The Influence of Seasons on Oocyte Parameters in ICSI Cycles

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Abstract
Objective: This study aimed to evaluate the effect of seasonal variability on the assisted reproductive technique (ART) success rate.
Materials and methods: This study was a descriptive – analytical survey performed on 91 infertile women undergoing intracytoplasmic sperm injection – embryo transfer (ICSI-ET) in different seasons. The patients aged less than 35 years old and had normal LH/FSH ratio. All patients entered long protocol down regulation treatment cycle and the picked up oocytes were transferred to Gili medium in the infertility laboratory. The cumulus characteristics, oocyte parameters including number of the retrieved oocytes, morphological characteristics, fertilization and degeneration rate and number of cleaved embryos were recorded. Data were analyzed by SPSS software.
Results: The number of embryos was significantly higher in autumn. Abnormal morphological parameters (color, size, zona thickness) and the degeneration rate were significantly higher in spring. The number of retrieved oocytes, MI, MII oocytes and fertilization rate had no significant seasonal variations.
Conclusion: The results of this study showed a significant seasonal variation in morphological parameters of the oocytes, degeneration rate and the number of formed embryos.

Keywords: Infertility, Seasonal variation, Oocyte, Assisted reproductive technique

Introduction

In spite of improvement of assisted reproductive methods and equipments that led to significant success in managing fertility problems of infertile couples, optimal results are not achieved. The rate of ART success depends on multiple factors such as infertility cause, type of treatment, induction ovulation method, drugs, female age, number of transferred embryos and environmental factors (1). Among mentioned factors, environmental factors that influence mammal’s fecundity such as seasonal variation and temperature are called as internal time – scheduling rhythm (2, 3).

Nevertheless, the seasonal effect on mammal’s reproduction has been absolutely proven but seasonal variation data and its mechanism in human being and primate is not completely cleared. ART makes available exclusive administered condition to study seasonal effect on human reproductive process (4).

In one study, ovaries of cows with ages under 30 months were collected and oocytes were picked up during a 2 year period. After maturation, in vitro fertilization and culture, cleaved embryos that became blastocysts were higher in autumn (5). In the other study on honeschtine cows, oocyte retrieval rate and cleaved embryos that became blastocyst on eighth
day were lower in summer. This could be related to the effect of cold seasons and reduction in oocyte quality in summer but sustaining cold conditions for 42 days for these cows didn’t omit seasonal influences (6).

In another study, effects of age on oocyte development from FSH-induced and non FSH – induced Rhesus were studied. In this study ova that were retrieved from FSH – induced immature rhesus showed more blastocyst development in fertility season (%16) than non fertility season (%0) and retrieved ova from matured FSH – induced rhesus in fertility seasons showed significant developmental growth in comparison to non fertility season too (p < 0.05). This information shows that animal age and fertility season influence oocyte maturation (7).

Studies showed that there is a relationship between temperature and seasonal pattern of birth in human beings. Seiver found in recent decades that American had more birth rate reduction in May in comparison to April due to consuming air conditioners. Also the relationship between climate and fertility has been noted in United Kingdom. It has been estimated that temperature influence on birth rate appears 7 to 11 months later. Also it has been proven that very cold or very hot weather tends to reduce the birth rate (8).

New documents suggest reduction of sperm quality, lower fertility rate and birth rate in hot summers near equator but in northern countries where diminished seasonal variability and hypophysis ovary axis activity are observed, fertility rate in dark months of winter is reduced. In these areas, fertility rate is maximal in summer that causes a maximum birth rate in spring. It is believed that seasonal variability in ovulation rates, and other mechanisms such as variability in oocyte quality or endometrial receptivity rate in special times of year are responsible (9).

Confirming variability of fertility in response to light, some studies have found seasonal variability in nocturnal melatonin and luteinizing hormone (LH) levels. Natural melatonin level in winter months was higher than other months and inversely, level of LH serum in summer was higher than winter (%76). It is considered that a higher level of melatonin in follicular fluid in winter has an inhibitory effect on serum LH and in summer when melatonin level is lower, inhibitory effect is lowered (10).

