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Delayed transfer of embryos from 2 to 3 or 4 days after oocyte retrieval and the pregnancy rate in ICSI

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Abstract

Background: Embryo transfer (ET) has traditionally been performed two days after oocyte retrieval. Delaying transfer from day two to day three or four would allow for further development of the embryo, might therefore optimize the selection of viable and best quality embryos for transfer and may be closer to the physiological time of the entry into the uterus than transfer on day two, and might have a positive effect on pregnancy outcomes.

Objective: The study aimed to determine whether delayed transfer of embryos from 2 to 3 or 4 days after oocyte retrieval and the pregnancy rate in ICSI (intra cytoplasmic sperm injection) procedure.

Materials and Methods: In this descriptive study we evaluated infertile couples who were referred to the Mehr Infertility Institute between 2006 and 2008 for ICSI, according to the day of embryo transfer. We compared embryo quality, pregnancy rate and implantation rate among day 2, 3 or 4 of transfers.

Pregnancy rate was confirmed by measurement of β-hCG in serum after 14 days. After data collection, analysis was carried out with the t-test and chi squared tests by using the statistical software package, SPSS.16.

Results: The overall clinical pregnancy rate (CPR) reported was 46.6%. The mean age of women and duration of infertility didn't differ on the day of embryo transfer ($p>0.05$). Overall CPRs were not statistically different for day 2 (50.3%), day 3 (46.5%) and day 4 (34.8%) transfers respectively, there were no significant differences in the age of transferred embryos between pregnant and nonpregnant women ($p>0.05$).

Conclusion: From the result of the present study there were no statistically significant differences in pregnancy rates according to the day of embryo transfer.

Key words: *Embryo transfer, Transfer day, Pregnancy rate, ICSI.*

Introduction

Embryo transfer (ET) has traditionally been performed two days after oocyte retrieval, when the embryos are at two to four cells (1), because the uterus is supposed to provide the best environment for the survival of the embryo (2). The question of optimal timing for embryo transfer arises when examining the differences between IVF procedures and what happens naturally in vivo. The timing of the arrival of the embryo in the uterus is premature compared with the situation in vivo, where the embryo enters the uterus at the morula stage 4-5 days after ovulation (3). Also, transfer of the embryo on day two is prior to the activation of the embryonic genome which occurs at the four to eight cell stages. Therefore measurement of embryo quality based on the embryonic genome is not possible and this selection is not precise (4). Transfer of a good

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quality embryo(s) is the most important factor for improvement of the implantation rate (5). Developments in culture media have allowed embryos to be maintained in culture for longer periods. Delaying transfer from day two to day three would allow for further development of the embryo, might therefore optimize the selection of viable and best quality embryos for transfer (6-8), and may be closer to the physiological time of uterine entry than transfer on day two.

Some studies have shown that extending the embryo culture period from 2 to 3 or 4 days had no effect on implantation and pregnancy rates (6, 9-12). Some have reported negative effects of day 3 transfer compared to day 2; for example a lower rate of pregnancy and implantation (13) or decrease of overall quality score in embryos which are kept in culture until day 3 (2) have been reported.

Other studies have demonstrated positive effects on implantation and pregnancy rate after transfer on day 3. In a retrospective study the pregnancy and implantation rates were found to be increased after transfer on day 3 (14). Another study concluded that the pregnancy rates were similar for day 2 and day 3 transfers but that the implantation rate in the day 3 group was higher (15).

Based on the above mentioned conflicting results the present descriptive study was designed to evaluate whether extension of the day of transfer from 2 to 3 or 4 days after oocyte retrieval has any effect on the outcome of ICSI-ET.

Materials and methods

Patient population, ovarian stimulation and oocyte retrieval

This study was performed at Mehr Institute of Rasht during a two year period. All patients (n=1028) underwent infertility treatment by intracytoplasmic sperm injection (ICSI). The female partner of the couples included in this study were <37 years old, and all of them were treated using a long protocol. This protocol began with pituitary desensitization using a GnRH agonist (Superfact, Aventispharma, Germany, or Decapeotyle, Ferring, Germany, or Dipherlin) in the midluteal phase of the preceding menstrual cycle. Administration of gonadotrophin (Gonal F, Serono, Switzerland, or Fostimon, IBSA, Lugano) was initiated on day 3 of the commencing cycle. 10000 IU of hCG (Pregnyl, Organon, Holland) was administered to trigger ovulation, when the leading follicle reached 18 mm in diameter and oestradiol concentrations were appropriate. At 35-36 hours after hCG injection a transvaginal ultrasound-guided ovum pickup was performed. Then oocytes were subjected to ICSI.

