



## Assessment of a maternal age on the early embryonic development followed Intracytoplasmic sperm injection (ICSI)

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### Abstract

The objective of this study aimed to investigate a different maternal age could have effect on the early embryonic development following (ICSI) or not. This study contained 150 ICSI patients divided to 3 groups. The first group includes females aged from 20-29 years old. The second group includes females aged from 30-39 years old. The third group includes females aged from 40-46 years old. The oocytes retrieved from each female were injected by normal sperm morphology >4%, the fertilization rate, cleavage, quality of the embryo, and the rate of blastocyst formation rate are the main outcome measures. The results refer to significant differences in the female's age, and the number of retrieved oocytes between three groups ( $p < 0.05$ ). The fertilization rate and cleavage rate are similar in all groups and the proportion of blastocyst formation was significantly decreased in the different groups (22.8% in group A versus 10.4% and 9.2% in groups B and C). In conclusion female age had no effect on the morphology of the embryo day 2 and 3 post insemination and ICSI procedure might make a negative effect on the process of extended culture to blastocyst stage.

**Keywords:** maternal age, aneuploidy, embryo morphology, ICSI

### Introduction

It is well recognized that the fertility potential of women decreases with increasing age. The decrease in female reproductive capacity with increasing age has two main causes: Gradual depletion of oocytes from the ovary, and a decrease in oocyte quality<sup>[1]</sup>.

A fully developmentally competent oocyte is an oocyte that through oogenesis achieves the ability to continue meiosis, become fertilized with a single spermatozoa, decondense the sperm head to create two pronuclei (PN), pass cleavage stages undergo maternal to embryonic transition, reach the blastocyst stage, hatch, and establish a pregnancy that results in the birth of a healthy baby<sup>[2]</sup>. That the reduction in the oocyte developmental competence contributes to lower fecundity with increasing age has been appeared by achieving comparable pregnancy rates among young women undergoing *in vitro* fertilization (IVF) treatment with their own oocytes and older women (>40 years) in IVF treatment with oocytes donated by the young women<sup>[3]</sup>. The aneuploidy rates increase in both oocytes and *in vitro* produced embryos with increasing female age<sup>[4-5-6]</sup>. In a recent study using array comparative genomic hybridization of polar bodies, the aneuploidy rate increased from 47% among women aged 27-37 years to 78% among those aged 38-47 years<sup>[6]</sup>. Pregnancy rates among women aged 19-26 years are twice those of women aged 35-39 years<sup>[7]</sup>. Furthermore, the recurrence of spontaneous abortion is 8.9% among women aged 20-24 years versus 74.7% among women aged 45 years or older<sup>[8]</sup>. Advancing maternal age has also been associated with increased rates of preterm delivery and low birth weight<sup>[9]</sup>.

The age related decline in fertility is also related to reduced

numbers and quality of oocytes, decreased embryo quality, and increased risk of spontaneous abortion owing to aneuploidy. Approximately 10%–30% of all fertilized human oocytes are aneuploidy, with most being either trisomic or monosomic; aneuploidy is implicated in approximately 33% of all spontaneous abortions, making this hereditary anomaly the leading cause of pregnancy loss. Trisomy influences roughly 2% of all clinically recognized pregnancies among women younger than 25 years; however, the rate of trisomy is around 35% among women aged older than 40 years<sup>[10-11]</sup>.

Intracytoplasmic sperm injection (ICSI) has considered the utilization of a single sperm for *in vitro* fertilization (IVF) in the idiopathic infertility patients. Fertilization by coordinating infusion of the sperm into the oocyte cytoplasm the standard procedure, for combining male and female genomes. While oocytes supply the cytoplasmic condition vital, necessary for sperm microtubule function, the sperm is responsible for the centrosome, which gives rise to microtubules<sup>[12]</sup>.

The aim of this study was to investigate whether individual oocyte, zygote, and embryo morphology and cleavage parameters of the developing embryo, as well as outcome measures are influenced by the age of the woman undergoing ICSI treatment.

