

Preimplantation Genetic Testing for Aneuploidy (PGT-A) on Blastocyst Quality, Vitricification Timing, and IVF Outcomes: A Comparative Study

Roya Rozati^{1*}, Muhammad Siddique Ahmed Khan², Wajeeda Tabasum³, Taalia Nazeer Ahmed⁴,
Ayapati Gautam Mehdi⁵, Vikram Aiman Ayapati⁶

1. MD (A.I.I.M.S, Delhi), F.R.C.O.G. (London), Professor and Head, Dept. of Obst & Gynecology, Shadan Institute of Medical Sciences, Medical and Research Director, Medical Health and Research Institute (MHRI), Hyderabad, India.
2. Professor HOD, Department of Biochemistry, Shadan Institute of Medical Sciences, Hyderabad, India.
3. M.Sc Genetics, Osmania University, Research Scholar, Medical Health and Research Institute (MHRI), Hyderabad, India.
4. M.Pharm, Osmania University, Research Scholar, Medical Health and Research Institute (MHRI), Hyderabad, India.
5. Scientist C, Medical Health and Research Institute (MHRI), Hyderabad, India.
6. Scientist C, Medical Health and Research Institute (MHRI), Hyderabad, India.

**Correspondence:* Roya Rozati

Received: 17 June 2024; *Accepted:* 25 June 2024; *Published:* 05 July 2025

Citation: Roya Rozati. Preimplantation Genetic Testing for Aneuploidy (PGT-A) on Blastocyst Quality, Vitricification Timing, and IVF Outcomes: A Comparative Study. AJMCRR. 2025; 4(7): 1-13.

Abstract

Background: Preimplantation genetic testing for aneuploidy (PGT-A) is increasingly applied in assisted reproductive technologies, particularly among individuals with advanced maternal age or a history of recurrent pregnancy loss. By enabling the selection of chromosomally normal embryos, PGT-A may improve implantation rates and pregnancy outcomes following the initial embryo transfer. This study aims to compare ICSI outcomes, blastocyst quality, and vitrification timing between PGT-A and control groups to assess the reproductive outcomes

Methods: A retrospective cohort study was conducted at a MHRT Hospital and Research Center, Hyderabad, involving patients undergoing Intracytoplasmic Sperm Injection (ICSI) cycles between March 2022 and March 2025. The study included 52 PGT-A and 52 control patients, with Baseline characteristics, embryological parameters, blastocyst quality, vitrification timing, and reproductive outcomes were assessed. The PGT-A group included embryos that underwent aneuploidy testing via trophectoderm biopsy. Blastocyst expansion, inner cell mass (ICM) quality, trophectoderm (TE)

grading, and vitrification day were compared between the two groups.

Results: Patients in the PGT-A group were older and had higher rates of primary and female-factor infertility. Hormonal profiles and oocyte retrieval outcomes were comparable between groups. The PGT-A group was older (mean age 36.2 vs. 33.7 years) and had higher proportions of primary infertility (71.15% vs. 59.61%) and female infertility (61.53% vs. 42.3%). Both groups had more or less similar oocyte retrieval numbers, but the Control group had more prior ICSI cycles. Regarding blastocyst quality, the PGT-A group exhibited higher proportions of grade 3 and grade 6 blastocysts, with a better ICM grade (B) and TE grade (A). The Control group had a higher proportion of grade 4 blastocysts. Additionally, more embryos in the Control group were vitrified on Day 5 (76.92% vs. 61.53%), while the PGT-A group showed a higher proportion vitrified on Day 6 (38.46% vs. 23.07%), these differences did not translate into superior clinical outcomes. The cumulative live-birth rate was slightly lower in the PGT-A group (75%) compared to controls (83.69%). Secondary outcomes, including biochemical, clinical, and ongoing pregnancy rates, were also lower in the PGT-A group. Singleton and twin birth weights were marginally reduced in the PGT-A cohort.

Conclusion: PGT-A is associated with higher-quality blastocysts, particularly in terms of ICM and TE grading. It also influences vitrification strategies, with PGT-A embryos more likely to be vitrified on Day 6. These findings suggest that PGT-A may improve embryo quality, potentially enhancing ICSI outcomes, especially in older women or those with recurrent implantation failure. However, further studies are necessary to refine patient selection criteria and evaluate cost-effectiveness and ethical concerns associated with PGT-A.

Keywords: Preimplantation Genetic Testing- Aneuploidy, Controls, Blastocyst, Trophoctoderm.

Abbreviations: ICSI, PGT-A, TE, ICM.

