ORIGINAL ARTICLE

Implantation Rates after Two and Five Days of Embryo Culture: A Comparative Study

Prabhleen Kaur, M.L.Swarankar, Manju Maheshwari, Seema Sharma

Abstract

The aim of our study was to compare the Implantation rates of embryos transferred after two and five days of culture. A Randomized, prospective study was conducted in Infertility clinic, Department of Obstetrics & Gynecology,Mahatma Gandhi Hospital, Jaipur on 300 patients aged 25-40 years undergoing in-vitro fertilization (IVF)/ intra-cytoplasmic sperm injection (ICSI) cycle from May 2010-April 2011. When three or more Grade-I embryos were observed on day 2 of culture, patients were divided randomly into two study groups, day 3 transfer group and blastocyst transfer group or day 5 transfer group having 150 patients each. IVF outcome in terms of Implantation rate was compared between the groups. The results were analyzed using proportions, standard deviation and chi-square test.Both the groups were similar for age, indication and number of embryos transferred. Embryo transfers on day 5 resulted in significantly higher ongoing pregnancy and implantation rates as compared with day 3 embryo transfers(44% and 35.17% versus 29.33% and 21.35%, respectively)($P< 0.001$). No significant difference was found in terms of multiple gestations in both the groups. Embryo transfers on day 5 of culture give significantly higher chance of ongoing pregnancy and implantation rates per cycle and per transfer than day 3 transfers.

Key Words

Implantation Rate, Blastocyst, Embryo Transfer, IVF

Introduction

Extended embryo culture together with amelioration of embryo selection methods and embryo culture conditions have allowed a substantial increase in both pregnancy and implantation rates. However, uterine embryo transfers are still performed after 2 to 6 days of egg retrieval.

Over the years, implantation rates and ongoing pregnancy rates after embryo transfers have increased from 10 % and 18% to current rates of 40% and 60% respectively (1). This significant increase in IVF outcome is due to improvements in embryo culture and laboratory conditions (2), and amelioration on embryo selection methods (3). Probably, embryo selection is the most important key involved on the improvement of implantation rates of a given cycle. A variety of morphological criteria such as number of blastomeres, fragmentation, symmetry among blastomeres, signs of multinucleation, presence of vacuoles, or aspect of the zona pellucida are used for that purpose.

A proportional relationship exists between number of embryos transferred and both, pregnancy and implantation rates. As pregnancy rates increase with the number of embryos replaced, so does the incidence of multiple gestations that are associated with both maternal and neonatal morbidity and mortality.

In order to decrease the number of embryos required for transfer, human embryos were grown in culture for two additional days. Embryo culture periods have been extended beyond 2-3 days to discard embryos incapable of expressing activated embryonic genome and therefore unable to implant.Uterine environment seems to be different from fallopian tubes. Premature contact of early cleavage stage embryos with the uterus should be inadequate to support further embryo development. However, successful human pregnancies have been obtained after uterine transfer of zygotes, day 2 embryos, day 3 embryos and blastocyst (4).

From the Infertility Clinic, Department of Obstetrics & Gynecology, Mahatma Gandhi Medical College & Hospital, Jaipur- India
Correspondence to : Dr Prabhleen Kaur, A-2/93 Janak Puri New Delhi- 110058

Materials and Methods

Three hundred patients aged 25-40 years undergoing in-vitro fertilization (IVF)/intra-cytoplasmic sperm injection (ICSI) cycle between May 2010 and April 2011 who met the inclusion criteria set namely,2-20 years of infertility,having minimum five oocytes at oocyte pick up and endometrial thickness of 7 mm and more indicating good ovarian response, having normal uterine cavity and basal Follicle stimulating hormone level (FSH <10mIU/ml), were included in our study. Complete work-up, baseline investigations and post-menstrual diagnostic hysteroscopy was done, and then patient was given Long Protocol, Gonadotropin releasing hormone (GnRH) agonists started on cycle day 21 daily doses given subcutaneously till cycle day 3. Hormonal evaluation-Serum FSH,Luteinizing hormone (LH),Estradiol (E2) and trans-vaginal sonography was done on day 3 to confirm down regulation.Induction with recombinant FSH (rFSH) was started once pituitary down regulation was confirmed, for five days (day 3 to 7). Follicular monitoring was initiated done from day eight.Women were scheduled for oocyte retrieval once at least 3 follicles reached 18 mm size and then Injection human chorionic gonadotropin (hCG) 10,000 IU was given. Transvaginal sonography guided oocyte retrieval was done 36 hours after giving hCG.

