Correlation between women age and oocyte quality, embryo formation and pregnancy outcomes in assisted reproductive technology cycles: A retrospective analysis

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Abstract

It is documented that there is a decline in female fertility as a function of age. To estimate the effect of women age on assisted reproductive technology (ART) outcomes, a total of 1560 in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles were initiated in different age-groups women. (Group 1: age ≤ 25, Group 2: age between 26-30, Group 3: age between 31-35, Group 4: age between 36-40, Group 5: age > 40). Patient’s data and pregnancy outcomes were ascertained by records review and face-to-face follow-up. Age, number of retrieved oocytes and matured oocytes, types of ART procedures, number of fertilized oocytes, zygote and embryo scores, as well as, pregnancy and delivery rates were evaluated. The results showed a significantly negative correlation between age of patients and the number of oocytes retrieved (P<0.01), mature oocytes (P<0.001), and fertilized oocytes (P<0.01) in five age-groups. We found no significantly differences in the number of mature oocytes in the 26–30 age group with the women who aged between 31 and 35 (P=0.066). The zygote and embryo scores only showed a significant negative correlation with age in women more than 40 years of age (P≤ 0.01 for each). However, a statistically negative correlation was found between the age of women with clinical pregnancy and delivery rates (P=0.031 and P=0.006, respectively). No striking differences found in pregnancy rate between IVF and ICSI procedures (P=0.095 and P=0.117, respectively). Our findings recommend that the age-related decline in female fertility by effect on oocyte and embryo quality. The women age negatively influence outcome of assisted reproductive technology treatment.

Keywords: Women age, Pregnancy rate, Intracytoplasmic sperm injection, In vitro fertilization

1. Introduction

Assisted reproductive technologies (ART) such as in-vitro fertilization (IVF) or Intra-cytoplasmic sperm injection (ICSI) are now widely accepted as effective and acceptable treatments for infertile couples both in the developed and developing countries (1, 2). The woman’s age is commonly accepted as the most important variable, affecting the success rate of ART procedures (3-7).

It is well established that there is a decline in female fertility as a function of age (4, 5, 7).

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This decrease in reproductive capacity has been found in a non-contracepting population, in ovulation induction, and also in infertile patients who undergo IVF or ICSI (3-6, 8).

Over the past decades, demographic and socioeconomic trends have resulted in an increase in the absolute number of women seeking pregnancy in their late 30’s and early to mid-40’s (5, 6, 9).

Earlier studies have shown that as age rises, ovarian responsiveness to stimulation declines, the number of oocytes and the number of available embryos for transfer to the uterus falls, pregnancy rates and birth rates decline, although fertilization rates are unchanged (4-8).
The purpose of this study was to compare and report our experience with IVF and ICSI treatment in infertile women who were more than 40 years of age with those of younger women to obtain more accurate information on the impact of the women’s age on the outcome of ART.

2. Materials and methods:

2.1. Study design

This study was approved by the ethics committee of Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

A retrospective analysis of all the treatment cycles between April 2007 and September 2012 was performed for women who referred to Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. A total of 1535 women aged between 18 and 49 years underwent IVF or ICSI treatment cycles during this period. The women were stratified into five groups by age from <25 to >40 years of age. Group 1: age ≤ 25, Group 2: age between 26-30, Group 3: age between 31-35, Group 4: age between 36-40, Group 5: age > 40.

Indications for IVF were in particular blocked fallopian tubes, severe endometriosis, unexplained infertility, and mild male-factor infertility. Indications for ICSI were obstructive and non-obstructive azoospermia, oligoasthenoteratozoospermia, and failed IVF cycles.

Patients’ data including age, number of retrieved oocytes and matured metaphase II (MII) oocytes, types of ART procedures (IVF and/or ICSI), number of Inseminated oocyte, zygote and embryo scores, as well as, pregnancy and delivery rates were recorded during the treatment phase. Outcomes of cycles were ascertained by clinic chart review or by face-to-face follow-up.

All women included in this study had a cycle day 3 FSH level of ≤15 IU/L measured within 3 months before the commencement of treatment. Only cases with homologous oocytes were included.

2.2. Ovarian stimulation and oocyte retrieval

Controlled ovarian hyperstimulation in ART cycles was accomplished using gonadotropin-releasing hormone (GnRH) agonist down-regulation combined with HMG/r-FSH or GnRH antagonist with HMG/r-FSH or only HMG protocols. When at least two follicles reached a mean diameter of 18 mm, using transvaginal ultrasonography, ovulation was induced by 10000 IU hCG injection. The Oocytes were collected about 34-36 hours after the hCG injection and conventional IVF or ICSI were done as appropriately.

