Original Article

Pregnancy rate and live birth rate are higher after blastocyst transfer than after day 2 and day 3 embryo transfer following Intracytoplasmic sperm injection (ICSI).

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ABSTRACT

Objective

To compare the differences in outcome when embryo transfer was performed on day 2, day 3 and day 5 following ICSI.

Subjects and Methods

A total of 644 subjects undergoing ICSI at Islamabad Clinic Serving Infertile Couples, with embryo transfers on day 2, day 3 and day 5 were included. It was carried out between February 2000 and February 2005. Main outcomes measured were fertilization, pregnancy and live birth rates.

Results

The percentage of Metaphase II oocytes retrieved and the fertilization rate were significantly ($P<0.001$) higher in blastocyst transfer subjects. Day 5 transfer subjects were observed with significantly ($P<0.01$) higher number of grade I embryos as compared to day 2 and day 3 embryo transfers. The mean number of embryos transferred was not significantly different in cleavage stage (day 2 and day 3) and blastocyst stage (day 5) embryo transfers. The cumulative pregnancy rate per embryo transfer and live

birth rate were higher in blastocyst stage subjects (51.3% and 39.6%), as compared to day 2 (42.5% and 19.9%) and day 3 (37.6% and 33.4%) embryo transfers.

Conclusion

Our data suggests that blastocyst transfer has a higher pregnancy rate with a higher live birth rate. (Rawal Med J 2010;35:).

Key words

ICSI, ART, blastocyst transfer, cleavage stage, pregnancy outcome, live birth rate.

INTRODUCTION

The delivery of a single, healthy child is the desired outcome of human assisted reproductive techniques.[1] The only guaranteed way to reach this goal is to restrict oneself to single embryo transfer.[2] The primary aim of elective single embryo transfer (eSET) is to reduce the multiple pregnancies while maintaining an acceptable overall pregnancy rate.[3] The question is, therefore, at what stage to transfer the human embryo is back to the uterus?[1] In human IVF, the embryo is routinely transferred to the uterus on day 2 or 3 of development, which is between 4 and 8 cells. The timing of the arrival of the embryo in the uterus, however, is premature compared with the situation in vivo, where the embryo enters the uterus at the morula stage 4-5 days after ovulation.[4,5] The endometrium, on day 5 post-conception, seems to be optimal for embryos to implant and mirrors the implantation time in natural conceptions, as it has been demonstrated that uterine contractility decreases at the time of blastocyst transfer when compared to early stage transfers on day two.[6]

Currently, the elective embryo transfer at the blastocyst stage (day 5) is the most important method available for attaining high implantation and pregnancy without the

risk of multiple pregnancies.[7-9] Laverage et al observed no difference in implantation and pregnancy rates between transfers on day 2 versus day 3 (23.8 versus 23.8% and 47.9 versus 46.8% respectively).[5] Coskun et al reported overall same pregnancy rate (39%) in day 3 and day 5 transfers and almost same implantation rates (21 and 24% for day 3 and day 5).[10] A randomized study in an unselected population observed a significantly higher clinical pregnancy rate (CPR)/embryo transfer (60% in day 5 subjects as compared to 35% in day 2 subjects).[11] The aim of our study was to compare the pregnancy outcome in subjects undergoing ICSI treatment by embryo transfer on day 2, day 3 and day 5.

SUBJECTS AND METHODS

This study included an analysis of 644 couples who underwent ICSI at Islamabad Clinic Serving Infertile Couples, Islamabad, Pakistan. It was approved by our Institutional Review Board and an informed consent was obtained. All underwent embryo transfers on day 2, day 3 or day 5, between February 2000 and February 2005. Only subjects whose cycles were abandoned for varying reasons (failure to retrieve oocytes or spermatozoa, failure of fertilization, unwillingness of the patient to continue with the treatment) or whose retrospective data was incomplete regarding any parameter being studied was excluded from the study.

