives

Several considerations apply to the present analysis. First, the relatively small oldest age group ($n = 29$ patients, $n = 126$ embryos) limits statistical power for this clinically important stratum, therefore, age-specific estimates for women ≥ 40 years should be interpreted cautiously even if they are consistent with the overall linear trend and external datasets. Second, the study was designed as a cross-sectional embryological analysis and did not systematically collect results on implantation, pregnancy, live birth, or offspring phenotype for all biopsied embryos; therefore, results cannot be directly translated into cumulative live birth probability or long-term child health. The linear dose-response assumption between maternal age and aneuploidy log-odds may oversimplify potential nonlinear trajectories. Unmeasured confounders including BMI, smoking status, ovarian stimulation protocol variations, trigger modality, and sperm source may independently influence results. The binary outcome classification simplifies the complex aneuploidy spectrum; separate stratification by whole-chromosome versus segmental abnormalities could provide mechanistically distinct insights. Selection bias from PGT-A clinical indication enrichment (age ≥ 35 years, prior failed IVF cycles, male factor infertility, and a history of genetic disease) may amplify observed age-aneuploidy effects compared to population-level estimates. International comparisons support generalizability (e.g. [32,33] analyzing 15,000 embryos reported similar whole-chromosome aneuploidy trajectories, while other study demonstrated comparable trisomy patterns. The present Vietnamese cohort thus demonstrates concordance with established international patterns while providing the largest and most comprehensively analyzed domestic PGT-A dataset.

In conclusion, this comprehensive analysis establishes maternal age as an independent predictor of embryonic aneuploidy operating through multiple converging molecular mechanisms affecting oocyte maturation, fertilization competence, embryonic developmental kinetics, and chromosomal segregation fidelity. The persistence of age effects after multivariate adjustment, coupled with age-independence of segmental and sex chromosome abnormalities, definitively demonstrates that maternal age effects operate through oocyte-intrinsic biological aging rather than quantitative ovarian reserve reduction. Day-5 blastocyst achievement emerges as a superior prognostic biomarker compared to oocyte quantity, establishing developmental competence as clinically meaningful for risk stratification. These evidence-based findings support individualized PGT-A counseling and fertility preservation decision-making across the reproductive lifespan, emphasizing that while reproductive aging is inevitable, strategic utilization of oocyte quality assessment and genetic testing can optimize reproductive outcomes across all age groups.

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Declaration of Competing Interest

The authors declare that the study was done in the absence of any financial or commercial relationships that could be perceived as a possible conflict of interest.

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Conflict of interest

The authors state that this research was carried out without any commercial or financial involvement that might be viewed as a potential conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.repbio.2026.101205](https://doi.org/10.1016/j.repbio.2026.101205).

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