Introduction: arrest of oocytes in prophase I, which begins Assisted reproductive technologies (ART), prenatally and continues until ovulation (Babariya particularly intracytoplasmic sperm injection D et al. 2017). This extended arrest period leads to (ICSI), have significantly transformed infertility progressive deterioration of the meiotic apparatus, treatment, enabling numerous couples to achieve contributing to an age-dependent increase in successful pregnancies (Retzloff MG et al.2003). aneuploidy rates in mature oocytes. Despite these advances, embryo aneuploidy remains a significant cause of implantation failure PGT-A has become an important adjunct to and pregnancy loss. traditional in vitro fertilization (IVF) and embryo transfer (ET) protocols. This technique involves The majority of aneuploidies in Preimplantation comprehensive chromosomal screening of embryos embryos arise from meiotic errors during to identify euploid candidates for transfer, thereby oogenesis. This is largely due to the prolonged reducing the likelihood of implanting aneuploid

embryos that have lower implantation potential and higher miscarriage risks (Viotti M 2020).

who exhibit a higher prevalence of embryo aneuploidy.

PGT-A is particularly indicated in couples with advanced maternal age (AMA), recurrent

Materials and Methods
Study design

implantation failure (RIF), severe male factor (SMF) infertility, and those with recurrent pregnancy loss despite normal parental karyotypes (Morales C.2020). The predominant method employed is blastocyst-stage biopsy-based PGT-A

This study was approved by the Institutional Ethical Review Board for Clinical Research at MHRI Hospital and Research Center, Hyderabad. All of the patients gave written informed consent.

(BB-PGT-A), which accounts for approximately 90% of PGT-A cycles. This approach includes culturing embryos to the blastocyst stage (day 5–6), followed by the removal of 5–10 trophoctoderm cells precursors to the placenta and extraembryonic tissues and subsequent comprehensive chromosome screening of all chromosomes (Stem HJ 2014).

This retrospective cohort study was conducted on patients who underwent Intracytoplasmic sperm injection (ICSI) cycles at a MHRT Hospital and Research Center, Hyderabad, between March 2022 and March 2025. The study included all single embryo transfer cycles following oocyte retrieval, where embryos were cultured to the blastocyst stage and vitrified. For cases involving

Approximately 50–60% of embryos tested via PGT-A are euploid and viable for transfer. The decline in oocyte quality with advancing ovarian age has been well documented and correlates with increased rates of embryonic aneuploidy (Anderson

preimplantation genetic testing for aneuploidy (PGT-A), only embryos that underwent aneuploidy testing via trophoctoderm biopsy were included, while those with cleavage-stage biopsies were excluded.

RE et al.2020). Proposed mechanisms for this decline include elevated meiotic errors during

Study protocol

oogenesis, diminished mitochondrial DNA content in oocytes, instability of the mitotic spindle, and telomere shortening in surrounding granulosa cells (Salame AA et al.2024). These factors collectively impair oocyte competence and contribute to the higher incidence of aneuploid embryos observed in older patients.

Ovarian stimulation was conducted using either long or short protocols involving GnRH agonists or antagonists (Yan J et al. 2021). In the long protocol, a gonadotropin-releasing hormone (GnRH) agonist is administered during the mid-luteal phase of the preceding menstrual cycle, with gonadotropin stimulation initiated following confirmation of adequate pituitary downregulation. In contrast, the short protocol begins with the administration of a

The principal objective of PGT-A is to enhance reproductive outcomes by increasing live birth rates and reducing adverse events such as miscarriage. Several studies have reported improved pregnancy success rates with PGT-A compared to ICSI, especially in patients of advanced maternal age, a combination of both, with oocyte retrieval

GnRH agonist on cycle day 2 or 3, followed by gonadotropin stimulation two days later. Final oocyte maturation was triggered using human chorionic gonadotropin (hCG), a GnRH agonist, or a combination of both, with oocyte retrieval

performed 34 to 36 hours later under transvaginal ultrasound guidance at least two follicles reached ≥ 18 mm in diameter. All retrieved oocytes underwent intracytoplasmic sperm injection (ICSI), and resulting embryos were cultured to the blastocyst stage. In the PGT-A group, three morphologically high-quality blastocysts per cycle were selected for trophectoderm biopsy, with a single euploid embryo chosen for transfer. In the control group, one high-quality blastocyst was selected for transfer without genetic testing. Endometrial preparation for frozen embryo transfer was achieved through natural, artificial, or ovulation induction protocols, followed by luteal-phase support in both groups.

Outcomes:

Embryo transfers were performed within one year of randomization, with up to three blastocysts transferred per patient until a live birth or pregnancy termination occurred. Only singleton live births were included in the final analysis, and all pregnancy and neonatal outcomes were systematically recorded. The Secondary outcomes included the rate of a good birth outcome, defined as a live birth at or beyond 37 weeks of gestation, with a birth weight between 2500 and 4000 grams, and the absence of major congenital anomalies.

Statistical Analysis:

All data were analyzed using SPSS software version 26.0. The distribution of continuous variables was evaluated to determine the appropriate statistical test. Parametric data were compared using independent t-tests, while nonparametric variables were analyzed using the Mann–Whitney U test. Categorical variables were assessed using the Chi-square test or Fisher's exact test when expected frequencies were less than five.

A two-tailed p-value of less than 0.05 was considered indicative of statistical significance. This methodology provided a comprehensive comparison of baseline characteristics, embryological parameters, and clinical outcomes between the PGT-A and control groups.