The hormonal estimations (serum FSH, LH, PRL, T3, T4, TSH) of all subjects were performed in the early follicular phase (Axsym System, Abbott, USA). Gonadotrophin-releasing hormone agonist long desensitizing protocol and short desensitizing protocol for down regulation was carried out with 3.75mg, or 0.1mg Decapeptyl (Ferring, Copenhagen NV, Denmark) or 1mg/ml Suprecur (Hoeecht Sonafi Oventis, UK) administered subcutaneously from the mid-luteal phase (Day-21) of the preceding

spontaneous menstrual cycle. Ovarian stimulation was carried out from the Day-3 of menstrual cycle using Human menopausal gonadotrophins (hMG) (Menogon; Ferring Pharmaceutical Germany or hMG Massone; Institute Massone SA, Buenos Aires, Argentina) or recombinant FSH (Puregon; NV Organon, Oss, The Netherlands), depending upon the age and serum FSH levels of the subject while ovulation was induced with 10,000 IU of Injection Pregnyl (Organon, Oss, Holland) given intramuscularly or 6500iu of Oviderell given subcutaneously. Ultrasound guided oocyte retrieval was performed 36-37 hours post hCG injection with 16G adapter and double lumen aspiration needle set (Cook Australia; Queensland, Australia). ICSI was only carried out on Metaphase II oocytes, using an inverted microscope (Leica DMIRBE, Leica Microsystems, Wetzlar Gmbh, Wetzlar, Germany) and a micromanipulation set on the heated stage of an inverted phase microscope under x200 magnification using micromanipulators (Research Instrument Inc. UK) and using 30º bend microinjection and holding pipettes (Research Instruments, UK or Hunter Scientific, ESSEX, UK).

Fertilization was considered normal when two clearly distinct pronuclei (2PN) were observed after 16 to 18 hours following microinjection under the stereo microscope at ×10. The embryo cleavage was evaluated after 24 hours of in-vitro culture. All the embryos were graded for the assessment of embryo quality[12] before embryo replacement. Embryo transfer was carried out using Rocket Echocath catheter system (R57630-EC-18) (Rocket medical, UK) on day 2, 3 or 5 of egg collection under ultrasound guidance, by one operator only.

Statistical analysis: Frequency of categorical variables (patient age group, pregnancy rate, live birth rate, clinical abortion rate, fertilization rate, cleavage rate, multiple

pregnancy rate, ectopic pregnancy rate) and numerical variables (age, mean FSH, number of oocytes retrieved, number of metaphase II oocytes and embryo grading) were calculated. All values were expressed as mean±standard error (S.E). Limit of minimum level was set as $P<0.05$. Mean values were compared using Student's *t*-test.

RESULTS

All 644 subjects were divided according to the day of the embryo transfer (Day 2, 3 and 5) and further subdivided into 3 groups on the basis of age (Table.1).

Table 1. Subjects undergoing ICSI treatment followed by embryo transfers.

Number of subjects	Day 2* No	Day 2* percentage	Day 3* No	Day 3* percentage	Day 5* No	Day 5* percentage
Group I (202) (20-29 yrs)	85	42.1	54	26.7	63	31.2
Group II (392) (30-39 yrs)	219	55.7	120	30.6	53	13.5
Group III (50) (40-49 yrs)	42	84	7	14	1	2
Total	346	100	181	100	117	100

* Day 2, Day 3 and Day 5 of embryo transfer following ICSI.

The mean age at presentation was comparable for day 2 and 3 (33.1±0.27 and 32±0.35) while being significantly ($P<0.05$) lower for day 5 (29.2±0.41).

Table 2. Pregnancy outcome.

Characteristics	Day 2*	Day 3*	Day 5*
Number (n)	346	181	116
Age at presentation (yrs)	33.1±0.27	32±0.35	29.2±0.41
Mean FSH (mIU/ml)	6.59±0.16	6.97±0.21	6.07±0.23
No. of Embryos transferred (n)	1522	893	477
No. of pregnancies (n)	130	77	60
Pregnancy rate/ET[b] (%)	37.6%	42.5%	51.3%
No. of live births (n)	105	34	33
Live birth rate/ET (%)	19.9%	33.4%	39.6%

*Day 2, Day 3 and Day 5 of embryo transfer following ICSI.

