The Impact of Maternal Age on Chromosomal Aneuploidy, Blastocyst Quality, and Pregnancy Outcomes During Intracytoplasmic Sperm Injection Cycles

Aghajani Shahrzad¹, Salehzadeh Ali²*, Ghasemian Fatemeh², Mehrfaża Marzieh³, Hosseini Ahmad¹
1. Department of Biology, Rasht Branch Islamic Azad University, Rasht, Iran
2. Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran
3. Mehr Fertility Research Center, Guilan University of Medical Sciences, Rasht, Iran

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Abstract

Background & Objective: Assisted reproduction techniques and preimplantation genetic testing (PGT) for aneuploidies help infertile couples achieve a healthy live birth. The objective of this study was to investigate if there is a correlation between chromosomal aneuploidy and maternal age.

Materials & Methods: We used 277 embryos with 6-8 cells and graded A derived from intracytoplasmic sperm injection cycles. There were two subgroups: fresh-PGT cycles (n = 38) and Frozen Embryo Transfer (FET) cycles (n = 38). The PGT cycles results were evaluated by fluorescence in situ hybridization and polymerase chain reaction assays. Couples (n = 76) with PGT on the third day were classified into four maternal age groups: ≤35 years (n = 35), 36-40 years (n = 24), 41-45 years (n = 11), and ≥46 years (n = 6). The rate of aneuploidy, blastocyst quality, and pregnancy was assessed in FET and fresh-PGT cycle.

Results: Aneuploidy rate in 13, 18, 21, X, and Y chromosomes in FET and fresh-PGT cycles are not significantly related to maternal age (p = 0.1). Significant differences were found in a decreased chemical pregnancy (p = 0.001), clinical pregnancy (p = 0.001), ongoing pregnancy (p = 0.001), and live birth rate (p = 0.001) among both groups. Decreased blastocyst rate increased maternal age in fresh PGT and FET PGT (p = 0.02). Early and clinical pregnancy loss was not related significantly to maternal age.

Conclusion: After evaluating cycle characteristics, an association was found between maternal age and declined pregnancy outcomes in embryos. There was a significant relationship between increasing female age with decreasing blastocyst rate.

Keywords: Blastocyst Quality, Pregnancy Outcomes, Intracytoplasmic Sperm Injection Cycles, Fluorescence in Situ Hybridization, Preimplantation Genetic Testing

Introduction

In modern society, postponing maternity has been rising continually. On the one hand, there were some educational, professional, economic, and personal reasons (1, 2). But, on the other hand, increasing aneuploidy rate with maternal age was the foremost hurdle to reproductive success (3, 4).

Maternal aneuploidy raises with female age, achieving up to 80% by 45 (4). Meiotic and primary mitotic errors may result in more rede of aneuploidy in higher maternal age. Embryonic early cleavage was affected by the oocyte attributes, whereas the 4-6 cell stage of embryo development was affected by both the sperm and the oocyte (5). The leading cause of the whole chromosomal aneuploidy in human embryos is meiotic errors.
Therefore, preimplantation genetic screening (PGT-A) of embryos is a widely used method for embryo selection and the possibility of gaining euploid blastocysts as a focus for clinicians’ consulting. Factors include the number of oocytes, mature follicles, and female age (3, 15).

This study investigated the possible correlation between 13, 18, 21, X, and Y chromosomal deviation and maternal age in embryos from frozen embryo transfer (FET) and fresh embryo transfer cycles. All results from the PGT-A procedure on day three single blastomere biopsy were obtained by fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR) assays for 13, 18, 21, X, and Y chromosomes. Finally, pregnancy and miscarriage results were evaluated.

Materials & Methods

Patients, study design, and study groups

This observational study was conducted at Mehr Fertility Center, Rasht, Iran, for about two years, from February 2018 to January 2020. This study was approved by the Research Ethics Board of Islamic Azad University, Rasht Branch (Ethics approval code no.1398.053).