Results:

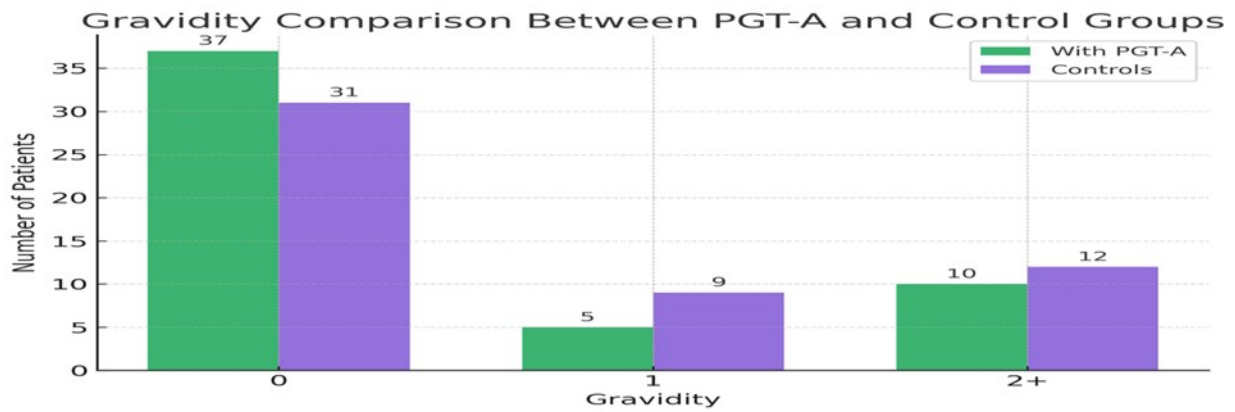
This study compares ICSI outcomes between two groups: those who underwent pre-implantation genetic testing for aneuploidy (PGT-A, n = 52) and those with Controls (n = 52). The PGT-A group was older (mean age at retrieval: 36.2 vs. 33.7 years) and had a higher proportion of primary infertility (71.15% vs. 59.61%) and female infertility (61.53% vs. 42.3%). The PGT-A group also had slightly lower BMI (24.1 vs. 25.1 kg/m²), fewer prior pregnancies (71.15% vs. 59.61%), and more prior miscarriages (15.4% vs. 9.7%). Oocyte retrieval numbers were similar between the groups (median 9), but the control group had a higher median of prior ICSI cycles (1 vs. 0). Hormonal profiles, including TSH, AMH, FSH, LH, estradiol, progesterone, testosterone, and prolactin, were comparable between the two groups. The distribution of gravidity and parity also showed slight variations, with a higher proportion of nulligravid (71.15%) and nulliparous (82.69%) individuals in the PGT-A group as shown in **graph1 and graph 2**. The number of prior miscarriages and ART cycles was similar across both cohorts, with the control group having a marginally higher median number of previous cycles. The control group also had slightly thicker endometrial lining at transfer (9.2 vs. 8.9 mm). The number of oocytes retrieved were consistent between groups, both reporting a median of 11 and 13 oocytes respectively in PGT- A and Control group as depicted in **graph 3**. The number of

mature (MII) oocytes and high-quality embryos on day 3 and day 5 were 7 and 8 in both the groups, suggesting comparable embryological outcomes despite demographic and clinical differences. These differences highlight the distinct patient profiles and clinical approaches associated with PGT-A in ICSI as depicted in **Table 1**

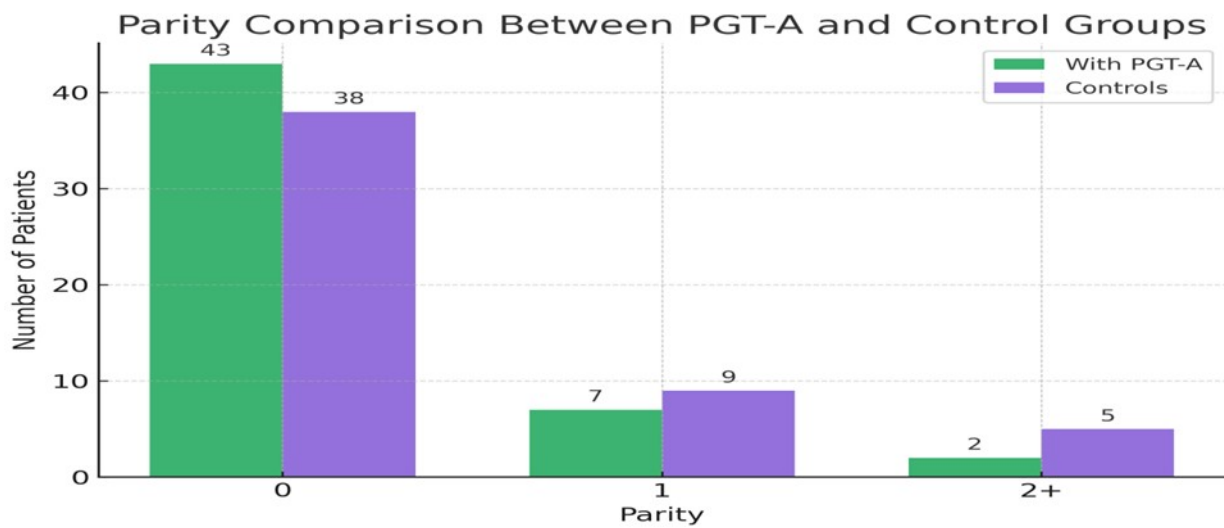
Table 1 Baseline Characteristics of All Vitrified-Warmed Single Blastocyst Transfers Stratified by Preimplantation Genetic Testing for Aneuploidy (PGT-A) Status