## 2. Material and methods

### 2.1. Patient and semen collection

This study analyses semen samples from 150 couples undergoing their ICSI attempt as a result of idiopathic infertility. The semens were assessed and associated with embryo quality and development from fertilization to the blastocyst stage (pre-implantation development). The study

conducted at the international Islamic center for population studies and research assisted reproduction unit, El-Hussain Hospital, Al-Azhar University, Cairo, Egypt. The study period started from December 2016 to May 2017. The cases were 150 men found to have normal semen parameters when their wives presented for investigation and treatment of infertility in the clinic. All the cases were identified from couples presenting to the clinic requesting investigation and treatment of infertility and each case had approved sheet.

Fresh semen samples were obtained from the 150 patients by masturbation after a sexual abstinence period of 2–5 days. The semen samples were collected in a sterile container on the day of oocyte retrieval and liquefied for 20 min at 37°C. The semen analysis was performed according to World Health Organization guidelines [13], and the following parameters were recorded: volume, sperm concentration, head and neck morphology, motility and vitality. The liquefied semen sample was processed by swim-up technique. The sample was diluted with sperm wash medium (Irvine) and centrifuged at 500g for 7 min. The supernatant was discarded and the pellet was recovered and pipetted in the bottom of a 5 mL tube (Falcon, USA) containing sperm wash (Irvine). After incubation, the upper layer was recovered for high magnification sperm analysis and ICSI.

## 2.2. Assessment of fertilization, and embryo development

Fertilization was confirmed at the time of 18–20 h after the ICSI. Normal fertilization was confirmed when two clearly distinct pronuclei were present. The fertilization rate was defined as the percentage of pronucleated oocytes to the number of injected oocytes. The embryo quality was evaluated under an inverted microscope equipped with a Hoffmann modulation contrast system under 400 × magnifications on the morning of days 1, 2, 3, and 5 of embryonic development according to [14]. The following characteristics were recorded for the pronuclear (PN) morphology: the presence of cytoplasmic halos, size and position of the PN, and the number and distribution of the nucleolar precursor bodies (NPBs) in the PN. Zygotes presenting abnormalities in any of these characteristics were considered to be of low quality [15–16]. Blastocyst formation rate was calculated by dividing the number of embryos that blastulated by the number of embryos in culture past day 3. The implantation rate was calculated by dividing the total number of fetal hearts by the total number of embryos transferred. High-quality embryos were defined as those possessing 4 blastomeres and 8–10 blastomeres on days 2 and 3 of development, respectively, less than 10% fragmentation, and symmetric and mono nucleated blastomeres. The following morphological characteristics were recorded at the blastocyst stage: the size and compactness of the inner cell mass (ICM), the cohesiveness

and number of the trophectoderm (TE) cells and the grade of expansion. The blastocysts were graded according to the Istanbul consensus workshop on embryo assessment [17].

Embryos were placed in a 50-μL drop of culture medium (Global®, Life Global, CT, USA) supplemented with 10 % protein supplement and covered with paraffin oil in a humidified atmosphere under 7.5 % CO<sub>2</sub> at 37 °C for 5 days. Embryo transfer was performed on day 5 of development using a soft catheter with transabdominal ultrasound guidance. Means and standard error were used to summarize continuous indices such as patient age, oocytes fertilized, number of embryos transferred and sperm concentration. Pronuclear pattern, syngamy and early cleavage and development were expressed as a percentage of embryos examined.

## 2.3. Statistical analysis

Statistical analysis was carried out using the software package SPSS 16. Results are expressed as Mean±SE. The paired student's t-test was applied for comparing means. Differences were considered to be statistically significant when  $p < 0.05$ .

## 3. Results

The present study showed the effect of different female age on the embryonic development. The male partners had a strict sperm morphology of an >4% normal sperm morphology. Maternal age groups and the outcome of ICSI cycle in regard to female age are shown in (Table 1 and 2).