In the other study, it was considered that melatonin takes part in secretion of steroid hormones such as progesterone from ovary; and melatonin maybe a factor influenced by season that causes seasonal variability in assisted reproductive (ART) results (11, 12).

In this study, we investigated the seasonal variability of quantity and quality of retrieved oocytes in ART.

Materials and methods

This cross sectional study was performed on 91 infertile women referred to Babol infertility and reproductive health research center from January 2004 to January 2005.

Infertile women aged 35 years or more, having abnormal FSH / LH ratio, polycystic ovary syndrome or endometriosis were excluded.

The patients underwent long – protocol down regulation regimen. At first, we performed a vaginal ultrasound in the second day of cycle. After a normal sonogram, Buserelin (superfact; aventis pharma Deutschnad GmbH Frankfurt am main, Germany) was administered 0.5cc subcutaneously from day of 21 of cycle for a week. After next menstruation and ultrasound evaluation of endometrial thickness and ovaries, ovulation induction was started with two daily intramuscular HMG (merional; IBSA – institute Biochimique SA – Lugano – Switzerland). By serial vaginal ultrasound and adjusting HMG dosage HCG (pregnly; Darou paksh – Mfg – Co. Iran under Technical Cooperation with Organon) was injected when at least two follicles reached to the size of 18–20 mm. After 36 hours of HCG injection, vaginally retrieved oocytes were transferred to the ART laboratory. Oocytes with zona pellucida and granulosa cells were washed in Ham’s f16 culture environment and placed in the GIII culture environment. Then, oocyte parameters and qualities were recorded. Oocytes were incubated at 37°C temperature and 5 percent CO2; then granulosa cells were omitted with hyaluronidase enzyme and transferred to the incubator for next stage. Then more detailed parameters of oocytes like oocytes’ size and color, zona pellucida thickness, oocyte evolution processes such as germinal vesicle stage (GV), metaphase I (MI), metaphase (MII) and other parameters such as being spherical, degenerated or other abnormalities were recorded. All oocytes at MII stage were injected by sperm and after 12 to 16 hours were incubated in GIII culture environment for the second time. The number of oocytes with pronucleus that overtook to fertilization stage and formed embryos at the third day were recorded. Women age, the number of oocytes, the number of
MI, MII and fertility rate, oocyte abnormalities, the number of degeneration, the number of embryos and different seasons when the cycles were started were the investigated factors in our study. All gathered data were analyzed using SPSS 10. Chi – square and ANOVA, Kruskal – Wallis and Mann-Whitney tests were applied.

Results

A total number of 91 women that have inclusive criteria entered our study. The average age of infertile women was 26.9±4.3 yr (mean± SD). The maximum age was 34 and the minimum was 16 yr. The duration of infertility was 6.0±4.2 year including 6 months as the shortest duration and 17 yrs as the longest duration. The average number of oocytes per cycle was 10.1 including 9.4 in maturity stage (MII) and 0.7 in MI stage, and the mean of fertilization rate and formed embryos was 4.4 and 5.1 in each treatment cycle (Table 1). Seventy four oocytes were small size and 870 (%96.2) had normal size. One hundred and one oocytes (%9.9) had dark, turbid color and 843 (%90.1) had natural color. Eighty three oocytes (%8.7) had abnormal zona thickness and 867 (%91.3) had normal zona thickness. The highest oocyte retrieval number was in spring and the lowest was in summer (11.0±5.49.5±5.9, respectively) without significant difference (P=0.812) (Table 2). The number of MII oocytes was more in spring (10.2±6.0) and the number of MI oocytes was less in winter but none of them had significant difference (Table 2).

Fertilization rate was higher in autumn (6.5±1.1) and lower in spring (4.5±0.8) than other seasons but there was no significant difference in fertilization rate regarding various seasons (P=0.363) (table 3).

The number of formed embryos was more in autumn (6.4±1.0) and less in winter (3.3±0.3) and there was a significant statistical difference between seasons (P-value 0.041) (Table 3).