The retrieved oocytes were then incubated for 3-4 hours in IVF-30 media and then depending on maturity of oocytes and previous IVF performance, IVF/ ICSI was performed. Short incubation insemination for two hours and group culture was followed for IVF.

Eighteen hours post-insemination, fertilization was confirmed. The fertilized oocytes were then transferred into a cleavage medium and incubated at 37 degree Celsius in atmosphere of 5.5 % carbon dioxide (CO2). Embryos were observed on day 2 and transfer was scheduled according to random allocation of patients into two groups based on availability of minimum three good quality embryos:-

Group-1 included patients undergoing embryo transfer on day 3, and Group-2 in which extended culture till day 5 was done in G2 plus media and blastocyst were transferred on day 5.

Random allocation of patients was done equally so that study population wascomparable.The number of blastocysts/embryos transferred was determined by the availability of embryos, patients age and previous clinical history. Not more than three embryos/blastocysts were transferred.All transfers were performedusing Edward-Wallace catheter.

Luteal support was given in form of micronized vaginal progesterone in dose of 200 mg thrice a day for eighteen days post retrieval and injection hCG 2000 IU was given intramuscular on days 5th,8thand 11th after retrieval. Serum beta-hCG was performed on day fifteen following embryo transfer and if positive then transvaginal sonography was performed fifteen days later to detect and confirm intra-uterine pregnancy. Positive cases were followed till six weeks to check for fetal cardiac activity.

Table 1. Distribution between Patients with Day 3 Versus Day 5 Transfer with Respect to Age, Duration of Infertility, Type of Infertility and Indication

Variable	Day 3 Transfer (n = 150)	Day 5 Transfer (n = 150)
Age (years, mean ± SD)	32.46 ± 4.3	32.04 ± 4.4
Range	25-40	25 – 40
Duration of Infertility (years)	8.9 ± 5.2	7.7 ± 4.7
Type of Infertility		
Primary	101	100
Secondary	49	50
Indication (n)		
Tubal	53	42
Endometriosis	10	9
Anovulation	15	21
Male	42	44
Mixed	15	19
Unexplained	15	15

No statistically significant difference was demonstrated between the two groups

Table 2. Results after Oocyte Aspiration with Respect to Number of Oocytes and Embryos

Variable	Day 3 Transfer (n = 150)	Day 5 Transfer (n = 150)
Oocytes at OR (n)	1094	1141
Total day 3 embryos (n)	447	549
Embryos transferred (n)	309	290
No of oocytes at OR (Mean ±SD)*	7.3±2.1	7.6±2.3
No of two-pronucleate embryos (Mean ±SD)*	3.9 ± 1.7	4.3 ± 1.5
No of Embryos per transfer (Mean ± SD)*	2.04 ± 0.74	1.93 ± 0.48

P > 0.05 The difference between the two groups was not significant for any of the variables shown*

Table 3. Reproductive Outcome in Patients after Oocyte Aspiration having day 3 or day 5 Embryo Transfer

Variable	Day 3 Transfer(n = 150)	Day 5 Transfer(n = 150)	X^2	df	P
Pregnancies per transfer	48	68			
% per OR	32	45.33	5.6	1	<0.01(S)
Ongoing Pregnancies (n)	44	66			
Ongoing Pregnancies (%)	29.33	44	6.3	1	<0.01(S)
Embryos transferred(n)	309	290			
Total Gestational Sacs on USG (n)	66	102			
Implantation Rate	21.35	35.17	14.12	1	<0.001(HS)
Total Multiple pregnancies	14	32			
Multiple Pregnancy (%)	31.81	48.48	2.36	1	>0.05 (NS)

S- significant, HS- highly significant, NS- not significant.

