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Original Investigation

Effect of Embryo Transfer on Days 2 and 3 on Pregnancy Rates in Patients Undergoing Controlled Ovarian Hyperstimulation/Intracytoplasmic Sperm Injection

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Abstract

Objective: The aim of this study is to investigate the effect of embryo transfer of days 2 and 3 on pregnancy rates in patients undergoing controlled ovarian hyperstimulation / intracytoplasmic sperm injection.

Methods: Individuals admitted to our clinic due to of male factor infertility between February 2006 and May 2010 and who underwent ICSI with the antagonist protocol were included in this study. Pregnancy rates between days 2 and 3 after embryo transfer were compared.

Results: Embryo qualities were similar between groups (p=0.828). Although pregnancy rates tended to be higher in the 2-day transfer group, it was not statistically significant (p=0.18). Azoospermia and the number of retrieved oocytes were predictive factors for a favorable outcome (p=0.001).

Conclusion: Retaining the embryos in culture for 3 days had no negative influence on pregnancy outcomes. Embryo transfer may be performed either on day 2 or 3 according to the work program of the clinic.

Keywords: Embryo transfer, pregnancy rate, controlled ovarian hyperstimulation

Introduction

Since the introduction of this technique, many steps of in vitro fertilization (IVF), which is an effective method for the treatment of infertility, have been standardized. However, the optimum time for embryo transfer (ET) is still controversial.

During the initial periods of IVF, Edwards et al. (1) performed ET 3 or 4 days after oocyte retrieval at the 8–16-cell stage (1). ET was then performed on the 2nd day at the 4-cell stage in many hospitals (2). During the past decade, extending embryo culture to 5–6 days has become a part of routine IVF in some clinics (3).

ET is generally performed 2 or 6 days after oocyte retrieval (4). The embryos that are transferred on the 2nd day are selected from those that completed embryonic genome activation at the 4–8-cell stage (5). Embryo quality measurement does not indicate that it is a normal embryo genome. Embryo selection becomes difficult when there are more than three embryos that can be transferred. A prolonged culture period allows embryo development for embryonic genome activation (blastocyst stage). This helps in the selection of suitable embryos for transfer and also acts in a positive way on pregnancy outcomes (6, 7).

Embryos whose development failed during ETs can be sorted out on the 3rd day and embryos that are more suitable for transfer can be selected (8). However, being exposed to the culture medium longer than that may cause embryo loss. For patients who failed to achieve pregnancy on the 2nd day, performing ET on the 3rd day might be helpful (9). The purpose of this study is to compare pregnancy outcomes between ET days 2 and 3.

Methods

Intracytoplasmic sperm injection/ET (ICSI/ET) cycles that were performed in our clinic between February 2006 and May 2010 were retrospectively evaluated. Only patients with an etiology of male factor and in which the female is younger than 40 years were included in the study. The patients with short uterine pathologies and autoimmune diseases and who were treated with short protocol, long protocol and microdose agonist protocols were excluded from the study. Only patients who had been undergoing antagonist protocols were included in the study. Ninety-eight patients who were in conformity with these criteria were included in the study.

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The cycles in which ET was performed on the 2nd and 3rd days were compared. Blastocyst transfers were not included in the study because ET was not performed at the clinic where the study was conducted on the 5th day during the aforementioned time. ET was performed on the 2nd day in patients who had low fertilization after OPU and for patients whose embryo selection could be performed on the 2nd day.

Grade 1, grade2, grade 3 embryos were selected on day 2 and day 3 (10). Two-Six cell stage embryos were transferred on day 2 and 6–10 cell stage embryos were transferred on day 3.

Following ET, patients who had serum β-human chorionic gonadotropin measurements after 12–14 days and those who had positive results were called for an ultrasonographic examination after 2 weeks. Patients whose ultrasonography findings showed gestational sac and fetal heart beat were included in the study.

Statistical analysis

Normality test was conducted by means of Shapiro–Wilk test and histogram charts. Data were presented in the form of mean, standard deviation, median, interquartile range (IQR), frequency, and percentage. Pairwise comparisons were conducted with t test, Mann–Whitney U test (for non-normally distributed variables), chi-square, and Fisher's exact test in independent groups. Analyses were performed using SPSS 17.0 software. The limit of significance was taken as $p<0.05$. Logistic regression analysis was performed to determine the risk factors affecting pregnancy (SPSS 17.0 Chicago, United States).

Ovarian stimulation and oocyte retrieval

In the antagonist protocol, while ovarian stimulation was continued with gonadotropins and dominant follicle size was identified as >11–14 mm, an antagonist (Orgalutran flacon 0 Organon, Istanbul, Turkey) of gonadotropin-releasing hormone (GnRH) 1×1 was subcutaneously started.