All women underwent a comprehensive fertility workup, including a detailed medical history, laboratory evaluation, and pelvic ultrasound before initiating the treatment. They also underwent an assessment before ICSI, including medical history, laboratory evaluation, and male semen analysis. The study analysis included infertility patients undergoing ICSI cycles involved in PGT-A in the fresh and frozen embryo transfer cycles. After centrifugation, this study used a standard swim-up technique to prepare all semen samples to determine a possible contribution to the sperm preparation method on embryo aneuploidy. Furthermore, the patients who underwent the ICSI cycle and requested PGT without embryos with 6-8 cells and grade A were excluded from the research process. A total of 277 embryos with 6-8 cells and grade A, derived from ICSI with single blastomere biopsy were combined with comprehensive chromosome screening using fluorescence in situ hybridization (FISH) and the polymerase chain reaction (PCR) methods 13, 18, 21, X, and Y chromosomes. Finally, pregnancy and miscarriage results were evaluated.
Patients aged from 21 to 47 years old for females. There were two subgroups in terms of type of cycle insist of Fresh PGT (n = 38) and PGT (n = 38) and then the patients were allocated into four maternal age groups: ≥35 years (n = 35); 36-40 years (n = 24); 41-45 years (n = 11); 46≥ years (n = 6). Patients’ clinical charts obtained demographic and clinical data, including paternal and maternal age, initial semen parameters (pre-wash), oocyte count, embryo count and quality, and PGT-A results. As a result of the natural difference of male age among the study groups, the researcher, depending on male age in the results, assessed male age and semen quality in each group to avoid the entrance of any compounding agent. The primary outcome measures were aneuploidy, euploidy, and blastocyst formation rates. Secondary endpoints compared chromosome aneuploidy and analyzed the overall percentage of pregnancy outcome and losses in each group. Then, the results were evaluated in terms of human embryos’ developmental potential in each group using the Gardner method (16). All applicants were informed about the procedure’s success rate, the cost, the probability of not finding the healthy embryo, and the possibility of not observing the desired signals from the embryos.

Embryo culture and evaluation

The cultured embryos were evaluated on the third day after ICSI. Based on Gardner’s Method, the embryos were classified into three groups; group A (6 to 8 blastomeres with the exact size and ≤ 10% fragmentation rate), group B (6-8 blastomeres with even or non-even size, and 10% to 20% fragmentation rate), and group C (low number and uneven blastomeres with more than 20% fragmentation). On day five, the blastocysts were scored based on the Gardner Method, which explains the extent of blastocoel expansion and establishment of trophectoderm (TE) and inner cell mass (ICM) development.

The rating of re-expansion was determined as follows: 1 = early blastocyst; cavity started to the formation, 2 = early blastocyst; cavity was less than 50% of the total embryo, 3 = full blastocyst; cavity thoroughly loaded with the embryo, 4 = expanded blastocyst; cavity volume became bigger than complete blastocyst, zona pellucida slimming, 5 = hatching blastocyst; TE was herniating via the zona, 6 = hatched blastocyst; blastocyst completely exited from the zona. ICM was graded as follows: A = many cells; firmly compacted, B = some cells; firmly compacted, C = some cells; disordered, D = few cells; disordered. The TE grading was defined as follows: A = forming a coherent epithelium in many cells, B = moderate cells making a weak epithelium, C = some cells making a weak epithelium, D = very few cells (16). Considering the accurate time of oocyte retrieval on day 0, all scored blastocyst were taken on the morning of day five.

Ovarian Stimulation and embryo culture

Ovarian stimulation for ICSI included a standard agonist-based protocol (17). The dosing regimen chosen by each physician was according to the pre-stimulation assessment of parameters, including anti-Mullerian hormone, antral follicle count, and prior stimulation cycle response. Oocyte collection was performed approximately 36 hours post-triggering. After ovum pick-up, mature (metaphase II) oocytes were fertilized by intracytoplasmic sperm injection (ICSI). Following ICSI, all fertilized oocytes were cultured individually from day one to day five in 25 mL droplets of a single culture medium with a serum protein supplement (Life Global) under oil. On day three, laser-assisted embryo biopsy was performed in the total global medium (Life Global) under oil. Embryos were evaluated on days three and five with the Gardner method (16).