The mean age of the females was showed significant differences in the female's age, and the number of retrieved oocytes between three groups ( $p < 0.05$ ). Furthermore, the number of oocytes retrieved decreased with increasing age. The analysis to reveal a potential age dependent trend in 2PN fertilization rate did not show any significant, and the fertilization rate between the groups are similar in all, and cleavage embryos rates on day 2 and 3 were unaltered by female age, also, no significant differences in fragmentation were present at the 4 and 8 cell stage. In addition to a significant decrease in cycle clinical outcome parameter (positive β-hCG). In the plate (1) showed the normal embryo morphology and different types of embryo Cleavage (two cells, four cells and as well as blastocyst stage)

## 4. Discussion

In the present investigation, observed that a typical indication of embryo viability and developmental potential is the cleavage rate and degree of fragmentation. Embryos with moderate development and high degree of fragmentation are generally thought to have a lower implantation potential than embryos with great morphology. The actual reason and mechanism of fragmentation is still not completely understood but

**Table 1:** Evaluation of clinical outcome after ICSI, with normal sperm morphology 4–15 % was injected oocytes with different maternal age 20–46 years

| Parameter                   | M ± SE     | Measure of individual variability |         |       | 95% confidence interval |
|-----------------------------|------------|-----------------------------------|---------|-------|-------------------------|
|                             |            | S.D                               | Range   | C.V.% |                         |
| Age/female for three groups | 26.08±0.36 | 2.57                              | 21 – 29 | 9.85  | 26.81-25.4              |
|                             | 33.8±0.40  | 2.83                              | 30 - 39 | 8.3   | 32.3-33.9               |
|                             | 41.9±0.24  | 1.71                              | 40 - 46 | 4.08  | 41.42-42.4              |
| No.of oocytes               | 6.16±0.37  | 2.63                              | 2 – 12  | 42.7  | 5.42-6.9                |

|                     |           |      |        |       |            |
|---------------------|-----------|------|--------|-------|------------|
| retrieved           | 5.52±0.50 | 3.6  | 1 - 17 | 64.5  | 4.82-6.3   |
|                     | 3.8±0.30  | 2.15 | 1 - 9  | 56.5  | 3.2-4.4    |
| MII                 | 4.18±0.3  | 2.06 | 0 - 8  | 49.3  | 3.6-4.8    |
|                     | 3.9±0.38  | 2.7  | 1 - 10 | 69.6  | 3.12-4.46  |
|                     | 2.47±0.20 | 1.44 | 0 - 7  | 58.3  | 2.07-2.9   |
| No. of inject.      | 5.38±0.36 | 2.53 | 1 - 11 | 47.03 | 4.7-6.1    |
|                     | 4.88±0.5  | 3.3  | 1 - 16 | 67.0  | 3.96- 5.8  |
|                     | 3.41±0.24 | 1.74 | 1 - 7  | 51.03 | 2.9- 3.9   |
| Embryo D1<br>(2 PN) | 4.29±0.30 | 2.15 | 1 - 19 | 50.1  | 3.69 - 4.9 |
|                     | 3.64±0.30 | 2.18 | 1 - 9  | 59.9  | 3.04-4.24  |
|                     | 2.64±0.19 | 1.42 | 1 - 6  | 53.8  | 2.24- 3.04 |
| Embryo D2           | 4.32±0.30 | 2.16 | 1 - 9  | 50    | 3.72- 4.92 |
|                     | 3.58±0.31 | 2.23 | 1 - 9  | 62.3  | 2.96 -4.2  |
|                     | 2.62±0.20 | 1.44 | 1 - 6  | 54.8  | 2.22- 3.02 |
| Embryo D3(GA)       | 3.32±0.3  | 2.12 | 0 - 8  | 63.9  | 2.7 - 3.92 |
|                     | 2.7±0.27  | 1.97 | 0 - 9  | 72.96 | 2.2 -3.24  |
|                     | 2.13±0.20 | 1.44 | 0 - 6  | 67.6  | 1.73- 2.53 |
| Embryo D3(GB)       | 1.02±0.16 | 1.13 | 0 - 4  | 110.8 | 0.7 - 1.34 |
|                     | 0.75±0.12 | 0.87 | 0 - 3  | 116   | 0.51-0.99  |
|                     | 0.48±0.10 | 0.73 | 0 -3   | 152.1 | 0.28- 0.68 |