	With PGT-A (n = 52)	Controls (n =52)	P-value
Age at retrieval (years)	36.2	33.7	0.002
Age at transfer (years)	36.6	34.6	0.010
BMI (kg/m2)	24.1	25.1	0.278
Primary infertility	37(71.15%)	31(59.61%)	0.212
Reason for ICSI			
Female infertility	32(61.53%)	22(42.3%)	-
Male infertility	9(17.3%)	12(23.07%)	-
Other	5 (13.46%)	6(11.53%)	-
Unexplained	6(11.53%)	12(23.07%)	-
TSH(mIU/mL)	2.34±1.51	2.17±1.12	0.494
AMH	6.39(4.17-10.12)	6.24(4.09-9.61)	0.798
FSH(IU/L)	5.87(5.01-7.91)	5.99(5.15-6.87)	0.642
LH(IU/L)	5.38(3.91-8.01)	5.69(4.15-8.24)	0.547
Estradiol (pg/ml)	33.58 (25.94-43.26)	37.15(28.75-48.61)	0.220
Progesterone(ng/ml)	0.40(0.23-0.67)	0.40(0.23-0.67)	1.000
Testosterone(ng/ml)	0.33(0.24-0.49)	0.33(0.24-0.49)	1.000
Prolactin(ng/ml)	15.75(11.38-20.96)	16.64(12.95-22.37)	0.391
Gravidity			
0	37(71.15%)	31(59.61%)	-
1	5(9.615%)	9(17.3%)	-
2+	10(19.23%)	12(23.07%)	-
Parity			
0	43(82.69%)	38(73.07%)	-
1	7(13.46%)	9(17.3%)	-
2+	2(3.84%)	5(9.61%)	-
Prior miscarriage			
0	44(84.6%)	47(90.3%)	-
1	5(9.61%)	4(7.69%)	-
2+	3(5.76%)	1(1.92%)	-
Number of prior cycles	0(0-2)	1(1-2)	0.027
Endometrial thickness	8.9(7.9-9.9)	9.2(8.1-10.8)	0.294
Number of Oocytes retrieved	11(11-16)	13(11-16)	0.924
Number of MII	7 (9-11)	8(9-11)	0.832
No. of high-quality embryos on day 3	5	6	1.000
No. of high-quality embryos on day 5	3	4	1.000

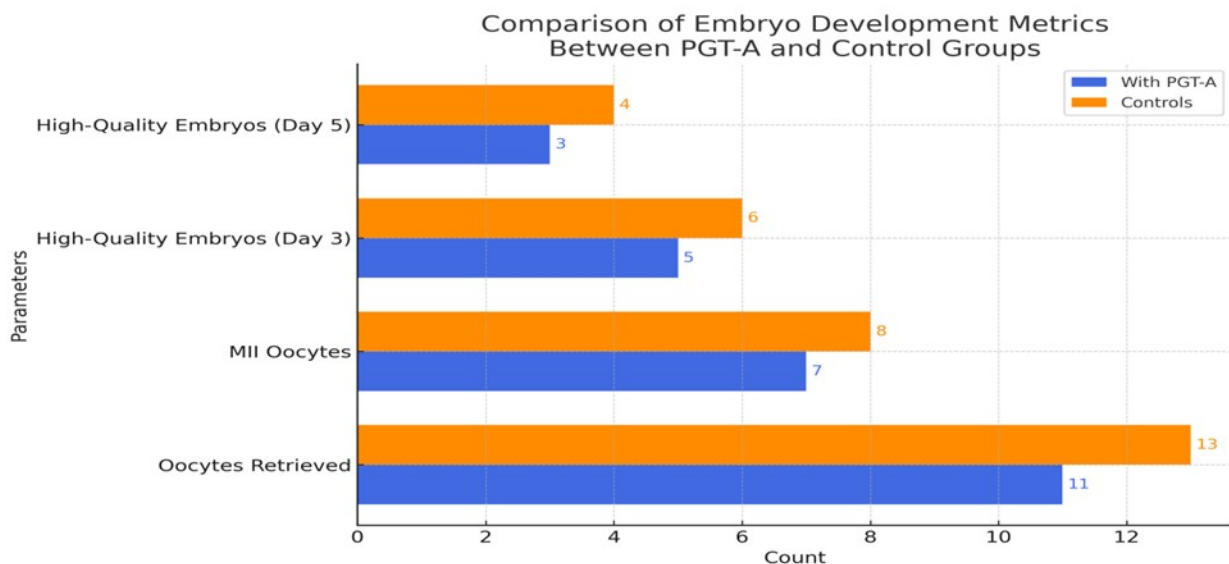
Statistical analyses were performed to compare baseline and clinical characteristics between the PGT-A and control groups (n = 52 each). Continuous variables were assessed for normality using distribution summaries. Variables following a normal distribution were compared using independent samples t-tests while non-normally distributed data were analyzed using the Mann–Whitney U test. Categorical variables were compared using the Chi-square test or Fisher’s exact test when expected frequencies were <5. A *p*-value < 0.05 was considered statistically significant. All analyses were two-tailed.