Semen and oocyte preparation/ ICSI/ embryo culture

All freshly ejaculated semen samples were allowed to liquefy for 25-30 minutes. Sperm count and motility analyses were performed. Then the semen was washed in medium supplement with HSA (5%) by centrifugation at 1600 rpm (round per minute) for 5 min. The sperm that swim up were used for ICSI.

After oocyte retrieval, oocytes were placed in hyaluronidase and the cells of cumulus and corona radiata were removed by gentle aspiration of the cumulus oocyte complexes by a mouth pipette with an inner diameter of 150 μm. The aspirated oocytes were rinsed several times in culture medium. Until the moment of injection, the oocytes were kept in 25μl drops of medium supplement with HSA in a Petri dish under mineral oil (Sigma-Aldrich, Germany) and stored in an incubator containing 5% CO_2 in air at 37˚C. Microinjection was carried out on the heated stage of an inverted microscope. The injected oocyte were then transferred to HFF, each embryo was kept in a 2 μl drop of HFF, under mineral oil and stored in an incubator containing 5% CO_2 air at 37˚C.

Embryo development and morphology

On the day of transfer embryonic development was assessed under the inverted microscope, and the number of blastomeres determined. Each embryo was classified based on its morphological criteria (symmetry and the extent of fragmentation of blastomeres). Briefly, grade A embryos contained unfragmented and equally-sized blastomeres, grade B were those that had slight cellular fragments and/or unequally-sized blastomeres, grade C had at least two or more fragments and unequally-sized blastomeres. Embryos of grade A were classified as excellent quality embryos, embryos of grade B as good quality embryos, and embryos of grade C as poor quality embryos.

Embryo transfer procedure

Embryo transfer was performed the 2nd, 3rd, and 4th day after oocyte retrieval with a Wallace catheter. Although, some reasons like personal problems, long distance to clinic and lack of complete embryo growth resulted in low number of cases in 4th day. The best quality embryos were selected for transfer. As a rule, three embryos were transferred in all patients, if three embryos were available. A number of factors, including the patient's age, cause and history of infertility and the number and grade of the available embryos, were taken into consideration when deciding how many embryos to transfer. Embryos remaining after transfer were cryopreserved in liquid nitrogen. Pregnancy rate was confirmed by measurement of β-hCG in serum after 14 days.

Statistical analysis

After data collection, analysis carried out with t-test and chi squared tests. In an effort to establish the factors associated with the success of ICSI, multivariate analysis was performed based on logistic regression by using the statistical software package, SPSS.16.

Results

During the study period, data from 1028 patients were analyzed. The overall clinical pregnancy rate (CPR) was 46.6% (479/1028 cycles).The mean age of the patients was 31.6±7.7 years old. The mean duration of infertility was 5.7±7.7years.A total of 9808 oocytes was obtained, 8196 (83.5%) mature oocytes were injected. The number of cleaved embryos, embryos transferred and embryo implantation rate were 6747 (82.3%), 3041 (45%) and 578 (19%) respectively. Data showed that the type and cause of infertility on the various days of embryo transfer were not significantly different (p=0.03).The patients who had 3 or more attempts at ART (IVF failure) showed no significant differences in pregnancy rates on 2nd, 3rd and 4th days (p=0.6).Overall CPRs were not significantly different on day 2 (50.3%), day 3 (46.6%) and day 4 (34.8%) transfers respectively. There were no significant differences on the day of transferred embryos between pregnant and nonpregnant women (p=0.1) (Table I). The mean age of women and duration of infertility didn't differ on each day of embryo transfer (p=0.3 and p=0.8).The implantation of embryos did not has statistically significant relation on day of embryos transferred (p=0.4) but the mean number of cleaved embryos and embryo transferred had statistically significant relation on day of embryos transferred (p=0.01).

There were no significant differences in mean number of embryo quality grades (grade A, B, C), among three groups (p=0.1) (Table II). Multiple logistic regressions showed no significant effect of female age, male age, cause and duration of infertility, number of ICSI attempt, day of embryo transfer, mean number of oocyte retrieval ,oocyte metaphase II, embryo cleaved ,embryo transferred and embryo quality in the outcome of IVF/ICSI (p>0.05).

Table I. Distribution of cause of infertility, number of IVF attempts and outcome of embryo transfer on days 2, 3 and 4.

