Advantage of day 3 over day 2 embryo transfer
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Objectives This study was done to compare embryo quality and pregnancy rate between days 2 and 3 embryo transfer, the effect of extended culture on embryo development potential also analyzed.

Methods In an 18-month period extending from January 2014 to June 2015, all couples were undergoing infertility treatment in the form of intracytoplasmic sperm injection (ICSI) whatever the cause and in whom fresh embryo transfer were done (258 cycle) were included in this prospective study. The patients were classified into two groups according to the day of embryo transfer. The policy of transfer was uniform in both groups and the transfer was done according to patient criteria or the number of embryos available.

Results The main outcome measures were embryo quality, embryo development potential and pregnancy rate. Our data suggest no significant statistical difference in pregnancy rate between days 2 and 3 embryo transfer (42.9% vs. 42.0% respectively, P > 0.05). The percentage of good quality embryos was slightly insignificantly higher in day 2 group than day 3 (86.03% vs. 84.7%, P > 0.05). The percentage of slow growing embryos was significantly higher in those cultured for 3 days than those remain for just 2 days in vitro (15.9% vs. 23.3%, P < 0.05).

Conclusion A similar pregnancy rate was obtained by doing embryo transfer on days 2 and 3 after ICSI in spite of slight regression in embryo quality and higher rate of developmental delay in cases of extended culture. So, no advantage for day 3 over day 2 embryo transfer.

Keywords embryo transfer, embryo quality, intracytoplasmic sperm injection

Introduction

Working in vitro associate with stressors that present in the culture system, which are absent in vivo within the female reproductive tract. Stressors evident in the embryology laboratory that have a negative influence on embryos include: frequent temperature fluctuation during manipulation, changes to the concentration of carbon dioxide with subsequent changes in pH, changes in atmospheric oxygen; and the accumulation of ammonium from amino acids.1

During early stages of embryonic life prior to activation of the embryonic genome, the embryo have only limited capacity at a molecular level to respond to a stress which can apply a major hazard on subsequent viability. In addition it is well known that the effects of a stress can be masked at the level of morphology and may only become evident at a subcellular level with the embryo having reduced metabolic activity, high rate of apoptosis, and lowering in pregnancy rate. Some of these stressors are unavoidable sequel of in vitro culture even in the best IVF laboratory and earlier embryo transfer is adopted to minimize this hazard.2

To maximize the chance of pregnancy, selection of human embryos with a good developmental competence for transfer is mandatory. Embryos are usually graded and selected depending on their morphology and progress rate. Transfer of human embryo (ET) in cleavage stage is commonly done on the 3rd day after oocyte pickup. The main reasons why ET conducted when embryos are at the morula stage is that the human embryo is normally located in the endometrial cavity on days 4–5 after fertilization. So, the intrauterine environment is physiologically more suitable for developing morula on the 3rd day than it is on the 2nd day.3 Also, extending the time of embryonic culture until activation of the embryonic genome at 4–8 cell stage might optimize the selection of viable embryos for transfer. The authors found that an extra 24 h of observation for embryonic development in vitro was possible by postponing and shifting the transfer until day 3 to recognize and discard the embryos that are developmentally arrested or retarded. Generally, delaying ET may therefore be appropriate, if embryo growth is satisfactory during in vitro culture and can increase the chance of successful implantation and facilitate the election of highest quality embryo for transfer.4 Previous researches found that day 3 transfer associate with higher pregnancy and implantation rate than day 2 ET.4–6 However, delaying ET and keeping embryos in in vitro culture conditions could have adverse effects on embryo development and could reduce the number of viable embryos available for transfer.6

A lot of studies found an elevation in the percentage of growth-retarded embryos on day 3 and there were morphological similarities between days 2 and 3 embryos.7 In a study by Laverge et al.,11 the implantation and pregnancy rates were identical between transfers done on day 2 vs. day 3; however, the overall quality score of the embryo decreased when the embryos were cultured up to day 3. Further studies have shown the benefit of ET in patients with poor response on day 2 compared with day 3. There have been an increase in clinical and ongoing pregnancy rates after ET on day 2 than on day 3 in poor responders, indicating that the miscarriage rate can be reduced by limiting embryo culture to only 2 days which could also provide an alternative management for poor responders.13 In addition, Racowsky et al.14 found that earlier embryo transfer can improve pregnancy rate for poorly progressed embryos suggesting that the in vivo environment can rescue less healthy embryos. However, the optimal day for ET of human cleavage-stage embryos remains a matter of debate and some recent studies have found no difference in IVF outcome between days 2 and 3 ET.

