Original Article
http://mjiri.iums.ac.ir

Medical Journal of the Islamic Republic of Iran (MJIRI)
Iran University of Medical Sciences

Embryo transfer in days 2 to 4 following intracytoplasmic sperm injection: a prospective cohort study

Atossa Mahdavi*[1], Ali Qashqaei[2], Ashraf Aleyasin[3], Marzieh Aghahosseini[4]
Leili Safdarian[5], Zahra Rezaeean[6], Parvin Fallahi[7]

Received: 9 January 2015 Accepted: 5 May 2015 Published: 12 September 2015

Abstract

Background: Improvement of assisted reproductive technique (ART) results in higher pregnancy rates from positive Beta HCG to take home baby statistics. Despite developments in culture media allowing blastocyst stage transfer, some centers apply second, third and sometimes fourth day post injection for embryo transfer. This study aimed to compare their reproductive outcomes.

Methods: This prospective cohort study conducted on 218 infertile couples with at least 4 oocytes retrieved and 2 good quality embryos. They were divided consecutively into 2nd (ET2) or 3rd (ET3) day embryo transfer. Some patients experienced 4rd (ET4) day embryo transfer due to weekend reasons, so we included them in our comparison as well. There were 98, 97 and 23 patients in the aforementioned groups, respectively. Reproductive and pregnancy outcomes were evaluated by Chi square and t-test with the significance level set at α=0.05.

Results: Totally, 73 patients (33%) had positive beta HCG and 39.7 percent of them (n=29) experienced pregnancy loss. Positive Beta HCG was detected in 31(31.6%) of ET2 patients, 38 (39.2%) of ET3 patients and 4 (17.4%) of ET4 group. Abortion or pregnancy loss was reported in 9 (29%) of ET2 patients, 18 (47.4%) of ET3 patients and 2 (50%) of ET4 group.

Conclusion: Our study demonstrated that there may be a higher pregnancy as well as higher abortion in day 3 embryo transfer.

Keywords: Infertility, Pregnancy, Intracytoplasmic sperm injection, Embryo transfer, assisted reproductive technology.

Cite this article as: Mahdavi A, Qashqaei A, Aleyasin A, Aghahosseini M, Safdarian L, Rezaeean Z, Fallahi P. Embryo transfer in days 2 to 4 following intracytoplasmic sperm injection: a prospective cohort study. *Med J Islam Repub Iran* 2015 (12 September). Vol. 29:262.

Introduction

Improvement of assisted reproductive technique (ART) has resulted in higher pregnancy rates. Achievement of oocytes with the best quality and subsequently the best embryos for uterine transfer, higher pregnancy rates from positive Beta HCG to take home baby statistics, remains the utmost concern of the all related specialists. One of the highlights of the process has been the estimation of the proper time for embryo transfer. Historically, at the first days of ART, embryos are transferred at the 8-16 cell stage (1), however, developments in culture media have allowed embryos to be transferred after longer periods of in vitro culture as in blastocyst stage (2). Despite improved laboratory and technical facilities and among different conclusions about the best time for embryo transfer more good quality trials are warranted in this regard to have a confident decision

[1]. (**Corresponding author**) Assistant Professor, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran. atossa_mahdavi@yahoo.com, at-mahdavi@tums.ac.ir
[2]. Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran. qashqaeia@yahoo.com
[3]. Professor, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran. ivf.shariti69@gmail.com
[4]. Professor. Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran. ivf.shariti69@gmail.com
[5]. Assciate Professor, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran. safdaria@tums.ac.ir
[6]. Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran. zahrarezaeean@yahoo.com
[7]. Embryologist, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran. parvin.fallahi@yahoo.com