2.3. Assessment of zygote quality, embryo cleavage and embryo quality

Oocyte fertilization was assessed 18 to 24 hours after IVF or ICSI by confirmation of the presence and location of two pronuclei (2PN) and the alignment of nucleolar precursor bodies (NPBs), concomitantly with assignment of previously described pattern by smith and scott (10) as A, B and C.

The observation of 2PN was performed using an inverted microscope at magnification of x400. Embryo cleavage and embryo quality were evaluated 48 hours after insemination. The embryos were scored as I, II, III and IV based on previously published method (10).

All embryo transfers were performed using a Labotect catheter (Labotect GmbH, Gottingen, Germany) on day 3 after oocyte retrieval based on the number and quality of obtained embryos.

As luteal support, the patient received daily i.m. injections of 100 mg of progesterone in oil (progesterone, Aburaian Co., Tehran, Iran) or 400 mg of vaginal progesterone (Cyclogest®; Actavis, Branstaple, UK), which was started from the day of oocytes pick-up and was continued until the negative pregnancy test or the end of the first trimester of pregnancy.

Follow-up of ART outcomes was done based on chemical pregnancy, clinical pregnancy, ongoing pregnancy and live birth, and was carried out by chart review and face-to-face communication. In order to identify chemical pregnancy in women, serum beta-hCG levels were measured two weeks after embryo transfer. Clinical pregnancy was confirmed by the presence of fetal cardiac activity on transvaginal ultrasonography at 6–8 weeks after positive beta-hCG.

2.4. Statistical analysis

The results were analyzed among the different age groups and also compared between the ICSI and the IVF groups. Data were initially assessed for normality with the Kolmogorov-Smirnov test. On the basis of these results, we decided to use nonparametric procedures to compare data among the five groups of patients. Therefore Kruskal-Wall test was used to test the differences between groups. Single posttest comparisons were performed by using the Mann-Whitney test for multiple comparisons. Correlations between parameters were examined with the Spearman rank correlation coefficient. All evaluations were performed with the SPSS statistical package (version 15; SPSS).
Significance level of $P < 0.05$ for comparative measurements was used throughout the study.

3. Results:

Detailed patients’ data are shown in Table 1. As noted, most of the women had aged between 26 and 30. Overall, one thousand five hundred sixty ART cycles were done in women in our center during that period of time. One hundred seventy five cycles (11.21%) of them were performed as conventional IVF, and one thousand three hundred eighty five cycles (88.79%) were performed as ICSI. Three hundred eighty seven of ART cycles (24.8 %) resulted in a successful pregnancy. We found a decreased mean number of oocytes retrieved based on women age. Logistic regression analysis showed a significant negative correlation between the age of patients and the number of oocytes retrieved ($P <0.001$).

There were significant differences between the number of oocytes retrieved in five age-groups ($P <0.01$ for each).

Moreover, there was a significant negative correlation between age and the number of mature oocytes ($P<0.001$). Statistical analysis showed that the women in the 26–30 age group had no significantly differences with the women who aged between 31-35 in the number of mature oocytes ($P=0.066$). On the contrary, the number of mature oocytes showed significantly differences between the other age-groups ($P<0.01$ for each).

Interestingly, we found a markedly negative correlation between women’s age and the number of Inseminated oocyte between all age-groups, independent of used ART procedures ($P<0.001$). Comparison between means revealed that the number of fertilized oocyte declines significantly with increasing age ($P<0.05$ for each).

The detailed data of the zygote morphology and embryo grading are listed in Table 2. The assessmen of zygote morphology showed a statistically significant decrease in class “A” configuration, and significantly increase in class “C” configuration in more than 40 years old patients in comparison to other aged group patients ($P\leq 0.01$ for each). The zygote scores only showed a significant negative correlation with age in women more than 40 years of age ($P=0.012$).

Embryo scoring also showed a significant decrease in grade “I” embryos in women with more than 40 years old, when compared to other groups ($P<0.05$ for each).

The data also revealed that the grade “III“ and “IV” embryos were significantly increased in each group, ($P<0.01$ for each). There was a significant negative correlation between ages of women and quality of embryos in patient who more than 40 years of age ($P=0.001$).