The GnRH antagonist were administered when the leading follicle reached to 11 mm dimension and used until to final triggering. 10,000 IU hCG was administered intramuscularly as soon as the leading follicle reached a mean diameter of 18 mm. Oocyte pick-up (OPU) procedure was carried out 36 hours after hCG injection by transvaginal ultrasound guided needle aspiration under general anesthesia.

Oocyte and Embryo Culture

Oocytes that were coated with Ovoil (Vitrolife Sweden AB, Gothenburg, Sweden), added with human serum albumin (HSA; Vitrolife, Gothenburg, Sweden) and collected in G-MOPS (Vitrolife, Gothenburg, Sweden) medium, were taken into HSA-added G-IVF medium (Vitrolife Gothenburg, Sweden) and put into incubators that were adjusted to 6% CO^2 at 37°C at the end of OPU. Two hours after OPU, granulosa cells were cleared in the G-MOPS medium that contained 80 IU/ mL of hyaluronidase. The oocytes were washed in the G-MOPS medium, and put back into the G-IVF medium and put into the incubator. Four hours after OPU, the mature oocytes on which ICSI was performed were coated with Ovoil, put into HSA-added G-1 medium droplets (Vitrolife, Gothenburg, Sweden), and placed into incubators that were adjusted to 6% CO^2 at 37°C. Eighteen hours after ICSI, fertilization control was conducted under an inverted microscope (Olympus IX71). During the fertilization control, the presence of two pronuclei in the zygote was taken into account. On the 2nd day, embryo control was conducted 24 h after the fertilization control, and on the 3rd day, the embryo control was conducted 48 h later. Prior to the fertilization and embryo controls on the 2nd day, the embryos were put into new G-1 medium droplets; on the 3rd day before the embryo control, the embryos were put into G-2 medium droplets (Vitrolife, Gothenburg, Sweden) and evaluated (11). Embryos exhibiting the best morphology and development in accordance with their stage were selected and transferred. One to three ETs were in accordance with patient's clinical symptoms.

Embryo morphology

The embryos were classified according to the number of cells on the morning of the transfer day and the symmetry of the blastomeres with the percentage of fragmentation. First quality (very good quality) embryos did not have fragmentation and had equal-sized blastomeres. Second quality (good quality) embryos had 1–20% of fragmentation and/or had blastomeres of unequal size. Third quality (medium quality) embryos had 21–50% of fragmentation and contained unevenly sized blastomeres (10).

Symptoms

A total of 98 patients (48 patients had ET on the 2nd day, 50 patients had ET on the 3rd day) were included in the study. For quantitative variables, normality and Shapiro–Wilk test were conducted, and leaf and steam and histogram charts were drawn. The comparison of normally distributed variables (age, number of oocytes, etc.) was performed using the t test for independent groups. Non-normally distributed variables (duration of infertility, number of transfers) were compared using the Mann–Whitney test. Categorical variables were evaluated with the chi-square and Fisher's exact tests. To determine the factors that influence pregnancy outcome, logistic regression analysis was performed. The clinical characteristics of the patients are shown in Table 1.

The number of oocytes collected, average age, duration of infertility, number of embryos transferred, and dose of drug were compared in both groups. Among the patient groups that had transfer on the 2nd and 3rd days, when the number of transferred embryos, age, number of oocytes collected, and embryo quality were compared, no significant difference was detected. However, the duration of infertility was found to be longer in the group that had ET on the 3rd day ($p=0.029$). When ET on the 2nd and 3rd days and the pregnancy rates were compared, the pregnancy rate was found to be higher in the group that had 2nd day transfer, but no statistical significance was present ($p=0.108$). There was no difference in terms of the quality of embryos transferred between the two groups (Table 2).

In patients who had male factor, azoospermia was evaluated as a subgroup. There was no statistical difference between the two groups in terms of distribution ($p=0.374$) (Table 3). It was observed that factors such as patient's age, duration of infertility, embryo quality, and number of embryos transferred did not have any effect on conceiving. P-values were respectively found to be $p=0.319$, $p=0.96$, $p=0.83$, and $p=0.14$.