without any conflicting ideas. This matters especially when the blastocyst transfer is not possible due to different reasons. Second day is the earliest time that morphological staging is possible for better selection of an embryo. Transfer of embryos prior to the activation of the embryonic genome (at the four to eight cell stages) decreases the precision of embryo selection (3). On the other hand, day 3 embryo transfer may eliminate arrested embryos and increases the desirable ART results (4). But this conclusion does not come true for all studies or at least for some categories of patients. There exists some studies supporting day 3 embryo transfer (5,6) and the others are in favor of day 2 (7,8). In a study among poor responder patients, clinical pregnancy rate per oocyte retrieval and embryo transfer were significantly higher in the day 2 embryo transfer group compared with day 3 (9). However; there is articles stating no significant difference exists between day 2 and 3 embryo transfer (10-12). According to a Cochrane review article, embryo transfer on day 3 showed more clinical pregnancies, but miscarriage rate was also increased, proving a lesser effect of one day delay in overall live birth rate (13). This review urged us to evaluate our outcomes in a university ART setting. As the most important issue for an infertile couple is having a live birth, the search for a method with minimum expense and maximum efficacy is mandatory. So, we conducted the following study to compare the results of different days of embryo transfer.

Methods

This prospective trial was conducted at the department of infertility, Shariati Hospital affiliated to Tehran University of Medical Sciences. The study was approved by the ethical committee of the university. In total, 218 infertile women undergoing ART with 4 or more oocytes and transfer cycles with at least two good quality embryos were included. All causes of infertility (except for uterine factor) were included. Also, couples requiring testicular sperm extraction and aspiration for sperm recovery and gamete or embryo donations, women aging more than 40 years or body mass index of 35kg/m^2 or those with systemic diseases or positive thrombophilia were excluded. For all the couples history was taken, gynecological examination plus semen analysis, hormonal profile, and transvaginal ultrasound were performed and documented. Standard long protocol of down regulation and standard protocol of ovarian stimulation individualized for each patient, and final oocyte maturation was commenced (14). After fertilization through intracytoplasmic sperm injection, good-quality embryos were transferred transcervically 2 or 3 days later. Good-quality embryos were defined based on standard embryologic definition (15,16). The day of Embryo Transfer (ET) was assigned according to computerized random allocation, but sometimes due to holidays or weekends it was not possible to transfer embryos at second or third day, so it was conducted on day 4. Because all of these ET timings were used routinely in our department depending on the situations, there existed no ethical concerned problem. On the other hand, we gathered information of the last group in order to have a comparison between our results in different days in our ward. Due to this reason, we could not claim our study as a well randomized study. Culture media and luteal phase support and other medications was similar for all patients and luteal phase support was started the day after ovum pick up by daily administration of the progesterone suppository according to our protocol.

It was emphasized that the culture media be the same for all embryos and our laboratory and embryologic measures were conducted by the same embryologists. Embryo quality determination was done based on standard embryologic definitions (17,18). Moreover, all medical prescriptions, surgical procedures including embryo transfers (performed by the catheters from the same company) and ultrasound scans were performed by attending physicians in prede-

fined protocols.

Pregnancy was detected by serum beta-hCG analysis 14 days after embryo transfer, and a transvaginal ultrasound scan was scheduled for 2 weeks later to detect the intra-uterine gestation sac. Each pregnant woman was followed by ultrasound scan until detection of fetal heart (clinical pregnancy), then ongoing pregnancy and pregnancy outcome were followed. Basal characteristics, stimulation parameters and ART results were gathered and reported.

Statistical Analysis

The sample size was calculated to be 190 participants with a power of 80% and a level of significance of 0.05. Statistical Package for the Social Sciences Version 12 (SPSS, Chicago, IL, USA) was used for data analysis. The chi square and t-test were performed. The significance level was set at α=0.05.

Results

The groups were well matched in terms of female and male age, infertility duration, type and cause of infertility, female body mass index, gravidity, basal gonadotropin level of female partner and total motile good sperm number (Table 1). As Table 2 shows, stimulation parameters of the patients were comparable in second day embryo transfer (ET2), third day embryo transfer (ET3) and fourth day embryo transfer (ET4). Totally 73 patients out of 218 participants had positive beta HCG and 39.7 percent of them experienced pregnancy loss.

