Day 5 expanded blastocysts transferred on same day have comparable outcome to those left for more extended culture and transferred on day 6

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Abstract

Objective To study the outcome of blastocysts showing expansion on day 5 and transferred on day 5 or 6, in comparison with those unexpanded and transferred on day 6.

Study Design Prospective cohort of 221 women prepared for BET classified into three groups according to timing of blastocyst expansion and day of embryo transfer. Group I; with expanded blastocysts on day 5 having day 5 transfer, group II; with expanded blastocysts on day 5 having day 6 transfer and group III ; with delayed expansion undergoing day 6 BET.

Results Implantation rates, pregnancy rates, ongoing pregnancy rates, and live birth rates in the first 2 groups were almost double the rates in the third group. The figures for

implantation rates were 40 % in the first two groups vs. 19 % in the third group ($P<0.05$). Pregnancy rates were 60.9 % and 64 % vs. 31.8 % ($P<0.05$) and ongoing pregnancy/ live-birth rates were 52.3 % & 56 % vs. 27.3 %.

Conclusion The current study reports better implantation and pregnancy rates with earlier expanding blastocysts regardless of the time of transfer.

Keywords Blastocyst expansion · Hatching · Blastocyst embryo transfer (BET)

Introduction

The best available evidence suggests that the probability of live birth after fresh IVF is significantly higher after blastocyst-stage embryo transfer (BET) as compared to cleavage-stage embryo transfer [1–3]. These higher live birth rates (LBR) are attributed to several factors; including natural selection of the best embryos, lower embryo expulsion rate, and better synchronization between the transferred embryos and the endometrium [4, 5]. Post-fertilization and early cleavage stage embryos are traditionally evaluated by morphology, while later cleavage stage embryos and blastocysts are commonly scored according to their developmental pace [6–8]. It must be noted that in fresh IVF cycle blastocyst scoring according to developmental pace may inadvertently measure embryo-endometrium synchrony along with inherent embryo viability [9, 10].

Previous studies reported higher implantation and pregnancy rates with day 5 versus day 6 BET. However; in all published studies, comparisons were made between blastocysts that were expanded by day 5 and transferred on the same day, and those who had shown one-day delay in expansion and postponed to be transferred on day 6 [11–13]. Therefore; it remains unclear whether this is due to embryonic factor, endometrial factor, or

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Capsule Expanded blastocysts on day 5 have good outcome whether transferred on day 5 or 6.

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better synchronization between them. In addition some of these studies were conducted on special groups of patients like those with repeated IVF failures, therefore, their results cannot be generalized [12]. In view of this uncertainty, this study was designed to address three basic questions. First; is delayed expansion indicates lower blastocyst quality? Second; is endometrium more receptive on day 5 than day 6? Third; what is the incidence of uniform delay in blastocyst expansion in good prognosis ICSI patients stimulated by long agonist protocol?

Materials and methods

This prospective study was conducted, between October 2008 to March 2011, in university IVF units following approval of the institutional review boards and written informed consent of participants. 221 ICSI cycles conducted. Baseline inclusion criteria included women's age ≤ 37 years, with regular cycles, day 3 follicle stimulating hormone (FSH) < 9.5 IU/L, and antral follicle count (AFC) > 8 . Patients' age, duration of infertility, and causes of infertility are shown in Table 1, and the three groups were comparable regarding all of these variables. Patients were stimulated using long agonist protocol with recombinant FSH (Puregon, Organon, The Netherlands) and HMG (Menogon, Ferring Pharmaceuticals, Switzerland) following pituitary down-regulation with triptorelin (Decapeptyl; Ferring, Kiel, Germany). HCG was given when at least 3 follicles were ≥ 17 mm. Oocyte retrieval was performed 35

hours after hCG administration. ART cycle inclusion criteria included endometrial thickness ≥ 8 mm on the day of hCG administration, and ≥ 4 good quality embryos on day 3 after retrieval. Cycle variables, including stimulation days, number of ampoules, number of retrieved oocytes and number of fertilized oocytes are shown in Table 1.