The rate of oocyte abnormalities was higher in spring (3.2±1.7) and lower in winter (0.4±0.3), but this difference was only significant between winter and spring (P=0.09). Oocyte degeneration rate was higher in spring and lower in winter (0.65±0.22 vs 2.0±0.4, respectively) (p = 0.042) (Table 3).

There was significant difference in oocyte parameters such as size, color, zona thickness in spring (P < 0.001). It means that small, dark, turbid oocytes and abnormal zona thickness were more common in spring (Table 4).

Discussion

About 10 to 20 percent of yearly birth rate is influenced by seasonal variation; depending on biography and environmental factors such as rhythmmic changes of weather or light and sexual activity changes (13). Since the IVF technique started to be used, scientists and biographers noticed to periodic time variability in success rate of treatment cycle relating to high rate or low rate of pregnancy. This

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**Table 1:** Oocyte characteristics, fertilization rate (FR) and number of embryos

<table>
<thead>
<tr>
<th>Oocyte Characteristics</th>
<th>N</th>
<th>Median</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocyte number</td>
<td>944</td>
<td>9</td>
<td>10.1</td>
<td>0.61</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>MII</td>
<td>875</td>
<td>9</td>
<td>9.4</td>
<td>0.64</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>MI</td>
<td>69</td>
<td>0</td>
<td>0.7</td>
<td>0.14</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>FR</td>
<td>486</td>
<td>5</td>
<td>5.1</td>
<td>0.37</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Abnormality</td>
<td>122</td>
<td>0</td>
<td>0.7</td>
<td>0.16</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Degeneration</td>
<td>105</td>
<td>0</td>
<td>1.0</td>
<td>0.14</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Number of embryos</td>
<td>397</td>
<td>4</td>
<td>4.4</td>
<td>0.36</td>
<td>0</td>
<td>19</td>
</tr>
</tbody>
</table>

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**Table 2:** Oocyte and MII retrieval in different seasons

<table>
<thead>
<tr>
<th>Season</th>
<th>Oocyte</th>
<th>MII</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Spring</td>
<td>151</td>
<td>10.7 ± 5.8</td>
</tr>
<tr>
<td>Summer</td>
<td>286</td>
<td>9.5 ± 5.9</td>
</tr>
<tr>
<td>Autumn</td>
<td>239</td>
<td>11.0 ± 5.4</td>
</tr>
<tr>
<td>Winter</td>
<td>268</td>
<td>9.9 ± 6.2</td>
</tr>
</tbody>
</table>

P-Value (ANOVA) 0.812 0.834
low pregnancy rate is not only due to technical problems, laboratory conditions or culture environment (4).

In this study number of oocyte per cycle was higher in spring and number of embryos was significantly higher in autumn and lower in winter. In a retrospective study that Revelli and et al. performed in Italy, the parameters that influence IVF outcome such as ovarian response to gonadotropin, oocyte and sperm quality, fertilization rate, embryo quality and pregnancy rate and implantation rate were investigated. None of IVF parameters showed significant variability with any specific season (14).

Vahid and et al. in Yazd infertility center (Shahid Sadooghi) had a study on 288 IVF – cycles and 821 ICSI cycles, pregnancy rate was maximum in early spring and was minimum in autumn. But there was no significant seasonal variation in fertilization rate, transferred embryos and sperm motility; however sperm number in spring was more than other seasons (1).

In the other study that Wunder and et al performed in Switzerland, fertilization rate (FR), Pregnancy rate and implantation were investigated in 7368 IVF cycles. There was no significant seasonal variation, but there was important statistical variables including age, IVF center, infertility cause and day of embryo transfer (15).

In Chang and et al. study in Taiwan, the effect of tropical weather on various ovarian responses to ART was investigated. Regarding embryo number and quality in the second day, ET in the third or fifth day of oocyte retrieval was performed. Clear relationship between maximum embryo quality in third and fifth day by temperature, humidity, atmosphere pressure was observed. Light hours had reverse relationship with fertilization rate and pregnancy outcome in embryos transferred in third day, but had direct relationship with the embryos transferred in the fifth day. This result shows, weather condition influences ART outcome and the patient with various ovarian response or various blastocyst culture and transfer may adjust the weather effect (16).