Data are presented as means ± SE S.E= Stander error S.D= Stander deviation  
C.V. % = Coefficient of variability MII: Metaphas II GA: Grade A GB: Grade B

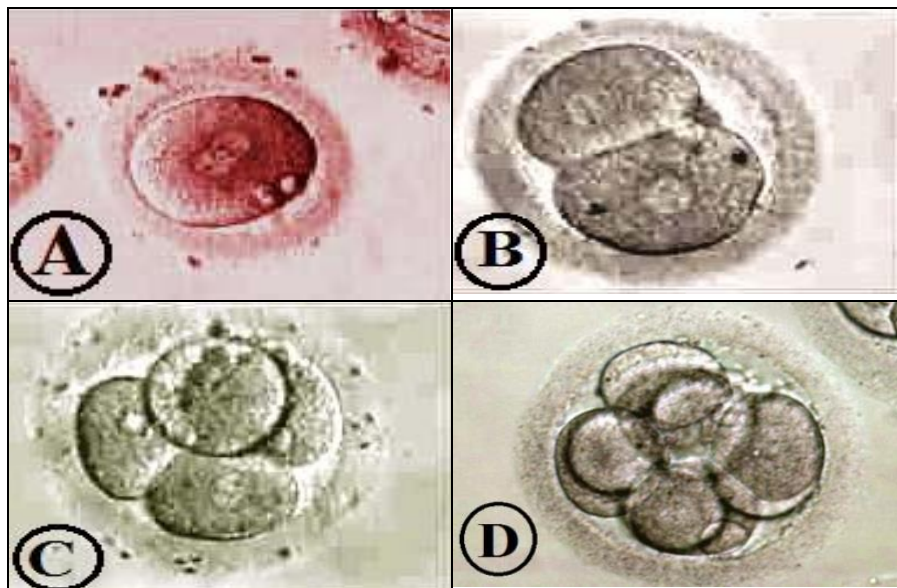
**Table 2:** Evaluation of Embryo development after ICSI

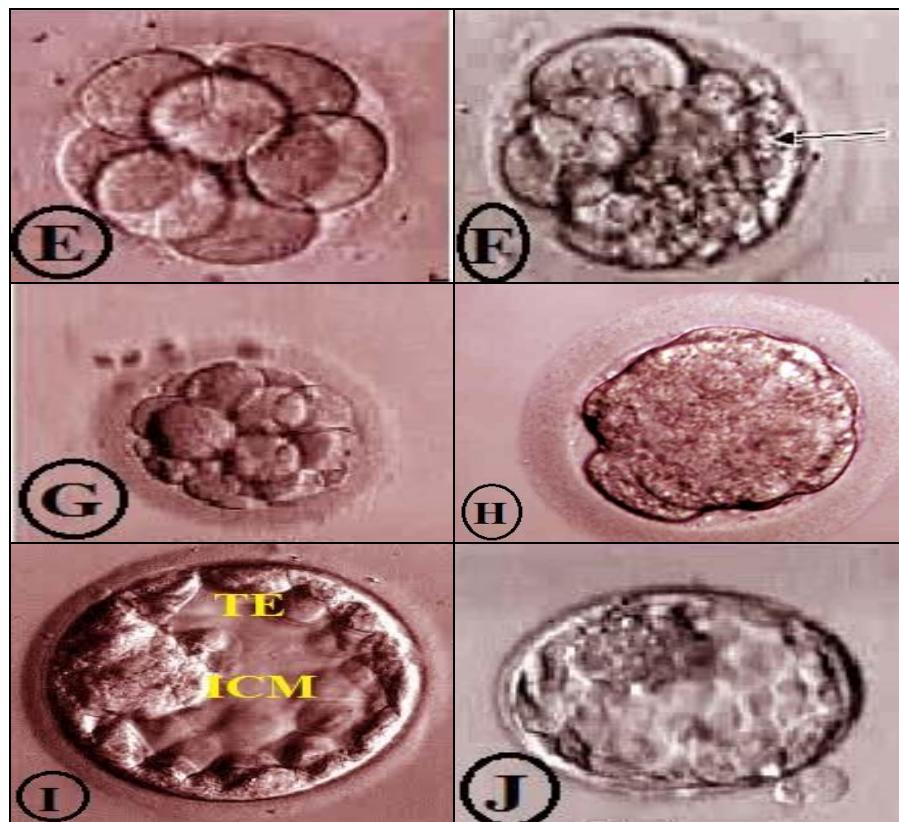
| Parameters               | Normal sperm morphology >4% NS |                 |                 |
|--------------------------|--------------------------------|-----------------|-----------------|
|                          | Group A                        | Group B         | Group C         |
| Female age/year          | 26.08±0.36                     | 33.82±0.39*     | 41.9±0.24*      |
| Fertilization rate (%)   | 203/269 (75.5)                 | 182/244 (74.5)  | 135/174 (77.6)  |
| Cleaved embryo (%)       | 177/203 (87.2)                 | 172/182 (94.5)  | 133/135 (98.5)  |
| High quality embryo (%)  | 158/177 (89.3)                 | 135/172 (78.5)* | 109/133 (81.9)* |
| Blastocyst formation (%) | 36/158 (22.8)                  | 14/135 (10.4)*  | 10/109 (9.2)*   |