Comparing treatment outcomes in the three groups, chemical pregnancy was detected in 31(31.6%) of ET2 patients, 38 (39.2%) of ET3 patients and 4 (17.4%) of ET4 group (Table 3). Abortion or pregnancy loss was reported in 9(29%) of ET2 patients, 18 (47.4%) of ET3 patients and 2 (50%) of ET4 group. Interestingly, these

Table 1. Ages and body mass indices (data presented as Mean±SD, basal characteristics of the participants

	Day 2 N=98	Day 3 N=97	Day 4 N=23	Total N=218	p
Female age (year)	29.8 ± 6.14	30.3± 5.3	31.4 ± 4.5	30.4 ± 5.27	0.456
Male age (year)	34.6 ± 6.08	35.2 ± 5.2	36.34± 4.2	35.20± 5.58	0.487
Female body mass index (kg/m2)	25.4 ± 3.5	25.6 ± 3.4	26.2± 3.8	25.6 ± 3.54	0.641
Infertility duration (year)	5.5 (1-20)	6 (1-18)	7 (2-20)	6 (2-20)	0.165
Infertility type	75	64	15	154	0.441
Primarsecondary	18	19	8	44	
Infertility cause					0.553
Aging	2	2	0	4	
Ovulation	4	4	1	9	
Unexplained	16	17	2	35	
Pcos	8	4	3	15	
Endometriosis	2	4	1	7	
Tubal	3	4	3	10	
Male	31	31	6	68	
Mixed	18	10	7	35	
Gravidity					0.136
0	70	67	15	152	
1	20	20	5	45	
2	7	4	1	12	
3	0	2	1	3	
4	0	0	1	1	
Antral follicle count	12 (4-20)	13.5 (4-20)	13.5 (5-20)	13 (4-20)	0.938
Follicle-stimulating hormone (mIU/ml) on 3rd day of cycle	7.0 (0.5-20)	6.4 (2-19)	6.9 (1-17.5)	6.7 (0.5-20)	0.812
Luteinizing hormone (mIU/ml) on 3rd day of cycle	5.0 (0.5-20.4)	5.5 (1.2-27)	6.5 (1.5-18.7)	5.0 (0.5-27)	0.400
Estradiol(pg/ml) on 3rd day of cycle	48.5 (3-173)	60.5 (5-188)	52.5 (17-132)	51(3-188)	0.728
Total good Sperm (Million) per ejaculate	4.1 (0.01-80)	6.8 (0.1-60)	4 (0.03-34)	5.9 (0.1-80)	0.474

Data presented as Mean (±SD) or Median (range) or frequency.

Table 2. Stimulation parameters of the patients

Parameter	Day 2 N=98	Day 3 N=97	Day4 N=23	Total N=218	p
Stimulation days	11 (10-15)	11 (10-17)	11 (10-14)	11 (10-17)	0.415
Gonadotropin injections (ampoule numbers)	36 (10-92)	38 (14-104)	29 (14-84)	36 (10-104)	0.507
Total rFSH (gonal F)	14 (0-56)	14 (0-33)	14 (0-56)	14 (0-56)	0.339
Total HMG	23.5 ± 14.9	22.9 ± 12.7	20.8 ± 13.9	22.9 ± 13.9	0.706
Estradiol on HCG day (pg/ml)	1607 (115-45000)	1879 (657-18440)	1357 (498-26600)	1777 (115-45000)	0.688
Total oocytes retrieved	11.9 ± 5.9	10.9 ± 5.1	11.3 ± 4.9	11.4 ± 5.4	0.463
Mature oocytes	7.3 ± 3.8	7.1 ± 3.9	7.2 ± 3.5	7.2 ± 3.8	0.937
Pronuclear number	4 (1-14)	5 (1-15)	13 (2-4)	5 (1-15)	0.675
Number of transferred embryos	3 (2-5)	4 (2-5)	3 (2-5)	4 (2-5)	0.087
Ovarian hyperstimulation syndrome	0	1	0	1	0.531
Having frozen embryos (%)	36.7	25.8	30.4	31.5	0.255

Data presented as Mean (±SD) or Median (range) or frequency.
Chi square and t-test were applied.