The clinical pregnancy rate was 27.9% among the 254 patients aged $\leq 25$ years. However, only 53 pregnancies terminated by delivery in this age, and delivery rate was 20.8% in patients aged $\leq 25$ group. Among these deliveries there were twelve twin pregnancies and two triplet pregnancies. Results showed clinical pregnancy rates of 26% among 526 women aged between 26 and 30. The pregnancy rate also showed a statistically negative correlation with the women’s age ($P=0.006$).

The pregnancy rate was significantly lower in women aged more than 40 when compared to other groups ($P=0.03$). In addition, we found that there was no striking differences in pregnancy rate between IVF and ICSI procedures ($P=0.095$ and $P=0.117$, respectively).

The delivery rate in 26-30 age groups was 19.5%, and there were 15 twin pregnancies and 3 triplet pregnancies (Table 1).

The rate of clinical pregnancy was 24.5% among the 444 patients aged 31 to 35. In this group , 83 (18.7%) terminated by delivery that We found 17 twin pregnancies and 2 triplet pregnancies (Table 1).

Among the 234 women aged between 36 and 40, 47 (20.1%) showed clinical pregnancies, and 16.6 % terminated by delivery. Four twin pregnancies were found. The clinical pregnancy rate was 7.8% among the 77 patients aged more than 41 years. However, only 5 (6.5%) pregnancies terminated by delivery.

Statistical analysis showed no significantly differences in the rates of clinical pregnancy and delivery in the women aged less than 25, and aged between 26 and 30 ($P=0.15$). However, the rates of clinical pregnancy and delivery showed statistically differences between the other age- groups ($P<0.01$ for each).

There was a negative relationship between age and rates of delivery and clinical pregnancy, using a Pearson’s correlation coefficient test ($P=0.031$ and $P=0.006$, respectively).

There were no statistically significant differences in the clinical pregnancy or in the delivery rates between the patients undergoing IVF or ICSI treatment.
Table 1. Distribution of the patients, controlled ovarian hyperstimulation, and pregnancy and delivery rates according to age. The values presented in mean ±SD and/or percentage

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cases (%)</th>
<th>26-30</th>
<th>31-35</th>
<th>36-40</th>
<th>&gt; 40</th>
<th>Total</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases (%)</td>
<td>153 (100)</td>
<td>77 (5)</td>
<td>234 (15.2)</td>
<td>77 (5)</td>
<td>1535 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>23.1±1.9</td>
<td>25.2±2.1</td>
<td>26.7±3.1</td>
<td>28.2±2.1</td>
<td>30.4±5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retrieved oocytes</td>
<td>12.2±7.6</td>
<td>10.8±7.2</td>
<td>9.7±6.1</td>
<td>7.8±5.4</td>
<td>5.7±4.3</td>
<td>10±6.8</td>
<td>P &lt;0.01*</td>
</tr>
<tr>
<td>Mature oocytes</td>
<td>8.8±5.6</td>
<td>7.9±5.4</td>
<td>7.3±4.9</td>
<td>6.1±4.4**</td>
<td>4.6±3.4**</td>
<td>7.4±5.1</td>
<td>P &lt;0.001</td>
</tr>
<tr>
<td>IVF</td>
<td>33</td>
<td>65</td>
<td>60</td>
<td>12</td>
<td>5</td>
<td>175</td>
<td>N.S. *</td>
</tr>
<tr>
<td>ICSI</td>
<td>223</td>
<td>469</td>
<td>395</td>
<td>226</td>
<td>72</td>
<td>1385</td>
<td>N.S. *</td>
</tr>
<tr>
<td>Inseminated oocytes</td>
<td>5.4±4.5</td>
<td>5±4.6</td>
<td>4.7±3.9</td>
<td>3.6±3.2</td>
<td>3±3</td>
<td>4.7±4.1</td>
<td>P &lt;0.01*</td>
</tr>
<tr>
<td>Pregnancy (%)</td>
<td>72 (28.3)</td>
<td>143 (27.2)</td>
<td>115 (26)</td>
<td>50 (21.3)</td>
<td>7 (9)***</td>
<td>387</td>
<td>P=0.03</td>
</tr>
<tr>
<td>Clinical Pregnancy</td>
<td>71</td>
<td>137</td>
<td>109</td>
<td>47</td>
<td>6</td>
<td>370</td>
<td>P&lt;0.01*</td>
</tr>
<tr>
<td>Chemical Pregnancy</td>
<td>1</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Delivery</td>
<td>53</td>
<td>103</td>
<td>83</td>
<td>39</td>
<td>5</td>
<td>283</td>
<td>P&lt;0.01*</td>
</tr>
<tr>
<td>Abortion</td>
<td>11</td>
<td>27</td>
<td>19</td>
<td>5</td>
<td>1</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>Multiple pregnancies</td>
<td>14</td>
<td>18</td>
<td>19</td>
<td>4</td>
<td>-</td>
<td>55</td>
<td></td>
</tr>
</tbody>
</table>