Graph 1 represents Gravidity comparison between PGT-A and Controls



Graph 2 represents parity comparison between PGT-A and Controls



Graph 3 represents the comparison of Embryo development metrics between PGT-A and Controls.

Our study compares blastocyst quality and vitrification day between two IVF groups: those with preimplantation genetic testing for aneuploidy (PGT-A, n = 52) and those without (n = 52). In terms of

blastocyst expansion, the PGT-A group had a higher proportion of grade 3 blastocysts (28.84% vs. 21.15%) and grade 6 blastocysts (13.46% vs. 5.76%), while the Control group had more grade 4 blastocysts (53.84% vs. 38.46%). For inner cell mass quality, the PGT-A group had a higher proportion of grade B (51.92% vs. 42.3%) but fewer grade A (44.23% vs. 53.84%) compared to the Control group. In trophoctoderm grading, both groups had similar distributions, with most blastocysts rated as grade B (57.69% in PGT-A vs. 59.61% in Control). Regarding blastocyst vitrification, more embryos from the Control group were vitrified on Day 5 (76.92% vs. 61.53%), while the PGT-A group had a higher proportion vitrified on Day 6 (38.46% vs. 23.07%) as given in **table 2**. These findings suggest differences in blastocyst quality and vitrification timing between the two groups, potentially reflecting the influence of genetic testing on embryo selection and cryopreservation strategies.

Table 2: Blastocyst Grading per Embryo Transfer, Grouped by PGT-A Testing

	With PGT-A (n = 52)	Controls(n =52)	P-value
Blastocyst expansion grade			0.203
3	15(28.84%)	11(21.15%)	
4	20(38.46%)	28(53.84%)	
5	10(19.23%)	10(19.23%)	
6	7(13.46%)	3(5.76%)	
Inner cell mass grade			0.623
A	23(44.23%)	28(53.84%)	
B	27(51.92%)	22(42.3%)	
C	2(3.84%)	2(3.84%)	
Trophectoderm grade			0.983
A	21(40.38%)	20(38.46%)	
B	30(57.69)	31(59.61%)	
C	1(1.92%)	1(1.92%)	
Day of blastocyst vitrification			0.085
Day 5	32(61.53%)	40(76.92%)	
Day 6	20(38.46%)	12(23.07%)	

All p-values were greater than 0.05, indicating no statistically significant differences between the PGT-A and control groups for the evaluated parameters. These findings suggest that the blastocyst morphology and timing of vitrification were comparable across both groups.

This comparison examines the vitrification day, blastocyst expansion, inner cell mass (ICM), and trophoctoderm (TE) grading in two IVF groups: those with pre-implantation genetic testing for aneuploidy (PGT-A, n = 52) and those without (n = 52). In terms of vitrification day, both groups had a similar proportion of embryos vitrified on Day 5 (58.33% in PGT-A vs. 47.5% in Control) and Day 6 (56.25% vs. 33.33%). Regarding pre-vitrification expansion grade, a higher proportion of PGT-A embryos were graded 3 (56.25% vs. 33.33%) and 5 (60% vs. 42.85%), while the Control group had more grade 4 blastocysts (45.45% vs. 55.55%). For inner cell mass quality, a greater proportion of PGT-A

embryos had grade A (63.63% vs. 44.44%) and grade B (57.69% vs. 38.88%) compared to the Control group. Similarly, trophoctoderm grading showed more PGT-A embryos with grade A (59.09% vs. 52.17%) and grade B (55.17% vs. 40.74%) compared to the Control group in **Table 3**. These findings suggest that PGT-A embryos may have a higher proportion of higher-quality blastocysts, especially in terms of ICM and TE grading, compared to Control embryos

Table 3: Impact of Blastocyst Quality on Live Birth Rates Following Vitrified-Warmed Transfers in Cycles in with and without PGT-A