Vitrification-thawing technique

On the day of hCG administration, the embryo transfers of patients with homogenous hyperechogenic endometrium and serum progesterone levels greater than two ng/mL were canceled. Embryos with grade A were vitrified on day three for subsequent cycles as a FET-PGT cycle. The vitrification and warming procedures were conducted according to the recommended protocol in Kitazato Vitrification Kit. In brief, the embryos were then placed in vitrification solutions in two steps for 15 minutes and 40-50 seconds.
First, embryos were picked up into the tip of a transfer pipette using a minimal volume of vitrification solution (0.1 μL or less) and then placed on a Cryotop strip. Then, the strip with the embryos was plunged into liquid nitrogen. For thawing, the frozen embryos were immediately immersed in a 37 °C warming solution for 1 minute. They were then recovered and rehydrated using a 3-step dilution protocol at room temperature. Finally, the embryos were transferred to embryo culture media (16).

**Evaluation of embryos after warming**

The embryos were thawed, and then their quality was determined by quantifying the proportion of morphologically intact cells in the embryo. Embryos were considered intact when all blastomeres had well-defined membranes and cytoplasm with no sign of degeneration. In this study, embryos were incubated for an additional two-day to assess blastulation potential. The embryos were then cultured in embryo medium (Global total, COOPER) covered by oil in a 37°C incubator (SANYO, MCO-5AC) with a 6% CO₂, 5% O₂, and 89% N₂ atmosphere to the blastocyst stage. Based on the degree of expansion and hatching status, blastocysts were given numeric scores from 1–6 (16).

**Embryo biopsy and PGT procedure**

A single blastomere biopsy was accomplished on the third day based on morphologic evaluation and hatching rate (18). Firstly, cleavage stage embryos (day three) were transferred into a cation (Na⁺ and Mg++) free medium. Then zona pellucid was hatched locally; so that just a single blastomere could be removed. The blastomere was then gently aspirated using an appropriate biopsy microneedle, and then the blastomere was removed from the embryos and remained biopsied embryos. The blastomeres were considered by the FISH technique (19). All biopsy samples were in hypotonic wash buffer and deferred for analysis, and all embryos were cultured again after the biopsy. PGT results were described as euploid or aneuploid established on the FISH method and PCR platform. Embryos with available PGT-A results were classified as euploid or aneuploid.

**Embryo selection for transfer**

Euploid blastocyst embryos with the expansion grades 2, 3, 4, and 5 were selected to be transferred. The embryo grade on day five was considered for embryo selection among the biopsied embryos by evaluating expansion, TE, and ICM morphology (20). FET was performed after the synthetic preparation of the endometrium. Embryo thawing and transfer were performed after five days of progesterone supplementation. Embryo transfer was performed with a Cook catheter under abdominal ultrasound guidance on day five.

**Outcome measures**

Chemical pregnancy, clinical pregnancy, ongoing pregnancy, and live birth were considered as the primary outcomes. Chemical pregnancy was confirmed by a positive βhCG test 14 days after the embryo transfer. The sonographic evidence of fetal heartbeat confirmed clinical pregnancy. Ongoing pregnancy was defined as a viable intrauterine gestation at the time of discharge from the practice, which occurred not earlier than eight weeks of gestation. Live birth was considered as the delivery of a live-born infant after 28 weeks gestation. The secondary outcomes included the clinical pregnancy rate, early pregnancy loss (EPL) rate, and clinical pregnancy loss (CPL) rate. EPL was defined as a pregnancy loss occurring before the detection of an intrauterine gestational sac on ultrasound. CPL was described as a loss following the detection of the fetal heartbeat on an ultrasound.