However, mean FSH levels were comparable in subjects for all 3 transfer groups (6.95±0.16 for day 2, 6.97±0.21 for day 3 and 6.07±0.23 for day 5 subjects) (Table. 2).

Table 3. Ovarian response to controlled super ovulation.

Characteristics	Day 2	Day 3	Day 5
Group I (20-29 yrs)			
No. of oocytes retrieved (n)	14.34±0.68	14.76±0.85	22.17±0.96[b***c***]
No. of metaphase II oocytes	10.96±0.52	12.8±0.77 [a**]	18.51±0.85[b***c***]
Oocyte maturity rate (%)	76.5%	86.7%	83.5%
Group II (30-39 yrs)			
No. of oocytes retrieved (n)	11.25±0.47	13.29±0.58[a**]	18.69±1.09 [b&c***]
No. of metaphase II oocytes	8.63±0.37	10.96±0.50[a***]	15.85±0.93 [b&c***]
Oocyte maturity rate (%)	76.4%	82.4%	84.8%
Group III (40-49 yrs)			
No. of oocytes retrieved (n)	7.74±0.85	8.57±2.06	20
No. of metaphase II oocytes	5.98±0.63	7.29±1.87 [a*]	19
Oocyte maturity rate (%)	77.2%	85%	95%

All values are given as mean±standard error (S.E). Values in parentheses are percents.
[a] Day 2 vs. day 3,[b] day 2 vs. day 5,[c]day 3 vs. day 5. *stands for statistically significant values P<0.05
** stands for statistically significant values P<0.01. ***stands for statistically significant values P<0.001

Across all age groups, the mean number of oocytes retrieved in day 5 embryo transfer subjects was significantly higher (P<0.001) than day 3 and day 2 subjects (Table. 3). The

oocyte maturity and the fertilization rate both were higher in day 5 subjects as compared to both day 2 and day 3 subjects, (Table. 3) although the cleavage rate varied (Table 4).

Table 4. Comparison of fertilization rate, cleavage rate and embryo grading.

Characteristics	Day 2	Day 3	Day 5
Group I (20-29 yrs)			
No. of 2-Pronuclei oocytes	7.1±0.41	8.87±0.57[a**]	13.65±0.63[b&c***]
Fertilization rate %	64.6%	69.3%	73.8%
No. of cleaved embryos	6±0.34	8.11±0.59[a**]	12.08±0.48[b&c***]
Cleavage rate %	84.7%	91.4%	88.5%
Embryo grading			
Grade 1	3.24±0.24	4.29±0.43	4.93±0.43
Grade 2	3.24±0.24	3.7±0.28	4.98±0.28
Grade 3	2.92±0.24	2.65±0.24	4.74±0.4
Grade 4	2.33±0.09	6.00	3.00
Group II (30-39 yrs)			
No. of 2-Pronuclei oocytes	5.6±0.24	7.31±0.36[a***]	11.58±0.57[b&c***]
Fertilization rate %	64.9%	66.7%	73.1%
No. of cleaved embryos	4.9±0.236	6.58±0.34[a***]	10.23±0.53 [b&c***]
Cleavage rate %	87.9%	90.1%	88.4%
Embryo grading			
Grade 1	2.88±0.15	4.05±0.24	4.61±0.38
Grade 2	2.80±0.13	3.26±0.18	4.37±0.36
Grade 3	2.67±0.11	2.65±0.15	4.53±0.47
Grade 4	1.40±0.01	-	6
Group III (40-49 yrs)			
No. of 2-Pronuclei oocytes	4.14±0.44	5±1.35	17±0.0 [b&c***]
Fertilization rate %	69.3%	68.6%	89.5%
No. of cleaved embryos	3.7±0.42	4.29±1.44	15±0.0 [b&c***]
Cleavage rate %	89.1%	85.7%	88.2%
Embryo grading			
Grade 1	2.16±0.21	3	5
Grade 2	2.50±0.26	2.50±0.51	8
Grade 3	2.43±0.22	1.40±0.72	2
Grade 4	1	-	-

All values are given as mean±standard error(S.E). Values in parentheses are percents.
[a] Day 2 vs. day 3,[b] day 2 vs. day 5,[c]day 3 vs. day 5. *stands for statistically significant values P<0.05
** stands for statistically significant values P<0.01. ***stands for statistically significant values P<0.001

There was only one patient in group III (40-49 yrs) and the highest number of grade 1 embryos was seen with day 5 embryo transfers (Table. 4).