We determined number of oocytes ($p=0.001$) and azoospermia as the factors affecting pregnancy ($p=0.04$) (Table 4). However, there

Table 1. Comparison of the clinical features of the 2nd and 3rd day embryo transfer patients

Transfer day	2nd day ET	3rd day ET	p-value
Duration of infertility	4 (1–21)	6 (2–21)	0.029*
Number of collected oocytes	8 (1–16)	10 (0–20)	0.21
Number of transferred embryos	3 (2–3)	3 (2-3)	0.42
Age	30 (18–40)	30 (18–40)	0.055
Used drug dose	300 (150475)	300 (225–450)	0.86
Azoospermia	8.3%	14%	0.37
Clinical pregnancy rates	47.9%	32%	0.10

ET: embryo transfer
*: p<0.05 statistical significance

Table 2. Distribution of embryo transfer days and embryo quality

Embryo quality	2nd day ET	3rd day ET	p-value
Grade 1	33 (68.8%)	27 (54%)	0.054
Grade 2	13 (27.1%)	23 (46%)	
Grade 3	2 (4.2%)	0	

ET: embryo transfer

Table 3. Transfer day and the distribution of indications

		Indications		
		Azoospermia	Male factor	p-value
Embryo transfer day	2	4	44	0.374
	3	7	43	
Total number		11	87	

Table 4. Distribution of the factors that affect pregnancy

		Pregnancy		
		Yes	No	p-value
Embryo transfer day	2	25 (42.4%)	23 (59%)	0.108
	3	34 (57.4%)	16 (41%)	
Embryo quality	1	35 (59.3%)	25 (64.1%)	0.78
	2	23 (39%)	13 (33.3%)	
	3	1 (1.7%)	1 (2.6%)	
Infertility duration	5 (1–21)	5 (1–15)	0.96	
Used drug dose	300 (150–475)	300 (225–450)	0.90	
Number of collected oocytes	7 (0–20)	11 (1–18)	0.001*	
Age	30±5.9	28±4.9	0.319	
Azoospermia	10 (16.9%)	1 (2.6%)	0.04*	

*: p<0.05 statistical significance

was no significant difference between the two groups in terms of the azoospermia distribution rate (p=0.374).

Discussion

In this study, we included 98 patients who had male factor infertility and who underwent the antagonist protocol. Following OPU, we investigated the effects of ETs that had been performed on the 2nd and 3rd days on pregnancy rates. Despite finding higher pregnancy rates on the 3rd day transfer, Ashraf et al. (9), Edwards et al. (12), and Dawson et al. (8) did not detect a statistically significant difference. In their comprehensive retrospective study that examined the 2nd, 3rd, and 4th days ETs, Huisman et al. (13) investigated implantation and pregnancy outcomes, and they found similar results in all three groups. They also did not observe statistically significant differences in the 2nd and 3rd days ETs in terms of pregnancy rates in other prospective studies (6, 14, 15).

Carrillo et al. (16) compared the 2nd and 3rd day ETs and detected significantly higher pregnancy rates on the 3rd day of transfer (44% against 26%). However, in this study, only patients with a high number of oocytes were included. Similar results were obtained in other studies (17, 18). In our study, when the 2nd and 3rd day ETs and pregnancy rates were compared, although the 2nd day transfer was found to be higher in in terms of pregnancy rates, there was no statistically significant difference. The 3rd day transfer having a lower pregnancy rate might be caused by the longer duration of infertility of these patients. However, in our study we found that pregnancy is not affected by the duration of infertility.

Compared to 2-day embryos, embryos to be transferred to the uterus on day 3 are closer to the physiological timing. Apart from this, extending the duration of ET allows the selection of more suitable embryos for the transfer (14).

Lavergne et al. (14) found that the number of good quality embryos on the 2nd day was greater in number than on the 3rd day. However, this did not affect pregnancy rates. In our study, the number of very good quality embryos we found on day 2 was 33 (68.8%) and on day 3 was 27 (54%), and there was no statistical difference in both groups in terms of distribution.

Keeping embryos in in vitro culture for 24 h more may allow the elimination of embryos whose development stopped and those with poor quality (19).

The weak points of this study are it being a retrospective study, the small number of patients, and the inability to perform ET on the 5th and 6th days at the time of this study.

Conclusion

There was no difference detected between the pregnancy rates on the 2nd and 3rd day ETs. These findings show that it is safe to plan ET when the patient and clinic are suitable. In our study, although there was no statistically significant difference between the patients who underwent 2nd or 3rd day ETs in terms of pregnancy rates, having a small number of patients, our study being a retrospective one, and not including the blastocyst transfer days affected the power of the study. Randomized controlled trials with extensive sampling are needed to resolve this issue.

Ethic Committee Approval: Ethics committee approval was not received due to the retrospective nature of this study.

Informed Consent: Written informed consent was not obtained from patients due to the retrospective nature of this study.

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