ICSI and Embryo Culture ICSI was performed in a standard way. Embryos were cultured in sequential media (Sage; Cooper Surgical, USA), in groups of four per 50 μ l micro drop. On days 2 and 3, embryos were scored according to the number, and symmetry of blastomeres, and the percentage of fragmentation. Cases who had at least four good-quality embryos (i.e. ≥ 8 regular symmetrical blastomeres with no fragmentation on day 3) were included in the current trial.

Embryos were examined at 24-h intervals and the cell number and developmental stage were recorded. 1–2 blastocysts were transferred to women after extensive counseling. The procedure is not governmentally sponsored and most couples chose to transfer 2 blastocysts to increase PR.

The study groups Women were classified into three groups according to the day blastocyst expansion and transfer:

Group I: Those with blastocyst expansion on day 5 undergoing day 5 BETs.

Table 1 Data of the three groups undergoing blastocyst transfer

	Group I (n=174)	Group II(n=25)	Group III (n=22)	p
Age(years)	28.2 $\pm$ 4.7	28.9 $\pm$ 4.2	26.3 $\pm$ 3.5	0.1
Cause of infertility				
- male	128 (73.6 %)	19 (76 %)	15(68.2 %)	
- tubal	30 (17.2 %)	4 (16 %)	4(18.2 %)	
- unexplained	16 (9.2 %)	2 (8 %)	3(13.6 %)	
Basal FSH(IU/L)	6.6 $\pm$ 1.7	6.9 $\pm$ 1.4	6.8 $\pm$ 0.9	0.6
AFC	13 $\pm$ 2.1	12.6 $\pm$ 2.2	12.1 $\pm$ 2.1	0.1
Stimulation days	11.4 $\pm$ 1.5	11.2 $\pm$ 1.2	11.6 $\pm$ 0.9	
Number of ampoules	33.6 $\pm$ 6.7	33.8 $\pm$ 7.1	31.8 $\pm$ 8.6	0.68
Retrieved oocytes	15.5 $\pm$ 5	17.1 $\pm$ 5.5	16.7 $\pm$ 4.9	0.2
Fertilized oocytes	11.2 $\pm$ 3.6	10.2 $\pm$ 3.6	10.9 $\pm$ 3.3	0.4
Blastocyst number	5.1 $\pm$ 1.9	5.7 $\pm$ 1.9	3.5 $\pm$ 1.3	< 0.001
Blastocyst formation rate (%)	46.2 % $\pm$ 0.09	56.4 % $\pm$ 0.05	33.2 $\pm$ 0.11	< 0.001
Number transferred	1.8 $\pm$ 0.4	2 $\pm$ 0.4	1.95 $\pm$ 0.6	0.07
Vitrification of excess embryos	138/174(79.3 %)	18/25(72 %)	6/22(27.3 %)	< 0.001
Implantation rate	128/320(40 %)	20/50 (40 %)	8/42 (19.04 %)	0.02
Clinical pregnancy rate	106/174 (60.9 %)	16/25 (64 %)	6/22 (31.8 %)	0.04
Live-birth rate	52.3 %	56 %	27.3 %	0.03
Spontaneous abortion rate	10/106(9.4 %)	2/16(12.5 %)	1/7(14.3 %)	0.67
Multiple pregnancy rate	22/106(20.7 %)	4/16(25 %)	1/7(14.3 %)	0.54

Data presented as mean $\pm$ SD. P value determined by analysis of variance, $P < 0.05$ =significant.