Outcome Measures:

1. Implantation Rate
2. Ongoing Pregnancy rate
3. Multiple Pregnancy Rate

Implantation Rate: defined as the number of gestational sacs determined by ultrasound by number of embryos transferred.

Statistical Analysis

The results were analyzed by using proportions, standard deviation and chi-square test.

Results

Total 300 patients were randomized for the study and were randomly allocated into two groups each of 150 patients. As shown in *Table 1* no statistically significant difference was found for age, duration of infertility, type of infertility and indication for IVF.

Asshown in *Table 2*, both the groups had a comparable mean number of oocytes at retrieval (7.3 2.1 and 7.6 2.3) and comparable mean number of embryos per transfer (2.04 0.74 and 1.93 0.48).

Table 3 shows higher ongoing pregnancies per oocyte retrieval was observed in blastocyst transfer group than in day 3 embryo transfer group (44% and 29.33%)(P<0.01) and higher implantation rate per embryo transfer in blastocyst transfer group (35.17%) than in day 3 embryo transfer group (21.35%)(P<0.001). No significant difference was found in terms of multiple gestations in both the groups.

Hence,women in blastocyst transfergroup had significantly higher clinical pregnancy rates, implantation rate and cleavage rates.

Discussion

Despite the different physiological environment existing between the uterus and the fallopian tubes, embryo transfers are performed after the 2 to 3 days of egg retrieval (4,5).Young patients having good ovarian response can have upto 55 per cent of clinical pregnancy rates after embryo transfers. These results indicate that premature embryo contact with the endometrial tissue is not as detrimental as expected, and that embryos can survive and grow into this virtual cavity until the

implantation window is opened (6). Several advantages are expected from delaying embryo transfer until blastocyst stage. First, it is believed that blastocyst transfer will improve embryo-uterine synchrony (7) favouring the metabolic environment of late cleavage human embryos. Second, it will facilitate the embryo transfer by itself, because of the decrease of uterine contractility and cervical mucus at the moment of the embryo transfer and third, blastocyst transfers will allow selecting higher viable and stronger embryos that have demonstrated the potential to continue developing and differentiate in vitro and with less chromosomal abnormalities (8,9).

In some centres the introduction of blastocyst transfers have successfully resulted in higher pregnancy rates in both non-selective couples undergoing IVF (10) and patients having fair quality embryo cohort (11). Other approaches found that blastocyst transfers only yielded better results in selected patient populations (12,13).

There are various studies in this field (14-19). Indeed, certain proportion of patients, especially those with less number of oocytes retrieved or poor embryo quality on day 3, will have a risk of embryo cleaving arrest on day 5 and therefore will not get the chance of having blastocyst transfer (18,19).

In our study, no significant difference was found between both the study groups in terms of age, duration of infertility, indication and type of infertility. This was in agreement with the study conducted by Auwera IV *et al* (10). The mean number of embryos transferred in both groups showed no significant difference [2.04 and 1.93, P>0.05].

Embryo transfers on day 5 resulted in significantly higher ongoing pregnancy and implantation rates as compared with day 3 embryo transfers (44% and 35.17% versus 29.33% and 21.35%, respectively)(P< 0.001) which was very similar to the rates observed in studies conducted by Mangalraj AM *et al* 2009 (15) and Auvera IV *et al* 2003 (12).

No significant difference was found in terms of multiple gestations in both the groups (19). These resultsagree with other studies performed by Schoolcraft et al2000 and Gardner *et al*, 2004 (13, 14).