Variable	Day 2	Day 3	Day 4	Total
Patients (%)	159 (15.5)	823 (80.1)	46 (4.5)	1028
Infertility (%)				
Primary	126 (82.4)	671 (84)	40 (88.9)	837 (84)
Secondary	27 (17.6)	128 (16)	5 (11.1)	160 (16)
*Diagnosis (%)				
Male	91 (58.3)	443 (56.3)	24 (52.2)	558 (56.4)
Female	51 (32.7)	246 (31.3)	14 (30.4)	311 (31.4)
Both	9 (5.8)	61 (7.8)	1 (1.4)	71 (7.2)
Unexplained	5 (3.2)	37 (4.7)	7 (15.2)	49 (5)
No. of ICSI cases (%)				
1	113 (72)	565 (69.5)	31 (67.4)	709 (69.8)
2	38 (24.2)	202 (24.8)	14 (30.4)	254 (25)
≥ 3	6 (3.8)	46 (4.5)	1 (0.1)	53 (5.2)
Chemical Pregnancy (%)				
Positive	81(50.9)	403(49)	18(39.1)	502(48.8)
Negative	78(49.1)	420(51)	28(60.9)	526(51.2)
Clinical Pregnancy (%)				
Positive	80(50.3)	383(46.5)	16(34.8)	479(46.6)
Negative	79(49.7)	440(53.3)	30(65.2)	549(53.4)

*p-value < 0.05.

Table II. Age of patients and duration of infertility and quality of embryo and their impact on outcome of embryo transfer on day 2, 3 and 4.

Variable (±SD)	Day 2	Day 3	Day 4	p- value
Female age (years)	31.3±5.6	31.7±5.7	32.7±6.1	p=0.3
Infertility duration (years)	7.5±5.3	7.8±5.4	7.8±5.1	p=0.8
Cleaved Embryos	5.1±3.5	6.1±3.8	5.7±3.5	p=0.01
ET	2.7±0.9	3±0.8	2.9±0.8	p=0.04
Implantation	1.3±0.5	1.3±0.6	1.2±0.7	p =0.4
No. of good embryos				
A	2.7±0.8	2.7±0.8	2.5±0.8	p=0.1
B	1.2±0.4	1.2±0.5	1.2±0.4	p=0.1
C	2	1.2±0.4	-	p=0.1

Discussion

Our results showed that clinical pregnancy rates on days 2, 3 and 4 ET were not significantly different in 1028 cycles.

Data showed that the patients' ages, duration of infertility, number of previous ART attempts, mean number of oocytes retrieved at MII, cleavage rates and implantation rates were also similar for each group.

Several studies comparing embryo transfer on day 2 versus day 3 after oocyte retrieval have been performed but the conclusions conflict. Ashrafi *et al* indicated that although the pregnancy rate was slightly higher after transfer on day 3 than on day two, these differences were not significantly different (11). These results are in agreement with the findings of Ertzeid *et al* (16), and Dawson *et al* (15), in which the pregnancy rates following transfer on day 3 were higher, but not significantly higher than day 2 transfer. Aboulghar et al showed that there is no significant difference in the pregnancy rate between ET on day 2 (50.9%) and ET on day 3 (50.5%). They concluded that embryo transfer could be done on days 2 or 3 according to the convenience of the patient and the medical team (17).

Goto *et al* compared day 2 *versus* day 4 and 5 embryo transfer and reported that the pregnancy rate per ET in 307 patients on Days 2 to 4 were not significantly different, whereas Day 5 ET produced a significantly lower pregnancy rate (Day 2, 29.6%; Day 3, 32.9%; Day 4, 30.4%; Day 5, 10.7%). Their results suggested that the day of ET does not fundamentally affect the pregnancy rate in human IVF-ET provided that transfer is made before Day 5 (12). Some studies have reported more positive effects of embryo transfer on day 3 than day 2. Carillo *et al* reported that the pregnancy and implantation rates increased after transfer on day 3 (14).

Patients with a large number of oocytes were selected for embryo transfer on day 3. It should be mentioned that glucose- and phosphate-free media were used, which is different from the culture condition in all other studies. Bahceci *et al* showed that the clinical pregnancy rates per embryo transfer were significantly higher in the day 2 embryo transfer group compared with day 3 (38.9% vs. 24.1% respectively p<0.05) (18). In the present study, there was no statistically significant difference between day 2, 3 and day 4 of embryo transfers and the implantation and pregnancy rates in any of the categories analyzed.

Although there were not any statistically significant differences in pregnancy rate according to the day of embryo transfer, the pregnancy rate after day 4 of embryo transfer was less than that of days 2 and 3. We think that it is related to the embryo nutrient requirement. It is believed that gene expression of human embryos is switched on around the 8-cell stage immediately before compaction (4).

Therefore, nutrient requirements of embryos are different after this stage. Early embryos can grow in a simple salt solution, whereas they require more complex media after they reach the 8-cell stage. These changes also correspond to environmental changes *in vivo* since the embryo reaches the uterus from the Fallopian tube at the stage when compaction begins. It is better that it should be in a physiological environment. Since we did not change the culture media on day 3, it seems that we may not have achieved a better outcome with day 4 embryo transfer, but this would need to be demonstrated.

Conclusion

As a result of the present study there were not any statistically significant differences in pregnancy rates according to the day of embryo transfer. The current study is based on existence data; further studies with larger sample sizes are needed.

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