**Statistical Analysis**

Distribution and the normality of cycle characteristics were evaluated using the Kolmogorov-Smirnov test. Continuing variables were expressed as mean with SD if normally distributed and median with interquartile range (IQR), if not normally distributed.
Categorical variables were expressed as frequencies with percentages. Continuing variables were compared between age groups using analysis of variance if normally distributed, or Kruskal-Wallis, if not normally distributed. Chi-square or Fisher’s exact test was used whenever proper to compare categorical data. Due to multiple patients (n = 76) providing multiple eggs (n = 1,093) in our data set, we performed a generalized linear mixed model to account for embryo-level variability for outcomes of aneuploidy and euploidy. Maternal and paternal age groups, semen volume, semen concentration, semen motility, oocyte count, and quality were considered fixed effects, whereas patients were considered a random effect. Nelder-Mead optimization was used to determine parameter estimates.

Continuing variables were scaled for regression analysis. Output beta-coefficients and 95% confidence intervals (CIs) limits exponentiated to raw odds ratios and corresponding 95% CIs for clinical interpretability. Statistical analysis was done using SPSS version 23. P < 0.05 was considered statistically significant.

Results

The study included 1093 oocytes from women aged 21–47 undergoing ICSI antagonist cycles, in which PGT-A was performed. Thus, a total of 277 embryos from 76 patients met the inclusion criteria. After excluding no detected embryos 62/277 (22.38%), a total of 215 embryos were included in the final analysis (Figure 1).

**Figure 1.** In-situ hybridization with X, Y, 13, 18, and 21 chromosome-specific DNA probes. X-specific probe is shown labelled in blue, chromosome Y in green, chromosome 13 in orange, chromosome 18 in purple, and chromosome 21 in red. (a) normal male blastomere (X, Y, 13, 13, 18, 18, 21, 21); (b) trisomy 13 female blastomere (X, X, 13, 13, 18, 18, 21, 21); (c) normal female blastomere (X, Y, 13, 13, 18, 18, 21, 21); trisomy 21 female blastomere (X, X, 13, 13, 18, 18, 21, 21, 21)
There were 153/215 (71.16%) euploid embryos and 62 (28.84%) aneuploid in the 13, 18, 21, X, and Y chromosomes in embryos with the detected signal. According to maternal age distribution, no clear correlation was found between embryo aneuploidy rates ($p = 0.1$). Therefore, the age range groupings were used for the detailed analysis.

Patients were allocated into four maternal age groups: ≥35 years ($n = 35$); 36-40 years ($n = 24$); 41-45 years ($n = 11$); 46≥ years ($n = 6$). A detailed comparison of baseline cycle characteristics among the study groups is presented in Table 1. There was no significant difference in affective cycle characteristics among the study groups.

Table 1. Comparison of ICSI cycle characteristics for 76 patients. Male age ($p = 0.001$, 0.023), number of oocytes retrieved ($p = 0.001$) and number of matured oocytes ($p = 0.001$) was significant