In Weigert and et al. study, seasonal effect on IVF and ET was investigated. The pregnancy rate in cold months of year (September – April) was a little higher than warm months (May – August) but this subject was not significant and other factors such as age and number of transferred embryos had more powerful effect on positive IVF outcome (4).

About the cause of seasonal variation in pregnancy and birth rate, there are many opinions suggested based on evidence and studies. For example, Lam and Miron investigated temperature effect on human fertility. They gathered data relative to monthly birth and temperature since 1942 for 46 years to investigate temperature effect on seasonal variation of birth. The result showed temperature had important effect not only on seasonality but also on non-seasonality of birth. So, temperature explains only a part of seasonal variation in birth pattern. High temperature can decrease sperm quality and coitus frequency (17,18).

On the other hand, some studies compared light

### Table 3: Fertilization rate and number of embryos and degenerated oocytes

<table>
<thead>
<tr>
<th>Season</th>
<th>Fertilize oocyte</th>
<th>Embryo</th>
<th>Abnormal oocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>Mean ± SD</td>
<td>n</td>
</tr>
<tr>
<td>Spring</td>
<td>76 (42)</td>
<td>4.5±0.8</td>
<td>60</td>
</tr>
<tr>
<td>Summer</td>
<td>145 (50)</td>
<td>4.8±0.5</td>
<td>107</td>
</tr>
<tr>
<td>Autumn</td>
<td>146 (59)</td>
<td>6.5±1.0</td>
<td>144</td>
</tr>
<tr>
<td>Winter</td>
<td>119 (47)</td>
<td>7.4±0.6</td>
<td>86</td>
</tr>
<tr>
<td>P-Value (Kruskal-Wallis)</td>
<td>0.363</td>
<td>0.041</td>
<td>0.042</td>
</tr>
</tbody>
</table>

### Table 4: Oocytes with abnormal size, color and zona thickness

<table>
<thead>
<tr>
<th>Season</th>
<th>n</th>
<th>Size</th>
<th>Color</th>
<th>Zona thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>151</td>
<td>31 (20.5)</td>
<td>54 (35.8)</td>
<td>34 (22.5)</td>
</tr>
<tr>
<td>Summer</td>
<td>286</td>
<td>13 (4.5)</td>
<td>1 (0.3)</td>
<td>43 (15.0)</td>
</tr>
<tr>
<td>Autumn</td>
<td>239</td>
<td>9 (7.8)</td>
<td>7 (14.6)</td>
<td>1 (1.9)</td>
</tr>
<tr>
<td>Winter</td>
<td>268</td>
<td>21 (3.8)</td>
<td>39 (2.9)</td>
<td>5 (0.4)</td>
</tr>
<tr>
<td>P-Value (Chi-square)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>
variations between day and night and seasonal variations of birth rate. Rojansky and et al. in a study, from 657 women undergoing IVF – ET cycle, investigated fertilization rate and embryos with A – quality. In this study we observed significant seasonal variation in fertilization rate and embryos with A – quality so that maximum rate of fertilization and A – quality embryos was related to spring and minimum rate was related to autumn. This variation had direct relationship with absolute light of day and neither with temperature and humidity or other environmental parameters (19).

Also, to explain light and time effect on circadian secretion of melatoniningonad otinop and prolactin in women, Kivela et al. performed a study. According to its result, night level of serum melatonin in winter was higher than summer and in turn night level of serum LH in summer was higher than winter. There was no seasonal variation in daily FSH, prolactin and melatonin level (10).

In other study which Ronnberg and et al. performed seasonal variation of melatonin level of follicular liquid in some of the women undergoing IVF and laparotomy were measured and significant daily and yearly variability was observed that shows melatonin may set human reproduction in follicular fluid competence (20).

It suggests that if seasonal variation was observed in an infertility center, and if these result was confirmed with more detailed complementary researches, a definite seasonal pattern in fertilization, pregnancy rate and implantation and birth rate will be gained that can be applied in locally ART protocol – treatment scheduling.

Acknowledgment
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References