- Group II: Cases with expanded blastocysts on day 5 for unsuitable circumstances related to patients and weekends had day 6 BET.
- Group III: Cases who did not have expanded blastocysts on day 5 left for one day, and had day 6 expanded BET

In all groups, serum β -hCG tests were performed two weeks after ET and transvaginal ultrasound (US) was scheduled three weeks later to confirm a clinical pregnancy. The implantation rate was determined by dividing the number of gestational sacs by the number of embryos transferred. Spontaneous abortion was defined as the spontaneous loss of a clinical pregnancy before 20 completed weeks of gestational age. Ongoing pregnancy rate was defined as the number of clinical pregnancies, continuing beyond 20 weeks of gestation and live-birth rate as the number of deliveries after 24 weeks of gestation with a live fetus

Sample size calculation The reported clinical pregnancy rate (CPR) among women undergoing day 5 BET and those showing delayed expansion and undergoing day 6 BET was 59.3 % and 32.3 %, respectively [9]. Using uncorrected chi-squared statistics (power 80 %, α -error 0.05), 52 women were needed to be included in each arm.

Statistical analysis Comparison of quantitative variables among the three study groups was done using one way analysis of variance (ANOVA) test with post hoc multiple two-group comparisons. For comparing categorical data, chi-square (χ^2) test was performed. Fisher Exact test was used instead when any expected frequency was <5 . A probability value (P value) <0.05 was considered statistically significant. Relative risk (RR) and 95 % confidence interval (CI) were calculated. All statistical calculations were done using SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL) statistical program for Microsoft Windows.

Results

A total of 221 ICSI cycles, were included in the study. 10 % of included participants showed a delay and had blastocyst expansion on day 6 (22 versus 199, $P<0.001$). Among the 199 women showing expanded blastocysts on day 5, 174 patients had day 5 BET (group I) and 25 women had day 6 BET with more expanded, hatching or hatched blastocysts. (group II). Group III comprised the 22 participants that did not have expanded blastocysts on day 5 and were left for one more day in culture. All of group III cases had expanded blastocysts on day 6 that were transferred.

There were no statistically significant differences in basal characters among the three groups (Table 1). Similar numbers

Table 2 Relative risk of clinical pregnancy in the three groups undergoing blastocyst transfer

Clinical pregnancy rate	Relative risk	95 % confidence interval	P value
Group II vs. group I	1.05	0.77–1.44	0.76
Group I vs. group III	1.91	1.03–3.57	0.009
Group II vs. group III	2.01	1.02–3.97	0.02

$P<0.05$ =significant

of oocytes were retrieved and a similar percentage of these oocytes were fertilized among the three groups of patients, however, the number of blastocysts formed were significantly higher in groups I and II than group III ($P<0.001$), while no differences were present between groups I & II ($P=0.17$). The number of transferred blastocysts was comparable among the 3 groups, however, groups I & II had more excess embryos available for vitrification than group III ($P<0.001$ & 0.002 respectively), while no differences were present between groups I & II ($P=0.41$). Similar implantation rates were found in groups I & II (40 %) which were nearly twice the rate of group III which was 19 % ($P<0.05$).

Pregnancy rates were also almost twice as high in groups I & II {106/174 (60.9 %) & 16/25(64 %)} than in group III {7/22(31.8 %)} with no differences between groups I & II. Similarly, ongoing pregnancy/ live-birth rates were also higher in groups I & II {91/174(52.3 %) & 14/25(56 %)} than in group III{6/22(27.3 %)}. Regarding miscarriage rates, in group I, 10 women had spontaneous miscarriage before 20 weeks gestation, while 5 extra losses occurred between 20–24 weeks of gestation, while, all losses in groups II and III were miscarriages before 20 weeks. The relative risk was used for Quantification and assessment of occurrence of pregnancy among our three groups in relation to each other. Moreover, there is estimation of upper as well as lower limits for the approximate occurrence of pregnancy through calculation of 95 % confidence interval. Tables 2 and 3 provide relative risk of clinical pregnancy rate and ongoing pregnancy/ live-birth rates in women undergoing BET in the three groups.