<table>
<thead>
<tr>
<th>Type of cycle</th>
<th>Freeze cycle PGT ($n = 38$)</th>
<th>Fresh cycle PGT ($n = 38$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female age groups</td>
<td>&lt;=35 36-40 41-45 &gt;=46</td>
<td>&lt;=35 36-40 41-45 &gt;=46</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Male age (years)</td>
<td>40.38± 4.46 41.63± 3.38 48.53± .77 48.20± .77</td>
<td>39.97± 9.67 41.93± 4.35 42.57± 3.69 52.00± 0.00</td>
</tr>
<tr>
<td>Count of semen (million)</td>
<td>30.5 ± 14.11 32.6 ± 17.45 26.2 ± 23.2 ± 13.12</td>
<td>24.3 ± 18.91 30.6 ± 23.33 27.3 ± 20.10 20.10 ± 0.00</td>
</tr>
<tr>
<td>Motility of semen (%)</td>
<td>55.7 ± 22.15 55.5 ± 25.64 49.8 ± 31.99 48.7 ± 13.12</td>
<td>56.2 ± 17.01 58.2 ± 12.91 60.9 ± 25.50 50.00 ± 0.00</td>
</tr>
<tr>
<td>Morphology of semen (%)</td>
<td>10.93± 4.70 9.54± 5.76 6.67± 7.24 11.60± 1.40</td>
<td>8.58 ± 3.67 11.13± 3.92 10.71± 7.33 8.00 ± 0.00</td>
</tr>
<tr>
<td>Oocytes retrieved (n)</td>
<td>18.33± 6.20 24.0± 22.71 10.80± 7.19 9.07 ± 1.75</td>
<td>14.29± 5.69 10.80± 3.15 11.21± 2.83 16.00 ± 0.00</td>
</tr>
<tr>
<td>Matured oocytes (n)</td>
<td>15.58± 4.86 19.2± 22.71 10.27± 6.70 8.00 ± 1.60</td>
<td>12.05± 5.12 9.46± 2.68 9.43 ± 2.50 16.00 ± 0.00</td>
</tr>
<tr>
<td>Cleavage embryo (n)</td>
<td>9.99± 4.13 12.8± 10.10 7.47± 4.67 8.00 ± 1.73</td>
<td>8.50± 3.78 8.28± 2.12 7.71 ± 3.24 10.00 ± 0.00</td>
</tr>
<tr>
<td>Transferred embryo (n)</td>
<td>1.61± .70 1.86± .69 2.00± .82 1.33 ± .58</td>
<td>1.64± .67 1.73 ± .79 1.33 ± .58 1.00 ± 0.00</td>
</tr>
<tr>
<td>Biopsy embryo (n)</td>
<td>5.70± 2.11 5.33± 2.92 4.20± 2.68 5.25 ± 2.75</td>
<td>5.20± 2.61 5.38 ± 2.36 4.50 ± 2.38 5.00 ± 0.00</td>
</tr>
</tbody>
</table>

Note: data are presented as mean ±standard deviation; P<0.05, t-test or Fisher’s test, as appropriate
The rate of aneuploidy in 13, 18, 21, X, and Y chromosomes, embryo development in FET, and fresh cycle are not significantly related to maternal age (p = 0.1) (Table 2). There was a significant difference in the blastocyst rate among maternal age groups (P-value 0.002).

Significant differences among maternal age groups were found in Chemical pregnancy (p = 0.001), Clinical pregnancy (p = 0.001), Ongoing pregnancy (p = 0.001), and Life birth rate (p = 0.001) (Chart 1).

![Chart 1. Pregnancy outcome in four maternal age groups: There is a significant difference in decreasing pregnancy results in both groups (Chemical pregnancy, Clinical pregnancy, Ongoing Pregnancy, and Life birth rate (p-value: <0.001))](image)

There was no significant relationship between maternal age and EPL and CPL (p = 0.08). There was also no significant difference between embryo development in FET and fresh cycle with maternal age.

Generalized analysis at the embryo level showed that oocytes retrieved (p = 0.001) and matured oocytes (p = 0.001) were significantly associated with female age (Table 2).

### Chart 1. Pregnancy outcome in four maternal age groups: There is a significant difference in decreasing pregnancy results in both groups (Chemical pregnancy, Clinical pregnancy, Ongoing Pregnancy, and Life birth rate (p-value: <0.001))

### Table 2. Developmental and pregnancy outcomes concerning maternal age. Decreasing blastocyst rate (p = 0.002) and pregnancy outcomes (p = 0.001) was significant.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Maternal age range (%) (years)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age range</td>
<td>≥35</td>
<td>36-40</td>
</tr>
<tr>
<td>Aneuploidy rate</td>
<td>24.2%</td>
<td>20.8%</td>
</tr>
<tr>
<td>Blastocyst rate</td>
<td>38.8%</td>
<td>25.0%</td>
</tr>
<tr>
<td>Euploid blastocyst</td>
<td>76.0%</td>
<td>80.0%</td>
</tr>
<tr>
<td>rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical pregnancy</td>
<td>54.4%</td>
<td>33.3%</td>
</tr>
</tbody>
</table>
There was a significant relation among Clinical pregnancy (p = 0.01), Ongoing pregnancy (p = 0.01), and Life birth rate (p = 0.01) especially in 41-45 age female group in the fresh cycle. For influential maternal factors, male and female age, oocyte count, semen concentration, semen motility, and semen morphology were significantly associated with aneuploidy rate, blastocyst rate, and pregnancy outcomes (Table 3).