\* Significant difference between patient groups p < 0.05

the rate of fragmentation on the third day of culture may be directly associated with chromosomal abnormalities, and about half of the fragmented embryos are aneuploidy [18].The women and oocytes age and the mitochondrial vitality creation decrease, that many of the processes of oocyte maturation, especially nuclear spindle activity and

chromosomal segregation, become impaired [19-20].The outcome is the increased rate of aneuploidy, especially trisomies observed in older women. Embryo chromosome studies show high rates of abnormalities, above 50% of embryos studied, in patients aged 35 and older.





**Fig 1:** Photomicrograph of normal embryo morphology, 2PN(A) 2-cells embryo (B) embryo 4-cells grade A (C), embryo 8-cells grade A (D) different form and number of blastomers with some fragmentation(E&F), many embryo showed with normal cleavage, morula and early compact embryo Grade A (G&H) and abnormal blastocyst formation (J) versus with normal (I).

The constant rate (80%) of nuclear matured oocytes indicates that the age of the woman does not impair the ovulation's trigger-induced signal to resumes meiosis. This is in contrast to a recent publication reporting a significantly higher rate of immature oocytes after controlled ovarian stimulation in older women over of age as compared to younger women and the present results is matched with [21], however, their finding was based on a limited number of observations with no alteration for referral diagnosis.

The present study showed that the fertilization rate (2PN) was not influenced by the increasing age of the women followed the ICSI technique in all groups confirming previous findings [22-23-24]. However, minor variation between ages groups were seen in ICSI cycles, the younger age group did not show a lower fertilization rate as compared to the other groups. Also we observed that, the competence to progress from 2PN to cleavage, showed no correlation with age, while the timing of the first cleavage (early cleavage) in ICSI 2PN zygotes, however, showed a tendency to be postponed by increasing age of the woman; from 40% of 2PN zygotes being early cleaved in the youngest age group to 25% in the eldest. The difference in timing of the evaluation relative to actual fertilization time, rather than the difference in the development of IVF and ICSI zygotes. The post-insemination difference in cleavage timing after ICSI is well-recognized [25], thus, splitting of the present embryo assessment information collection into ICSI probably provided the present investigation with a merely time differentiated picture of the cleavage events.

Recent studies assessed the impact of age on embryo arrangement (blastomeres), demonstrated that no relationship between age and a summed score of the embryo at day 2 and day 3 [22-23]. Neither of these studies assessed early cleavage, nor did they analyze the prevalence of the different various cell stages, which may explain the discrepancies. The age induced impairment of embryo competence is strongly associated with aneuploidy in human embryos, which begins prevalently from mis-segregation in meiosis [5-6], and to a minor degree from early mitotic errors [6].