DISCUSSION

The decision regarding what stage to transfer the human embryos is a source of great ongoing debate. Perhaps the reason why assisted reproductive technique (ART) clinics persistently perform embryo transfer on day 2 or 3 of culture, inspite of abundant evidence to suggest that there is very little chance of identifying and selecting a developmentally competent embryo is because, for blastocyst transfer to be accepted by the patients and doctors, it must be associated with acceptable pregnancy rates. Although the first clinical human pregnancy with in vitro fertilization transferred a blastocyst,[13] however, due to difficult maintenance of human embryo in culture for more than a few days, the cleavage-stage transfers became routine. The introduction of sequential culture media, facilitating successful extended culture, has refocused attention on human blastocyst transfer in ART. However, there is still the fear that at the end of the 5th day, the patient may have no embryo left to be transferred.

A study by Papanikolaou, in women under 36 years of age, indicated an advantage of the transfer of blastocyst stage embryo versus day 3 embryo transfer with a clinical pregnancy rate 33.1% as compared to 23.3%.[14] However, the age group was below 36 years of age while our study had an infertile female population between the ages 20-49 years. The same study strictly transferred only single embryos in either groups (blastocyst and cleavage stage) thus the CPR/ patient becomes same as CPR/ embryo transfer, bringing another very important point into focus. A limiting factor when comparing blastocyst transfer with cleavage-stage embryo transfer appears to be the point in time of randomization process

and the number of embryos being transferred in each group. Usually random allocation of the patients is done early in the study (as in our study) and the tendency for the patient to fall in either group depends upon the increased cancellation rate between oocyte retrieval and embryo transfer, again depending upon the quality of the embryos available in each cohort. It is not surprising that only those subjects have blastocyst stage transfers in whom a greater number of embryos have survived till day 5. This is especially true of our study where the blastocyst transfer subjects throughout show higher number of oocyte retrieval and fertilization rates together with the highest number of grade 1 and grade 2 embryos (Table 4). This is explained on the basis that only patients with higher number of oocytes retrieved were selected for blastocyst transfer. Although the cleavage rate varied amongst the subjects of all three days of embryo transfer, however, subjects with blastocyst transfer again showed the highest fertilization rates (Table 3). This can again be explained on the basis that the patients with the highest number of cleaved embryos are selected for blastocyst transfer.

Auwera et al also reported a trend towards a higher clinical pregnancy and live birth rate per embryo transfer in blastocyst stage (BS) transfers (46%) as compared to cleavage stage (CS) transfers (29%).[11] Geuriff et al[15] reported a higher implantation rate with single blastocyst transfer (SBT) (43.6% versus 29.6% for cleavage stage) while Zech et al[16] reported a higher pregnancy rate (32.8% for day 5 versus 23.2% for cleavage stage). All these studies support our results (BS: 51.3% and CS 37.6%) and also the theory that blastocyst culture might increase opportunities for choosing more viable and genetically normal embryos.

An extensive literature search by Papanikolaou[17] yielded initially 282 studies from which 8 randomized controlled trials were studied for live birth rate, clinical pregnancy rate and multiple pregnancy rates among other outcomes. A total of 1654 patients were reviewed and live birth rate and clinical pregnancy rate per randomized patient was significantly higher in patients who had a blastocyst-stage transfer as compared to patients with cleavage-stage embryo transfer. As a further argument in support, it has been shown that an embryo having undergone Pregenetic Diagnosis (PGD) and then used for single blastocyst transfer (SBT) yields even better pregnancy rate.[18]

CONCLUSION

In conclusion, blastocyst transfer leads to increased implantation and pregnancy rates with an overall better live birth rate compared to day 2 and 3 transfers. Advancing age had a detrimental effect on the outcome of ICSI treatment in embryo transfers on day 2, 3 or blastocyst transfers.

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