**Table 3.** Developmental and pregnancy outcomes related to maternal age, oocyte count (Mean no ± SD, P-value), chemical pregnancy in FET cycles with PGT and clinical pregnancy, ongoing pregnancy, and live birth rate in Fresh cycles with PGT was significant due to female age

<table>
<thead>
<tr>
<th></th>
<th>Aneuploidy rate</th>
<th>Blastocyst rate</th>
<th>Chemical pregnancy</th>
<th>Clinical pregnancy</th>
<th>Ongoing pregnancy</th>
<th>Live birth rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FET Cycle With PGT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=38)</td>
<td>Mean ± SD</td>
<td>34.96±5.31</td>
<td>36.30±5.22</td>
<td>36.38±5.30</td>
<td>35.91±5.78</td>
<td>36.67±6.29</td>
</tr>
<tr>
<td>Female age</td>
<td>p-value</td>
<td>NS</td>
<td>NS</td>
<td>0.04</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Oocyte count</td>
<td>Mean ± SD</td>
<td>15.80±6.39</td>
<td>11.75±7.14</td>
<td>13.36±5.81</td>
<td>14.86±3.98</td>
<td>15.67±3.67</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>NS</td>
<td>NS</td>
<td>0.017</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Fresh Cycle With PGT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=38)</td>
<td>Mean ± SD</td>
<td>35.35±5.21</td>
<td>32.60±4.19</td>
<td>35.33±5.07</td>
<td>36.21±4.66</td>
<td>36.21±4.66</td>
</tr>
<tr>
<td>Female age</td>
<td>p-value</td>
<td>0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Oocyte count</td>
<td>Mean ± SD</td>
<td>9.25±3.59</td>
<td>10.60±5.40</td>
<td>10.11±4.73</td>
<td>8.43±33.69</td>
<td>8.43±33.69</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>NS</td>
<td>NS</td>
<td>0.011</td>
<td>0.011</td>
<td>0.011</td>
</tr>
</tbody>
</table>

*P<0.05, t-test or Fisher’s test exact test, as appropriate. NS = not statistically significant
The odds ratio of pregnancy outcomes based on composite expansion grade and maternal age were analyzed compared to expansion five embryos. Achieving expansion grade 3 was significantly higher in group A compared with other groups with referencing expansion 5 embryos (odds ratio = 1.38(0.33-5.78) vs 0.38 (0.08-1.77), 0.76 (0.05-10.48), and 0.57 (0.07-4.55)); and in expansion 5 group B was pioneer (odds ratio = 1.53(0.13-17.33) vs references).

Table 4. Odds of pregnancy outcomes based on composite expansion grade and maternal age compared with expansion five embryos. Expansion 3 in ≥35 groups and expansion 5 in 36–the 40-year-old group had more OR

<table>
<thead>
<tr>
<th>Age range</th>
<th>OR (99% CI)</th>
<th>OR (99% CI)</th>
<th>OR (99% CI)</th>
<th>OR (99% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expansion grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.38(0.33-5.78)</td>
<td>0.38(0.08-1.77)</td>
<td>0.76(0.05-10.48)</td>
<td>0.57(0.07-4.55)</td>
</tr>
<tr>
<td>4</td>
<td>0.68(0.20-2.33)</td>
<td>0.15(0.02-1.06)</td>
<td>Zero sample size</td>
<td>0.86(0.18-4.14)</td>
</tr>
<tr>
<td>5</td>
<td>Reference</td>
<td>1.53(0.13-17.33)</td>
<td>Small sample size</td>
<td>Small sample size</td>
</tr>
</tbody>
</table>

*Note: CI = confidence interval; OR = odds ratio;

Discussion

By developing techniques used for PGT, chromosome aneuploidy could be tested in the embryos in a preimplantation stage. However, to date, the contribution of maternal age to embryo aneuploidy, developmental potential, and pregnancy outcomes remains uncertain.