The second meiotic division may be impacted by age, since a recent publication comparing two age groups reports increased time to second polar body extrusion after ICSI in women aged 38 years as compared to younger women [26]. This is not matching with the effects of age on initial cleavages in the present study. Previous reports have shown no age [23-24], or aneuploidy [6], dependent effect on summed morphology score that included blastomere size (even; uneven) without including cell stage specificity. The present data take the morphology investigation further, and show no association between maternal age and the blastomere size, regularity in 2- and 4-cell embryos, indicating normal arrangement of cleavage furrow and cytokinesis during the first cleavages. Time-lapse studies have elucidated that fragments are created dynamically during cytokinesis [27-28], in the present information; no age impact was found in single time point fragmentation degree in neither 2-cell, nor 4- cell (ICSI) embryos.

In the study by [29], found no difference in implantation and

pregnancy rates among a group of young women versus a group of older women ( $\leq 42$  years) following selective transfer of euploid (typical number of chromosomes) embryos. However, patients who choose to undergo pre-implantation hereditary screening should be made aware of the fact that no fresh transfer could be available owing to the absence of a euploid embryo. For instance [30] found that only 61% of women aged 40–42 years with four or less blastocyst had at least one euploid embryo available for transfer. Pre-implantation genetic screening is rapidly becoming an integral part of all IVF cycles in some centers; however, no convincing evidence is available to support the notion that this novel technique has enhanced live birth rates.

In the present study the lower rate of blastocyst stage is agree with a review published in 2016 [31], concluded that at least eight metaphase II oocytes are required to make reasonable success rates, with a live birth rate of 22.9% recorded among patients aged 36 years or older. Given that poor outcome after *in vitro* fertilization connect with age, and [32], concluded that maternal age at retrieval influences the number of euploid blastocysts but does not impair the implantation capability of chromosomally and morphologically-selected embryos. The assessment of morphodynamic attributes of blastocysts, by assessing blastocyst grading and the pace of embryo development, may reflect the epigenetic health of the conceptus and is paramount to select among euploid embryos, other approaches should be considered to check the pattern of increasing maternal age. Relationship status, relationship stability, personal situation, and financial security are factors known to make substantial contributions to delayed motherhood [33-34]. Additionally, numerous women are putting great emphasis on their career trajectory, often completing higher education and pursuing careers only as they enter their fourth decade of life. The societal acceptability and normality of delayed childbearing further potentiate individual decision making [34].

The majority of the studies that assessed knowledge of the implications of delayed childbearing showed that women underestimate the effect of aging on the oocyte. A study of 863 Danish women aged 20–40 years who were working in a healthcare setting. [35] Found that 50% underestimated the impact of age on fertility and overestimated the success rates of ART [26], interviewed 2000 women without children about their reproductive desires and found that 31.3% would be distraught if they were unable to have a child. These women felt that the perfect age for pregnancy was 26.8 years; however, they did not expect to conceive until the age of 32.4 years [26]. Another study assessed fertility knowledge among college undergraduates; only one-third of these women could accurately pinpoint the age at which fertility declines [36]. Various studies have identified advanced maternal age (usually defined as women older than 35 years) as a hazard factor for reduced fertility as well as multiple for adverse maternal and perinatal outcomes [37, 38, 39, 40, 41, 42]. These adverse events included (but are not limited to) hypertensive disorders of pregnancy, gestational diabetes mellitus (GDM), cesarean deliveries, preterm delivery, and fetal. Further, among women of advanced maternal age, pregnancy represents a greater risk of maternal morbidity and mortality compared with the younger patient's demise [43, 44, 45]. Finally,

a study by [46] showed that participants had knowledge deficits about the implications of aging on fertility and pregnancy. In conclusions: Female, age patients ICSI assisted reproduction might not influence the embryo development ability during the period of cleavage stage, the impact of maternal age may be masked in ICSI cycles however, ICSI assisted reproduction might make a negative effect on the process of extended culture to blastocyst stage, and the number of euploid blastocysts negatively correlated with maternal age.

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