* P ≤ 0.05, comparison between all aged groups
** P ≤ 0.05 compared to group aged ≤ 25
*** P ≤ 0.05 compared to other aged groups
° Not statistically significant both for maternal age and for insemination technique

Table 2. Zygote morphology and embryo cleavage scores related to the women age. The values presented in number and percentage

<table>
<thead>
<tr>
<th>Zygote Morphology</th>
<th>Less than 25</th>
<th>Less than 25</th>
<th>26-30</th>
<th>36-40</th>
<th>31-35</th>
<th>More than 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>78 (30.7)</td>
<td>178 (33.8)</td>
<td>153 (34.5)</td>
<td>70 (30)</td>
<td>70 (30)</td>
<td>17 (22)</td>
</tr>
<tr>
<td>B</td>
<td>137 (53.9)</td>
<td>265 (50.4)</td>
<td>215 (48.4)</td>
<td>124 (53)</td>
<td>124 (53)</td>
<td>44 (57.2)</td>
</tr>
<tr>
<td>C</td>
<td>39 (15.4)</td>
<td>83 (15.8)</td>
<td>76 (17.1)</td>
<td>40 (17)</td>
<td>40 (17)</td>
<td>16 (20.8)</td>
</tr>
<tr>
<td>I</td>
<td>58 (22)</td>
<td>106 (20.2)</td>
<td>77 (17.3)</td>
<td>44 (18.8)</td>
<td>44 (18.8)</td>
<td>10 (13)</td>
</tr>
<tr>
<td>II</td>
<td>121 (47.6)</td>
<td>256 (48.7)</td>
<td>230 (51.8)</td>
<td>115 (49.6)</td>
<td>115 (49.6)</td>
<td>34 (44.2)</td>
</tr>
<tr>
<td>III</td>
<td>62 (24.4)</td>
<td>137 (26)</td>
<td>114 (25.7)</td>
<td>57 (24.4)</td>
<td>57 (24.4)</td>
<td>26 (33.8)</td>
</tr>
<tr>
<td>IV</td>
<td>15 (6)</td>
<td>27 (5.1)</td>
<td>23 (5.2)</td>
<td>17 (7.2)</td>
<td>17 (7.2)</td>
<td>7 (9)</td>
</tr>
</tbody>
</table>

4. Discussion

The present study was undertaken to compare the number and quality of retrieved oocytes, the quality of fertilized oocytes, pregnancy and delivery rates, as well as, IVF and ICSI outcomes between infertile women who were in the range less than 25 and more than 40 years of age to demonstrate the impact of the women’s age on the outcome of ART.

Infertility is a common problem for couples and is defined as not being able to get pregnant after one year of trying (1, 11, 12). ART is a group of different methods used to help infertile couples (11, 12).

Success rate of ART varies and depends on many factors. The age of the woman is the most important factor in determining the success of an ART cycle (3-7). According to the previous reports, success rates decline as women age, and drop off even more dramatically after about age 37 (4-7). This decline is partly due to a lower chance of become conceive from ART, and also due to a higher risk of miscarriage with increasing age, especially over age 40 (4, 7).

The outcome of this study showed that the woman age had a negative correlation with the number of retrieved oocytes, oocyte maturity, number of Inseminated oocyte, clinical pregnancy and delivery rates. There is no doubt that the results in older patient groups are lower.

There are several lines of evidence demonstrating abnormalities of the oocyte as a major cause of age associated infertility. The effects of women age on cytoskeleton, organelle distribution, fertilization, and development in vitro and in vivo of mammalian oocytes/embryos have been investigated (13).

There are a limited number of studies analyzing the ultrastructure of oocytes from middle-aged
women. Women age is associated with an increased proportion of germinal vesicle oocytes exhibiting undulations in the nuclear membrane (14). The nucleoplasm appears more dense and the nuclear membrane thicker than those of younger women oocytes. On the other hand, Fiber network of the zona pellucid in aged oocytes is arranged into continuous bands and the cytoplasmic projections of the cumulus cells which penetrate the zona pellucida are retracted (15).