Materials and Methods

This study was a cohort retrospective study performed on infertile couples attending Infertility Treatment Center in
AL-Sadr Medical City in Najaf, from January 2014 to June 2015. No written/verbal informed consent was provided from the patients. They underwent intracytoplasmic sperm injection (ICSI) according to the standard protocols. Demographic, clinical and laboratory informations about patients were collected. The subjects were couples undergoing ICSI with fresh embryo transfer as a treatment option for various fertility problems (258 cycle) Female partners was <42 years old and had normal endometrial thickness (7–12 mm) on ET day; no apparent endometrial pathology and less than three failed previous cycles. Controlled ovarian hyper stimulation was done either by pituitary down regulation in the early follicular phase with a GnRH agonist (Decapeptyl 0.1 mg/day) or mid-follicular pituitary down regulation with a GnRH antagonist (Cetrotelix, 250 mcg/day). 150–300 IU/L of recombinant FSH (Gonal-F, 75 IU/L) were used to induce multiple follicle growth and the response was evaluated with serial ultrasound monitoring. When two or more follicles grew up to 17 mm or more, 10,000 IU/mL of human chorionic gonadotropin was taken as an ovulation trigger and oocytes were collected 34–36 h later through transvaginal ultrasound guided aspiration under general anesthesia.

Demuatation of the oocytes was achieved both mechanically and enzymatically. After that all mature metaphase II oocytes are injected with husband sperm (intracytoplasmic sperm injection). Oocytes were examined for signs of fertilization 16–18 h post injection, the two pronuclei zygotes were cultured for 24–48 h in Fertipro Cleavage Medium supplemented with 10% human serum albumin. Two to three embryos at the four or eight cell stage were transferred to the uterus depending on the patient conditions and prognosis (particularly female age and previous unsuccessful attempts) on the 2nd or 3rd day after insemination. ET performed using soft catheter (Cook Ob/Gyn) on day 2 or 3 after oocyte retrieval. Luteal phase support was started on the day of ovum pick up and patients used vaginal progesterone, 400 mg every 12 h. A positive pregnancy test was considered when β-HCG levels above 25 mIU/mL, 2 weeks after ET; fertilization rate, cleavage rate embryo quality and their developmental potential with the average number of embryos transferred were assessed.

Embryo grading depended on degree of fragmentation and morphology of blastomer. Grades 1 and 2 embryos described as good quality embryos while Grades 3 and 4 described as bad quality embryos.

Rate of embryo development also evaluated, embryo less than four cells on day 2 or less than eight cells on day 3 was regarded as slow growing embryos (days were calculated from time of oocyte injection). Embryos with normal fertilization and of good quality (grades 1–2) were chosen for transfer. Day 3 transfer was done on 1, day two fall on a holiday or 2. The patient cannot undergo day 2 transfer. Embryo quality, embryo progression and pregnancy rate were compared between days 2 and 3 ET.

**Statistical Analysis**

Statistical analysis was done using Statistical Package for Social Science, version 20.0. Numerical data expressed as mean ± SEM. Categorical data are expressed as frequencies and percentages. To compare between the parameters of two groups, independent samples, Student’s t-test was used and Chi-square was used for the comparison of categorical variables. The difference between the values were considered statistically significant at \( P < 0.05 \).

**Results**

About 258 intracytoplasmic sperm injection cycle were included in this study. In 189 cycle embryo transfer was done in day 2 post-injection while day 3 transfer was done in 69 cycle.

Table 1 shows the main criteria of patients represented by their age, body mass index, duration of infertility, number of attempts, number of oocyte collected, number of mature injected oocyte and number of transferred embryos. No significant statistical difference in these parameters between days 2 and 3 groups (\( P > 0.05 \)).

Table 2 shows fertilization rate of injected oocytes (number of zygotes/total number of injected oocytes), percentage of good quality embryos, percentage of bad quality and slow growing embryos.

No significant statistical differences in fertilization rate and embryo quality between both groups (\( P > 0.05 \)), while the ratio of slow growing embryos is significantly higher in group of day 3 ET (\( P < 0.05 \)).

In Table 3, pregnancy rate was calculated and compared between patients included in days 2 and 3 embryo transfer. No significant statistical difference in positive pregnancy rate was found between both groups (\( P > 0.05 \)).

**Table 1. The main criteria of patient included in the study**

<table>
<thead>
<tr>
<th>Variable (%)</th>
<th>Day 2 (No. = 189)</th>
<th>Day 3 (No. = 69)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.6 ± 0.5</td>
<td>31.37 ± 0.8</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.5 ± 5.2</td>
<td>27.7 ± 4.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Infertility period</td>
<td>7.4 ± 5.7</td>
<td>5.1 ± 6.6</td>
<td>0.3</td>
</tr>
<tr>
<td>No. of attempts</td>
<td>1.3 ± 0.7</td>
<td>1.4 ± 0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>No. of oocyte</td>
<td>9.4 ± 0.4</td>
<td>9.04 ± 0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>MII</td>
<td>7.8 ± 0.3</td>
<td>7.39 ± 0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>No. of zygote</td>
<td>5.2 ± 0.2</td>
<td>4.75 ± 0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>No. of embryo</td>
<td>4.9 ± 0.2</td>
<td>4.51 ± 0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Transferred embryo</td>
<td>2.94 ± 0.81</td>
<td>2.77 ± 0.85</td>
<td>0.5</td>
</tr>
</tbody>
</table>

BMI: body mass index.