Table 3.Comparison of outcomes

Parameter	Day 2 N=98	Day3 N=97	Day 4 N=23	Total N=218	p
Chemical pregnancy	31(31.6)	38 (39.2)	4 (17.4)	73 (33.5)	0.12
Clinical pregnancy	20 (20)	18 (18)	1(4)	39(18)	0.218
Ongoing pregnancy	17 (17)	15(15.4)	1(4)	33(15)	0.299
Abortion	9(29)	18(47.4)	2(50)	29(39.7)	0.306
Ectopic pregnancy	2(6.5)	2 (5)	1(25)	5(6.8)	0.291
Premature labor	1(3.2)	1(2.5)	0	2(2.7)	0.888
Term	15(48.4)	12(31.6)	1(25)	28 (38.4)	0.362
Multiple pregnancy	3(9.7)	3(7.5)	1(25)	7(9.6)	0.948

Values are presented as frequency and percent. Chi square was applied.
Chemical, clinical and ongoing pregnancy rates are calculated per embryo transfer.

results beside clinical and ongoing pregnancies did not differ significantly. Fertilization rate and implantation rate did not differ statistically between the aforementioned groups. Fertilization rate was 70%, 78% and 25% in ET2, ET3 and ET4 groups, respectively. It was 68 percent in all the patients. Implantation rate did not differ significantly between groups as well. Orderly ongoing pregnancy were reported in 17% in ET2, 15.4% in ET3, 4% in ET4, respectively. There were only seven sets of twins in our study groups.

Discussion

Our study demonstrated that there may be a higher pregnancy as well as a higher abortion in day 3 embryo transfer. Although not statistically significant, it was in accordance with previous articles (10-3,19). Although this study aimed to evaluate the effect of timing of embryo transfer on implantation and subsequently on pregnancy, there is a wide range of important factors affecting pregnancy occurrence including stimulation response, oocyte maturity, embryo morphology or quality, uterine milieu or endometrial receptivity, and culture conditions.

It is documented that switching of human embryos gene expression happens around the 8-cell stage immediately before compaction stage (3). One important factor that should be considered is nutrient requirements of the embryo, since the embryo reaches the uterus from the Fallopian tube at the stage when compaction begins. We did not find the reason why pregnancy loss may be higher in third day embryo transfer. Early uterine replacement may be advantageous for the embryos because of the shorter in vitro time.

In a large retrospective study by Huisman et al., comparing IVF results after day 2, 3 and 4 of ET, the implantation rate on day 4 was higher (41%). It seemed that transfer on day 4 may help to recognize embryos with very high implantation potential (20). However our study was not designed for day 4 and we could not make a definite

conclusion in this regard.

Our study had some limitations including its randomized structure. Blindness was not any matter of concern because day 2 or 3 or 4 was routine in our department and neither the researchers nor the patients had any weighted idea about the day of embryo transfer. We calculated our sample size according to the fact that the abortion was higher in the day 3 ET in comparison with day 2 ET by 15 percents. Prospective nature of the study, comparable basal and stimulation parameters, considering the ET4 group, and follow up until delivery were the positive points of our study. Although the study was not designed for ET4 group, we considered them for a raw estimation in our department.

Our findings, in accordance with previous results, revealed no difference between day 2 and 3 embryo transfer (10-12). We strongly recommend considering individualization of proper embryo transfer timing for different group of patients such as normal patients, poor responders, recurrent abortion, and patients with polycystic ovary disease. Maybe embryo transfer should not be done upon convenience of the patients and the medical team. So, future prospective especially randomized clinical trials in a multicenter design with large sample sizes considering especial group of patients are strongly warranted.

Acknowledgements

The authors would like to thank the patients as well as staff of Infertility department of Shariati Hospital for all their help and concern.

Conflict of Interest

There exists no conflict of interest to declare.

References

1. Edwards RG, Steptoe PC, Purdy JM. Establishing full-term human pregnancies using cleaving embryos grown in vitro. Br J Obstet Gynecol 1980;87:737.

2. Gardner DK, Vella P, Lane M, Wagley T, Schlenker T, Schoolcraft WB. Culture and transfer of human blastocyst increases implantation rates and reduces the need for multiple embryo transfer. Fertil Steril 1998;69:84-88.

3. Braude P, Bolton V, Moore S. Human gene expression first occurs between the four and eight cell stages of preimplantation development. Nature 1988; 332: 458-461.

4. Dawson KJ, Conaghan J, Ostera GR, Winston ML R, Hardy K. Delaying transfer to the third post-insemination, to select non-arrested embryos, increases development to the fetal heart stage. Hum Reprod 1995; 10: 177-182.