It is well documented that oocytes from women in their early 40’s exhibit a high incidence of abnormalities in microtubule and chromosome placement at the metaphase stage of meiosis II (13). Higher prevalence of abnormalities has been reported in oocytes derived from older infertile cases following ovarian hyperstimulation (16).

Zenzes, et al (1997), using the regressions of oocyte number on age, found that both the numbers of retrieved oocytes and of mature oocytes decreased by approximately 50% between the ages of 24 and 42 years. They estimated that, on average, the number of mature oocytes drops from approximately 7.1 at age 24 years to approximately 3.4 at age 42 years. The same work showed that the women aged over 40 years had a mean number of 3.4 mature oocytes, and of these 70% were fertilized, concluding an average women age is associated with an increased proportion of germinal vesicle oocytes exhibiting undulations in the nuclear membrane (14). The nucleoplasm appears more dense and the nuclear membrane thicker than those of younger women oocytes. On the other hand, Fiber network of the zona pellucid in aged oocytes is arranged into continuous bands and the cytoplasmic projections of the cumulus cells which penetrate the zona pellucida are retracted (15).

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Other physiologic factors may contribute to age-related decline in fertility include decline in ovarian sensitivity to gonadotropins, evidenced by higher serum levels of FSH (18).

Previous investigation demonstrated that the older women are less responsive to gonadotropins, producing lower numbers of developing follicles and oocytes in spite of higher dose requirements of gonadotropins (19). The mechanism underlying this phenomenon is almost certainly related to the progressive follicular depletion of the ovarian that occurs with age (20).

Data on the effects of women age on fertilization rate is contradictory (14). Though, sperm binding capacity of zona pellucida and oocyte quality may be influenced by ageing remains unknown (21). Whereas, some studies have reported no effect of oocyte ageing on cleavage rate and/or embryo morphology after ICSI (22), others have showed increased fragmentation following conventional IVF methods (23). Most authors point out that aged oocytes have a reduced potential for implantation and development (24). There is also evidence demonstrating a negative impact of women age on zona pellucida quality. Suzuki et al. (1988) reported that zona pellucida function was influenced by the age (25).

The evaluation of the zygote and embryo characteristics has been proposed as an indicator of embryo development and chromosomal complement in human fertilized oocytes (26). The analyses of zygotes seem to provide important information about arrangements of chromosomes in embryos, even if, to date, there is no definitive scientific evidence about its clinical efficacy (27).

In the present study, we have evaluated the zygote-score related to maternal age in patients submitted to ART cycles. We observed that all parameters analyzed were generally uniformly distributed in patients <40 years old. Nonetheless, a significantly increase in class “A”, with a significant decrease in class “C” zygotes was showed, demonstrating a notable effect of maternal ages on zygote quality.

In a study by nicoli and coworkers (28), they showed a statistically significant decrease of zygote scores in patients 38-41 years old. Their results are in agreement with our findings.

In agreement with previously reported data (26) we observed a statistically significant decrease in grade "I" configuration, with a concomitant significant increase in grade "III" configuration, in patients >40 years old in comparison to younger women. This result is in contrast with previously published data showing no correlation between pronuclear morphology and maternal ages (28). We believe that these discrepancies could be related to the different sample size and to the different maternal age between our patients and those analyzed by others.

In the present study, the rates of clinical pregnancy and delivery showed significantly decrease in patients >30 years old in comparison to younger patients. Moreover, we found a negative correlation between women age with clinical pregnancy and delivery rates. There are several lines of prior studies demonstrating the negative impact of age on pregnancy and delivery rates (5, 29-32).

Some researchers established a gradual decrease in the cumulative pregnancy rate. However, the starting point of this decrease was established at different ages: 27 years (33), 28 years (34), 30 years (29), 31 years (35), 35 years (36), 37 years (32), and 40 years(31).

5. Conclusion
The results of this study confirm that the women’s age strongly influence outcomes of assisted reproductive technology treatment. Our study results added additional data indicating a negative impact of
woman age on the number of retrieved oocytes, the quality of oocytes, and number of fertilized oocyte, as well as, zygote and embryo scores. In addition, Age strikingly influence the pregnancy and delivery rates independent of ICSI or IVF procedures.

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References