Thus, it is concluded that in younger patients with good ovarian response extended culture to day 5 can be offered as blastocysts have good implantation rates and ongoing pregnancy rates, which will confidently allow transfer of not more than two good quality blastocysts and allow women to enjoy the benefits of limiting numbers for transfer (16,17)

References

1. RienziL, Ubaldi F, Iacobelli M, *et al*. Day 3 embryo transfer with combined evaluation at the pronuclear and cleavage stages compares favorably with day 5 blastocyst transfer. *Hum Reprod* 2002;17: 1852-1855.
2. Hill DL. Role of the in vitro fertilization laboratory in a negative pregnancy outcome. *Fertil Steril* 2001;75: 249-51.
3. Racowsky C, Jackson KV, Cekleniak NA, *et al*. The number of eight-cell embryos is a key determinant for selecting day 3 or day 5 transfer. *Fertil Steril* 2000;73: 558-564.
4. Dale B, Fiorentino A, de Simone ML, *et al*. Zygote versus embryo transfer: a prospective randomized study. *J Assist Reprod Genet* 2002; 19: 456-61.
5. Laverge H, De Sutter P, Van der Elst J, Dhont M. A prospective, randomized study comparing day 2 and day 3 embryo transfer in humanIVF. *Hum Reprod* 2002; 16: 476-80
6. Nardo LG, Sabatini L, Rai R, Nardo F. Pinopode expression during human implantation. *Eur J Obstet Gynecol Reprod Biol* 2002;101:104-08.
7. Milki AA, Hinckley MD, Fisch JD, Dasig D, Behr B. Comparison of blastocyst transfer with day 3 embryo transfer in similar patient populations. *Fertil Steril* 2002;73:126-129.
8. Margreiter M, Weghofer A, Kogosowski A, Mahmooud KZ, Feichtinger W.A prospective randomized multicenter study to evaluate the best day for embryo transfer: does the outcome justify prolonged embryo culture. *J Assist Reprod Genet* 2003;20: 91-94.
9. Balaban B, Urman B, Alatas C, Mercan R, Aksoy S, Isiklar A. Blastocyst-stage transfer of poor-quality cleavage-stage embryos results in higher implantation rates. *Fertil Steril* 2001;75: 514-18.
10. Auwera IV, Debrock S, Spiessens C, *et al*. A prospective randomized study: day 2 versus day 5 embryo transfer. *Hum Reprod* 2002;17:1507-12.
11. Frattarelli JL, Leondires MP, McKeeby J, Miller B, Segars JH. Blastocyst transfer decreases multiple pregnancy rates in in vitro fertilizationcycles: a randomized controlled trial. *Fertil Steril* 2003; 79: 228-30.
12. Santos MJ, Mercader A, Galan A, Albert C, Romero JL, Pellicer A. Implantation rates after two, three, or five days of embryo culture. Placenta 2003; 24: S13-S19.
13. Schoolcraft WB, Gardner DK. Blastocyst culture and transfer increases the efficiency of oocyte donation. Fertil Steril 2000;74: 482-486.
14. Gardner DK, Surrey E, Minjarez D, Leitz A, Stevens J, Schoolcraft WB. Single blastocyst transfer: a prospective randomized trial. *Fertil Steril* 2004; 81: 551-5.
15. Mangalraj AM, Muthukumar K, Aleyamma TK, *et al*. Blastocyst stage transfer versus cleavage stage embryo transfer. *J Hum Reprod Sci* 2009; 2(1): 23-26.
16. Sakkas D. Reflections on the number of embryos to replace at the dawnof the 21st century. *Curr Opin Obstet Gynecol* 2001;13: 293-97.
17. Salha O, Dada T, Levett S, Allgar V, Sharma V. The influence of supernumerary embryos on the clinical outcome of IVF cycles. *J Assist Reprod Genet* 2000; 17: 335-43.
18. Sandalinas M, Sadowy S, Alikani M, Calderon G, Cohen J, Munne S. Developmental ability of chromosomally abnormal human embryos to develop to the blastocyst stage.*Hum Reprod* 2001; 16: 1954-1958.
19. Ombelet W, De Sutter P, Van der Elst J, Martens G. Multiple gestation and infertility treatment: registration, reflection and reaction- the Belgian project. *Hum Reprod Update* 2005;11: 3-14.