Category	PGT-A (n = 52)	Controls (n = 52)	P-Value
Day of Vitrification			
Day 5	21 (58.33%)	19 (47.5%)	0.0933
Day 6	9 (56.25%)	4 (33.33%)	
Pre-Vitrification Expansion Grade			
Grade 3	9 (56.25%)	3 (33.33%)	0.1474
Grade 4	10 (55.55%)	15 (45.45%)	
Grade 5(n=10)	6 (60%)	3 (42.85%)	
Grade 6(n=7)	3 (37.5%)	1 (33.33%)	
Inner Cell Mass Grade			
Grade A	14 (63.63%)	12 (44.44%)	0.5749
Grade B	15 (57.69%)	7 (38.88%)	
Grade C	1 (25%)	1 (14.28%)	
Trophoctoderm Grade			
Grade A	13 (59.09%)	12 (52.17%)	0.5886
Grade B	16 (55.17%)	11 (40.74%)	
Grade C	1 (50%)	0	

Categorical variables such as day of vitrification, pre-vitrification expansion grade, inner cell mass (ICM) grade, and trophoctoderm (TE) grade were analyzed using the Chi-square test for multi-level variables and Fisher's exact test for 2x2 comparisons. The resulting p-values for all comparisons were greater than 0.05, indicating no statistically significant differences between the PGT-A and control groups across the assessed parameters. This suggests that the embryological characteristics were comparable between the two groups.

The cumulative live-birth rate was slightly lower in the PGT-A group (75%) compared to the control group (83.69%). Among these, singleton births accounted for 73.07% in the PGT-A group and 76.92% in the control group, while twin births were less frequent in both groups (1.92% vs. 5.76%, respectively) as depicted in **Graph 4**. Regarding secondary outcomes, the cumulative rates of biochemical pregnancy

(78.57% vs. 92.30%), clinical pregnancy (80.76% vs. 90.38%), and ongoing pregnancy (78.84% vs. 86.53%) were consistently lower in the PGT-A group compared to controls. The average birth weight of singleton newborns was slightly lower in the PGT-A group (3300±250 g) than in the control group (3500±280 g). Similarly, mean twin birth weights were lower in the PGT-A group (2250±380 g) compared to controls (2450±450 g) as given in **Table 4**

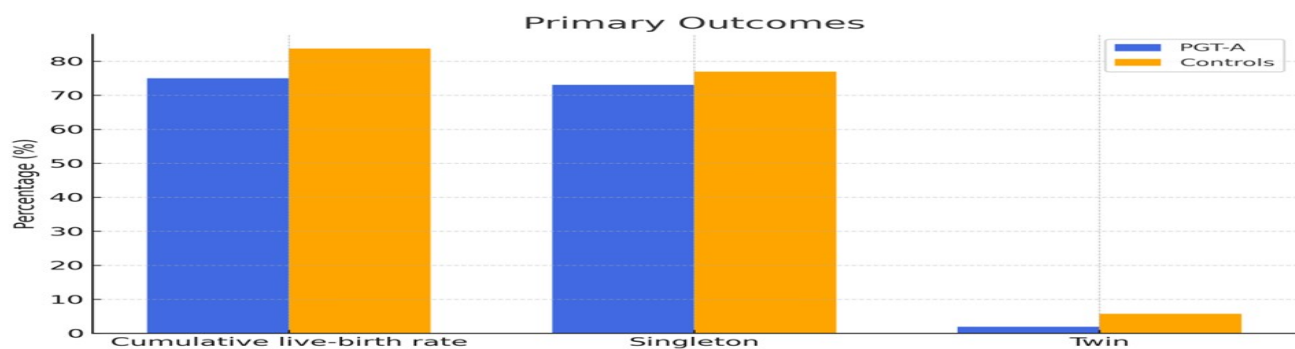
Table 4: Cumulative Live-Birth Rate and Secondary Outcomes

Outcome	PGT-A (n = 52)	Controls (n = 52)	P-value
Primary outcome			
Cumulative live-birth rate no. (%)	39(75%)	43(83.69%)	0.273
Singleton	38(73.07%)	40(76.92%)	0.654
Twin	1(1.92%)	3(5.76%)	0.617
Secondary outcomes			
Cumulative biochemical pregnancy — no. (%)	44(78.57%)	48(92.30%)	0.049
Cumulative clinical pregnancy — no. (%)	42(80.76%)	47(90.38%)	0.181
Cumulative ongoing pregnancy — no. (%)	41(78.84%)	45(86.53%)	0.293
Mean weight (Singleton)	3300±250	3500±280	0.005
Mean weight (Twin)	2250±380	2450±450	0.522

Categorical variables such as cumulative live birth rate, biochemical pregnancy rate, clinical pregnancy rate, and ongoing pregnancy rate were analyzed using the Chi-square test or Fisher’s exact test, depending on expected cell counts. Continuous variables, including birth weights of singleton and twin deliveries, were compared using the independent samples t-test after confirming normal distribution.

A p-value < 0.05 was considered statistically significant. The cumulative biochemical pregnancy rate was significantly higher in the control group compared to the PGT-A group (p = 0.049). Similarly, mean singleton birth weight was significantly greater in the control group (3500 ± 280 g) compared to the PGT-A group (3300 ± 250 g) (p = 0.005). No other comparisons reached statistical significance.