Table 3 Relative risk of live birth rate (LBR) in the three groups undergoing blastocyst transfer

LBR	Relative risk	95 % confidence interval	P value
Group II vs. group I	0.93	0.64–1.36	0.72
Group III vs. group I	1.92	1–3.85	0.02
Group III vs. group II	2.05	1–4.42	0.04

$P<0.05$ =significant

Discussion

Embryo implantation remains one of the least understood aspects of human reproduction. While ovarian follicular development, fertilization, and embryo development are readily observed and studied, embryo implantation is impossible to observe and remains an important frontier for research and future improvements in IVF outcomes [13]. Human embryo implantation is the end result of successful embryo-endometrial dialogue which in turn relies on three factors namely embryo quality, endometrial receptivity, and embryo-endometrial synchrony. The first question of this study was whether delayed blastocyst expansion indicates lower embryo quality, and it seems that the answer is YES. In the study population, delayed blastocyst expansion has been significantly correlated with lower blastocyst number, lower blastocyst formation rate, lower rate of excess embryo vitrification, and most important of all lower live birth rates. It remains however to be excluded that impaired day 6 endometrium or even closure of implantation window [13] might have contributed to the reduced ongoing pregnancy rates after day 6 BET, and here comes the second question. The second group of this study was used to address this question. In this group, early expanding, probably better quality, blastocysts were not transferred on day 5 for exceptional situations and patients were therefore postponed to have day 6 BET. The comparable implantation and pregnancy rates between group I and II probably indicates that the answer to the second question is NO, meaning that day 6 endometrium remains receptive and that the implantation window remains open. It should be admitted that the size of group II is small and not matching the calculated required number of 52 in contrast to group I which is almost triple that number. However, we thought that randomization of women with day 5 expanding blastocysts between group I and II would be ethically unjustifiable in view of the reports about decline in endometrial receptivity. The assuring results of the present study might relieve these ethical concerns and therefore, open the door for larger studies which are definitely warranted.

These results might also have positive implications on practice. In women undergoing pre-implantation genetic screening (PGS), embryos can be transferred when a result becomes available, on day 5 or on day 6 [14]. These patients might also be encouraged to have their tested blastocysts transferred one at a time whether on day 5 or day 6 to avoid multiple pregnancies [15]. These results are also potentially useful to busy units as it allows flexibility in scheduling patients for BET to avoid fluctuations in work load and working in weekends. Another indication for more extended culture may arise when there are many early expanding blastocysts. One day delay in transfer will provide a mechanism for a better selection of the healthiest embryos which

will be more expanded, hatching, hatched or larger diameter blastocysts [16].

The third question of this study was addressed by the third group. It was calculated that 10 % of properly selected patients for extended culture did not show blastocyst expansion by day 5, fortunately however, all of them had expansion by the next day and transfer was satisfactorily done. It should be emphasized that this study was not terminated and that recruitment continues to achieve bigger sample size for the second and third groups, however, we wanted to report these preliminary data in order to relieve concerns about day 6 transfer and to stress the importance of timing of blastocyst expansion as a marker of embryo quality. These results should not be considered contradictory to the studies conducted on frozen embryo transfer cycles (FET) that reported equivalent performance of early and late expanding blastocysts [17–19]. This discrepancy between fresh and FET cycles can be explained by the fact that blastocysts that are vitrified on day 6 are required to be expanded and, must have survived the warming process. These requirements might have selected better quality embryos than blastocysts expanding only on day 6 transferred in the fresh cycle.

In Conclusion; earlier expanding blastocysts are probably superior to later ones as they have better implantation and pregnancy rates whether transferred on day 5 or day 6. Further, about 10 % of properly selected patients for extended culture show expansion only on day 6 with lower outcome.