**Table 2. Fertilization rate, percentage of good and bad quality, and slow growing embryos**

<table>
<thead>
<tr>
<th>Variable (%)</th>
<th>Day 2 (No. = 189)</th>
<th>Day 3 (No. = 69)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>FR</td>
<td>70.3 ± 1.6</td>
<td>68.5 ± 2.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Good</td>
<td>86.03 ± 1.8</td>
<td>84.7 ± 3.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Bad</td>
<td>14.8 ± 1.9</td>
<td>15.3 ± 3.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Slow growing embryos</td>
<td>15.9 ± 1.7</td>
<td>23.3 ± 4.0</td>
<td>0.049</td>
</tr>
</tbody>
</table>

**Table 3. Pregnancy rate in day 2 vs. day 3 embryo transfer**

<table>
<thead>
<tr>
<th>Day 2, No. (%)</th>
<th>Day 3, No. (%)</th>
<th>Total</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT Positive</td>
<td>81 (42.9)</td>
<td>29 (42.0)</td>
<td>110 (42.6)</td>
</tr>
<tr>
<td>Negative</td>
<td>108 (57.1)</td>
<td>40 (58.0)</td>
<td>148 (57.4)</td>
</tr>
<tr>
<td>Total</td>
<td>189 (100)</td>
<td>69 (100)</td>
<td>258 (100)</td>
</tr>
</tbody>
</table>
Discussion

A lot of research has documented that the implantation of the zygote was comparable to that of cleaved embryo.15,16 Quinn et al.17 reported that the culture conditions used for the fertilized oocytes affected pregnancy rate. That is, in presence of suboptimal culture conditions, pregnancy rates can be increased by earlier transfer. The authors found that if culture environment is optimized, pregnancy rate would be the same after ET on day 1 or 2. Further studies, mentioned that embryos transferred on day 2 were comparable to day 3.3,18,19 However, in these studies, patients with good prognostic factor, fewer embryos were transferred, while for us the number of embryos transferred remained fixed.

In a study done by Laverge et al.,12 to compare pregnancy outcome between days 2 and 3 ET, they observed that the clinical pregnancy and implantation rates were the same between days 2 and 3, but overall embryo quality scores were lower on day 3. Shen et al.4 found that ongoing pregnancy rate were increased and abortion rate were decreased on day 2 ET in cycles with low numbers of embryos in poor responders younger than 40 years. Findings of these studies encourage earlier embryo transfer suggesting that in vivo environment will be healthier for early growing embryo.

Regarding our study, we found no differences in clinical outcomes regarding pregnancy rate and overall embryo quality. While, slow growing or arrested embryos were significantly higher in day 3 group. Bahceci et al.7 found that pregnancy rate per transfer was significantly higher in day 2 vs. day 3 ET for poor responder. A Cochrane meta-analysis failed to prove any improvement in clinical outcome on day 3 transfer. Shifting ET from days 2 to 3, associated with an elevation in clinical pregnancy rates but due to a higher miscarriage rate with the day 3 ET, the live birth rate remained the same. This may be due to unavoidable adverse effects of culture system on early stages of embryonic development causing elevation of miscarriage rate on day 3 transfer.2 However, in another study it was suggested that extended culture to day 3 may lead to selection of best quality embryos for transfer.7

In a retrospective study, Dawson et al.6 showed that the pregnancy rate was insignificantly higher with day 3 ET and on postponing ET from days 2 to 3, 16% of embryos stopped growing. So, waiting until day 3 allowed us to recognize these growth retarded or arrested embryos and avoid their transfer thus lowering miscarriage rate. While certain studies suppose that earlier embryo transfer can rescue less healthy embryos.

In our study, pregnancy rate was the same but slow growing embryos was significantly higher in day 3 group. This confirming Dawson finding and suggesting that extended culture can be used as a non-invasive method to identify embryos with poor developmental potential. Under most ideal conditions, the culture media could exert a negative impact on the developing embryos and led to a higher miscarriage rate with poor embryo quality. In our study, overall embryo quality was identical for both groups, which may be due to the restricted control on culture condition and laboratory environment.

Many studies have shown that in vitro cultured blastocystcs have higher implantation rates, so may be more effective for those who produce enough number of high quality embryos at the cleavage stage, promoting the selection of the top quality embryos11,12 and improving pregnancy rate and offer the opportunity of single embryo transfer.

Dar et al.21 found a considerably elevated risk of preterm delivery (<37 weeks) in singletons following blastocyst culture compared with day 3 transfer. They supposed that extended culture may have adverse effects on the future placentation.

The retrospective design of this study and random population are considered limitations which may have affected the outcomes.

Conclusion

We conclude that the same outcomes was obtained in days 2 and 3 embryo transfer. So we suggest that there is no need to extend in vitro culture an extra 24 h and depending day 2 transfer as a routine instead of traditional day 3 ET. This conclusion also offer to our patients the opportunity to select which day would be suitable for her to do ET.

Further studies are recommended to confirm the influence of earlier embryo transfer on live birth and miscarriage rate in comparison with the extended culture.

Conflicts of Interest

None.

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