Graph 4 represents Primary Outcomes Comparison Between PGT-A and Control Groups



Discussion:

(Guo L, et al., 2024).

This study aimed to evaluate the clinical utility of preimplantation genetic testing for aneuploidy (PGT-A) in intracytoplasmic sperm injection (ICSI) cycles by comparing embryological and pregnancy outcomes with a control group undergoing conventional embryo selection. The comparison of two ICSI groups those undergoing pre-implantation genetic testing for aneuploidy (PGT-A) and those without (non-PGT-A) reveals significant differences in patient demographics, clinical parameters, blastocyst quality, and vitrification outcomes. These differences highlight the potential impact of genetic testing on embryo selection, vitrification timing, and overall IVF strategies. Preimplantation genetic testing for aneuploidy (PGT-A) involves an invasive embryo biopsy, making the timing of the procedure critical to minimize potential harm to embryonic development. Currently, biopsies can be performed at the polar body, cleavage, or blastocyst stages, with blastocyst-stage biopsy being the most widely used due to its higher diagnostic accuracy and reduced risk to the inner cell mass. The PGT-A group was notably older (mean age at retrieval 36.2 years) compared to the non-PGT-A group (33.7 years). Advanced maternal age is a well-established factor associated with diminished oocyte quality, increased chromosomal abnormalities, and reduced IVF success rates (Mikwar M, et al., 2020). Additionally, the PGT-A group exhibited a higher proportion of primary infertility (71.15% vs. 59.61%) and female factor infertility (61.53% vs. 42.3%), which may reflect a more selective approach to offering genetic testing in cases with known infertility factors. Moreover, the PGT-A group had a lower BMI (24.1 vs. 25.1 kg/m²), which is consistent with studies showing that a lower BMI is associated with better IVF outcomes (Guo L, et al., 2024). The PGT-A group had fewer prior pregnancies and more prior miscarriages, reflecting the potential use of genetic testing in women with a history of recurrent pregnancy loss or implantation failure (Munné et al., 2017). These demographic factors may explain why the PGT-A group had more aggressive approaches like ICSI (51.92% vs. 40.38%), which is commonly employed in cases of male factor infertility or when there is a history of poor fertilization. The quality of blastocysts, as assessed by expansion grade, inner cell mass (ICM) grade, and trophectoderm (TE) grade, differed between the groups. The PGT-A group had a higher proportion of blastocysts with expansion grade 3 and 6, as well as a greater proportion with ICM grade B and TE grade A. Previous studies have shown that embryos with higher expansion grades and better ICM and TE quality are associated with higher implantation potential and clinical pregnancy rates (Zhao J, et al.2019, Fragouli et al., 2014, Fragouli E et al.2011). This suggests that PGT-A may be associated with better-quality embryos, possibly due to the selective embryo transfer after genetic testing, which ensures that only chromosomally normal embryos are transferred (Sanders KD 2020). However, the non-PGT-A group had more grade 4 blastocysts (53.84% vs. 38.46%), which could reflect differences in selection criteria or patient characteristics. A greater proportion of higher-grade blastocysts in the PGT-A group may also be indicative of a more stringent embryo selection process, with a focus on selecting embryos with the best developmental potential for transfer (Sordia-Hernandez, L.H., et al.2022). Regarding vitrification, both groups had similar proportions of embryos vitrified on Day 5 (58.33%

vs. 47.5%) and Day 6 (56.25% vs. 33.33%). While a higher proportion of embryos from the non PGT-A group were vitrified on Day 5, the PGT-A group showed a higher proportion vitrified on Day 6. The timing of vitrification is critical because it affects the viability and survival of embryos during the freezing and thawing process. Day 5 embryos are often considered more mature and have a higher chance of successful implantation, but Day 6 embryos can still yield good outcomes, particularly when they are genetically normal (Wu CQ et al., 2022). The differences in vitrification timing between the two groups may reflect the emphasis placed on genetic testing results, with a greater focus on selecting genetically normal embryos for later vitrification and transfer.

These findings underscore the potential benefits of PGT-A in improving IVF outcomes, especially in older women and those with a history of infertility or recurrent pregnancy loss. By selecting embryos with normal chromosomal content, PGT-A may

improve embryo quality and increase the likelihood of a successful pregnancy. Furthermore, the data suggest that PGT-A may lead to more optimized cryopreservation strategies, potentially improving long-term embryo survival and clinical pregnancy rates. However, the results also suggest that while PGT-A offers clear advantages in terms of embryo quality and genetic normality, the technique may also involve higher costs, patient burden, and ethical considerations, particularly in younger women with no known risk factors for aneuploidy (Forman et al., 2015). As such, further studies are needed to better define the patient populations that would benefit most from PGT-A.