5. Baruffi RL, Mauri AL, Petersen C, Felipe V, Franco Júnior JG. Day 2 vs. day 3 embryo transfer after intracytoplasmic sperm injection. A prospective, randomized study. J Reprod Med 2003; 48(8): 631-634.

6. Carillo AJ, Lane B, Pridman DD, Risch PP, Pool TB, Silverman IH, et al. Improved clinical outcomes for in vitro fertilization with delay of embryo transfer from 48 to 72 hours after oocyte retrieval: use of glucose-and phosphate-free media. Fertil Steril 1998; 69:329-334.

7. Lundin K, Bergh C, Hardarson T. Early embryo cleavage is a strong indicator of embryo quality in human IVF. Hum Reprod 2001; 16(12): 2652-2657.

8. Laverge H, De Sutter P, Van der Elst J, Dhont M. A prospective, randomized studycomparing day 2 and day 3 embryo transfer in human IVF. Hum Reprod 2001; 16(3):476-480.

9. Bahceci M, Ulug U, Nadir Ciray H, Akman MA, Firat Erden H. Efficiency of changing the embryo transfer time from day 3 to day 2 among women with poor ovarian response: A prospective randomized trial. Fertility and Sterility 2006;86(1):81-85.

10. Ertzeid G, Dale PO, Tanbo T, Storeng R, Kjekshus E, Abyholm T. Clinical outcome of day 2 versus day 3 embryo transfer using serum-free culture media: A prospective randomized study. J Assist Reprod Genet 1999; 16(10): 529-534.

11. Baruffi RL, Mauri AL, Petersen C, Felipe V, Franco Júnior JG. Day 2 vs. day 3 embryo transfer after intracytoplasmic sperm injection. A prospective, randomized study. J Reprod Med 2003; 48(8): 631-634.

12. Ashrafi M, Kiani K, Mirzaagha M, Shabani F. The Pregnancy Outcomes of Day 2 versus Day 3 Embryo Transfer: A Cross-Sectional Study. Iranian Journal of Fertility and Sterility 2007;1(2): 47-54.

13. Gunby JL, Daya S, Olive D, Brown J. Day three versus day two embryo transfer following in vitro fertilization or intracytoplasmic sperm injection (Review). 2009 The Cochrane Collaboration. Published by JohnWiley & Sons, Ltd.

14. Aleyasin A, Agha Hosseini M, Mahdavi M, Safdarian L, Fallahi P, Mohajeri MR, et al. Predictive value of the level of vitamin D in follicular fluid

on the outcome of assisted reproductive technology. European Journal of Obstetrics & Gynecology and Reproductive Biology 2011;159:132–137.

15. Sakkas D, Gardner DK. Evaluation of embryo quality: New strategies to facilitate single embryo transfer. In: Gardner DK, Weissman A, Howles CM, Shoham Z (eds). Textbook of Assisted Reproductive Technologies, Laboratory and Clinical Perspectives, 3rd edn. London: Informa Health Care. 2009;243–247.

16. Rao AK. Laboratory Manual in Assisted Reproductive Technology, 1st edn. New Delhi: Jaypee, 2006.

17. Sakkas D, Gardner DK. Evaluation of embryo quality: new strategies to facilitate single embryo transfer.In: Gardner DK, Weissman A, M Howles C, Shoham Z (eds) Textbook of assisted reproductive technologies, laboratory and clinical perspectives, 3rd edn. London: Informa health care, 2009; 243-247.

18. Rao AK. Laboratory manual in assisted reproductive technology, 1st edn. New delhi: Jaypee, 2006.

19. Aboulghar MM, Aboulghar MA, Mansour RT, Serour GI, Amin YM, Abou-Setta AM. Pregnancy rate is not improved by delaying embryo transfer from days 2 to 3. Eur J Obstet Gynecol Reprod Biol 2003; 107: 176-179.

20. Huisman GJ, Alberda AT, Leerentveld RA, Verhoeff A, Zeilmaker GH. A comparison of in vitro fertilization results after embryo transfer after 2, 3, and 4 days of embryo culture. Fertil Steril 1994; 61: 970-971.