References

1. Papanikolaou EG, Kolibianakis EM, Tournaye H, et al. Live birth rates after transfer of equal number of blastocysts or cleavage-stage embryos in IVF. A systematic review and metaanalysis. *Hum Reprod*. 2008;23(1):91–9.
2. Utsunomiya T, Ito H, Nagaki M, Sato J. A prospective, randomized study: day 3 versus hatching blastocyst stage. *Hum Reprod*. 2004;19:1598–603.
3. Bungum M, Bungum L, Humaidan P, Yding Andersen C. Day 3 versus day 5 embryo transfer: a prospective randomized study. *Reprod Biomed Online*. 2003;7:98–104.
4. Papanikolaou EG, Evangelos G, D'haeseleer E, et al. Live birth rate is significantly higher after blastocyst transfer than after cleavage-stage embryo transfer when at least four embryos are available on day 3 of embryo culture. A randomized prospective study. *Hum Reprod*. 2005;20:3198–203.
5. Sills ES, Palermo GD. Human blastocyst culture in IVF: current laboratory applications in reproductive medicine practice. *Rom J Morphol Embryol*. 2010;51(3):441–5.
6. Huisman GJ, Fauser BC, Eijkemans MJ, Pieters MH. Implantation rates after in vitro fertilization and transfer of a maximum of two embryos that have undergone three to five days of culture. *Fertil Steril*. 2000;73:117–22.
7. Ziebe S, Petersen K, Lindenberg S, Andersen AG, Gabrielsen A, Andersen AN. Embryo morphology or cleavage stage: how to select the best embryos for transfer after in-vitro fertilization. *Hum Reprod*. 1997;12:15459.

8. Hsu MI, Mayer J, Aronshon M, et al. Embryo implantation in in vitro fertilization and intracytoplasmic sperm injection: impact of cleavage status, morphology grade, and number of embryos transferred. *Fertil Steril*. 1999;72:679–85.
9. Shapiro BS, Richter KS, Harris DC, Daneshmand ST. A comparison of day 5 and day 6 blastocyst transfers. *Fertil Steril*. 2001;75:1126–30.
10. Shoukir Y, Chardonens D, Campana A, Bischof P, Sakkas D. The rate of development and time of transfer play different roles in influencing the viability of human blastocysts. *Hum Reprod*. 1998;13:676–81.
11. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Ross R. Contrasting patterns in in vitro fertilization pregnancy rates among fresh autologous, fresh oocyte donor and cryopreserved cycles with the use of day 5 or day 6 blastocysts may reflect differences in embryo-endometrium synchrony. *Fertil Steril*. 2008;89(1):20–6.
12. Barrenetxea G, de Larruzea A, Ganzabal T, Jiménez R, Carbonero K, Mandiola M. Blastocyst culture after repeated failure of cleavage stage embryo transfers: a comparison of day 5 and day 6 transfers. *Fertil Steril*. 2005;83:49–53.
13. Van Voorhis B, Dorkas A. Delayed blastocyst transfer: is the window shutting. *Fertil Steril*. 2008;89(1):31–2.
14. McArthur S, Don Leigh D, Marshal J, App B, de Boer K, Jansen R. Pregnancies and live births after trophectoderm biopsy and preimplantation genetic testing of human blastocysts. *Fertil Steril*. 2005;84:1628–36.
15. McArthur SJ, Leigh D, Marshall JT, Gee AJ, De Boer KA, Jansen RP. Blastocyst trophectoderm biopsy and preimplantation genetic diagnosis for familial monogenic disorders and chromosomal translocations. *Prenat Diagn*. 2008;28(5):434–42.
16. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Thomas S. Large blastocyst diameter, early blastulation, and low preovulatory serum progesterone are dominant predictors of clinical pregnancy in fresh autologous cycles. *Fertil Steril*. 2008;90(2):302–9.
17. Veeck LL, Bodine R, Clarke RN, Berrios R, Libraro J, Moschini RM, et al. High pregnancy rates can be achieved after freezing and thawing human blastocysts. *Fertil Steril*. 2004;82:1418–27.
18. Behr B, Gebhardt J, Lyon J, Milki AA. Factors relating to successful cryopreserved blastocyst transfer program. *Fertil Steril*. 2002;77:697–9.
19. Van Landuyt L, Stoop D, Verheyen G, Verpoest W, Camus M, Van de Velde H, Devroey P, Van den Abbeel E. Outcome of closed blastocyst vitrification in relation to blastocyst quality: evaluation of 759 warming cycles in a single-embryo transfer policy. *Hum Reprod*. 2011;26(3):527–34.