Conclusion:

This study assessed the influence of

Preimplantation Genetic Testing for Aneuploidy on embryological characteristics and clinical outcomes in patients undergoing ICSI. Although the PGT-A group exhibited improved blastocyst morphology reflected by better inner cell mass and trophectoderm grades, along with a higher frequency of Day 6 vitrification these enhancements did not lead to statistically superior clinical outcomes. Rates of cumulative live birth, clinical pregnancy, and ongoing pregnancy were similar between the PGT-A and control groups. Notably, the control group demonstrated a higher biochemical pregnancy rate and greater average singleton birth weight. These results indicate that while PGT-A may support enhanced embryo selection, its routine application in unselected IVF populations may offer limited clinical benefit.

Future large-scale, prospective studies are warranted to identify the specific patient populations most likely to benefit from PGT-A and to evaluate its cost-effectiveness and long-term reproductive implications.

Funding:

This study was funded by Indian Council of Medical Research.

Conflict of interest:

All authors declare that they have no conflict of interest.

Acknowledgement:

We acknowledge all the authors of the manuscript.

References:

1. Retzliff MG, Hornstein MD. Is intracytoplasmic sperm injection safe?. Fertility and sterility. 2003 Oct 1;80(4):851-9.

2. Babariya D, Fragouli E, Alfarawati S, Spath K, Wells D. The incidence and origin of segmental aneuploidy in human oocytes and preimplantation embryos. *Human Reproduction*. 2017 Dec 1;32(12):2549-60.
3. Viotti M. Preimplantation genetic testing for chromosomal abnormalities: aneuploidy, mosaicism, and structural rearrangements. *Genes*. 2020 May 29;11(6):602.
4. Morales C. Current applications and controversies in preimplantation genetic testing for aneuploidies (PGT-A) in in vitro fertilization. *Reproductive Sciences*. 2024 Jan;31(1):66-80.
5. Stern HJ. Preimplantation genetic diagnosis: prenatal testing for embryos finally achieving its potential. *Journal of clinical medicine*. 2014 Mar 17;3(1):280-309.
6. Anderson RE, Whitney JB, Schiewe MC. Clinical benefits of preimplantation genetic testing for aneuploidy (PGT-A) for all in vitro fertilization treatment cycles. *European journal of medical genetics*. 2020 Feb 1;63(2):103731.
7. Salame AA, Dahdouh EM, Aljafari R, Samuel DA, Koodathingal BP, Bajpai A, Kainoth S, Fakih M. Predictive factors of aneuploidy in infertile patients undergoing IVF: a retrospective analysis in a private IVF practice. *Middle East Fertility Society Journal*. 2024 Feb 23;29(1):12.
8. Yan J, Qin Y, Zhao H, Sun Y, Gong F, Li R, Sun X, Ling X, Li H, Hao C, Tan J. Live birth with or without preimplantation genetic testing for aneuploidy. *New England Journal of Medicine*. 2021 Nov 25;385(22):2047-58.
9. Mikwar M, MacFarlane AJ, Marchetti F. Mechanisms of oocyte aneuploidy associated with advanced maternal age. *Mutation Research/Reviews in Mutation Research*. 2020 Jul 1;785:108320.
10. Guo L, Li X, Guo A, Wang Y, Liang Y, Li Y, Xu X, Lv H. Comparative study on pregnancy complications: PGT-A vs. IVF-ET with gender-specific outcomes. *Frontiers in Endocrinology*. 2024 Nov 6;15:1453083.
11. Munné S, Wells D. Detection of mosaicism at blastocyst stage with the use of high-resolution next-generation sequencing. *Fertility and sterility*. 2017 May 1;107(5):1085-91.
12. Zhao J, Yan Y, Huang X, Sun L, Li Y. Blastocoele expansion: an important parameter for predicting clinical success pregnancy after frozen-warmed blastocysts transfer. *Reproductive Biology and Endocrinology*. 2019 Dec;17:1-8.
13. Fragouli E, Alfarawati S, Spath K, Wells D. Morphological and cytogenetic assessment of cleavage and blastocyst stage embryos. *Molecular human reproduction*. 2014 Feb 1;20(2):117-26.
14. Fragouli E, Wells D. Aneuploidy in the human blastocyst. *Cytogenetic and genome research*. 2011 Jan 19;133(2-4):149-59.
15. Sanders KD. Novel Insights into Preimplantation Genetic Testing for Aneuploidy and Non-invasive Prenatal Testing. University of Kent (United Kingdom); 2020.
16. Sordia-Hernandez, L.H., Morales-Martinez, F.A., González-Colmenero, F.D., Flores Rodriguez, A., Leyva-Camacho, P.C., Sordia-Piñeyro, M.O., Valdés-Martínez, O.H., García-Luna, S.M., Rodríguez-Guajardo, R. and Sordia-Piñeyro, L.H., 2022. The effects of preimplantation genetic testing for aneuploidy

(PGT-A) on patient-important outcomes in embryo transfer cases: a meta-analysis. *Journal of Reproduction & Infertility*, 23(4), p.231.

17. Wu CQ, Campbell M, Shmorgun D, Torrance S, Gale J, Leveille MC. Comparative embryo development outcomes following extending embryo culture to day 6: a retrospective cohort study. *International Journal of Fertility & Sterility*. 2022 Dec 25;17(1):40.