In this study, the data showed no relationship between maternal age and aneuploidy rate (determined using FISH and PCR assays for PGT-A) in 277 embryos derived from ICSI patients. In addition, the researchers observed a significant relationship between blastocyst rate and pregnancy outcomes. The results of this research are consistent with some previous studies, including the one recently published by Gonzalez et al. (21). They analyzed an outsized cohort of 1,549 embryos from IVF clinics using Next-Generation Sequencing (NGS) technology. Their results showed that maternal age does not have an impact on the prevalence of embryo aneuploidy. However, the aneuploidy rate they reported ranged is different from our result. It could be explained by differences in used technology, treatment protocols among centers, IVF culturing conditions, or interpretation of PGT-A results to include a report (22).

Given that aneuploidy rates often suffer from confounding factors, such as semen parameters, ovum donor, and cycle parameters (23, 24), we have included these factors in the statistical analysis with proper adjustments to control their effects. So far, no other study has used detailed clinical data on the oocytes and sperm parameters and ICSI cycle characteristics to regulate their effects on the results parameter rate.

Further supporting our findings, some studies using genotyping technology to determine the maternal origin of aneuploidy also reported no correlation between maternal age and aneuploidy (3, 24). For example, in their study, using single nucleotide polymorphism (SNP) array technology, Sills et al. (3) reported that there is no correlation between maternal age and embryo aneuploidy, McCoy et al. (24).

This study indicated that the frequency of aneuploidy in chromosomes would not increase significantly with maternal age. But the rate of embryos achieving blastocyst stage with expansion 3, 4, and 5 and pregnancy outcomes decreased significantly with parental age.
Aneuploidy was mainly higher in the older ages due to meiosis errors during the development of oocytes. Demco et al., in their study, found that there is an inverse relationship between maternal age and euploid embryos (3). This study showed that the average clinical pregnancy rate is 30.92%, and the live birth rate is 25.8%. As a result, based on age, pregnancy outcomes were statistically significant.

Evaluating maternal age and type of FET or fresh cycle by fluorescent in situ hybridization technology (FISH) and PCR assays on chromosomes is this research’s strength. The data were collected from one fertility center. Another emphasis of the study since all genetic analyses, embryo culture, and ovarian stimulation was operated at a single center, thus obliterating significant genetic analysis variation, embryo handling, and clinical protocols as confusing factors. Furthermore, to support the study results, standardized methods of semen analysis and oocyte screening were used. As another merit of this study, multiple regression analyses were conducted to analyze potential confounding effects from oocyte and sperm parameters and cycle characteristics.

The main limitation of this study was that the used genetic analysis technology was an old technology for genetic diagnosis. To have more effective and complete evaluations, whole-genome assessment methods are more appropriate. Interestingly, newer genotyping assays like SNP-NGS sequencing are emerging, which could allow us, within the future, to rise and characterize the origin of aneuploidy in embryos. Besides this, the older maternal age results should be interpreted with caution due to the smaller sample size of the group.

**Conclusion**

In the present study, the researchers found that after adjusting the semen quality, oocyte information, and ICSI cycle characteristics, there is a significant relationship between maternal age and pregnancy outcomes and blastocyst rates in embryos derived from ICSI cycles. Thus, our data add to the general knowledge of maternal age’s effect on human embryo aneuploidy and developmental potential. In addition, our findings may assist in providing presentational consulting to older women who wish to have children.

**Acknowledgement**

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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