

Research Article

Comparative Study of Pregnancy Outcome in Day 2 Embryo Transfer versus Day 5 Transfer

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Abstract

Background: Improvements in culture systems and laboratory performance have resulted in the ability to culture, the human embryo to the blastocyst stage. Therefore, the Assisted Reproductive Technology clinics are opting to blastocyst transfer as transfer at this stage results in a significant increase in the implantation rate, leading to reduction in the number of embryos transferred. Therefore we carried out a Comparative Study of Pregnancy Outcome in Day 2 Embryo Transfer Versus Day 5 Transfer.

Methods: A randomized controlled study of 100 cases undergoing in-vitro fertilization was conducted wherein the first study group of 50 patients would undergo embryo transfer on day 2 and the second group of would undergo transfer on day 5.

Results: It was observed that day 2 embryo transfer led to a pregnancy rate of 36% which was similar to pregnancy rate of 38% when embryos were transferred on day 5.

Conclusions: Even though blastocyst transfer entails more man hours, clinical embryology support and more equipment; the pregnancy rates are not significantly different from those achieved by day 2 embryo transfer. However day 5 embryo transfer would be the accepted norm in IVF cycles as we are moving towards single embryo transfer.

Keywords: embryo transfer, blastocyst, in vitro fertilization.

Introduction

Improvements in culture systems and laboratory performance over recent years have resulted in the ability to culture, the human embryo to the blastocyst stage. Therefore, the Assisted Reprod-

uctive Technology clinics have to decide which day to perform the embryo transfer after an in vitro fertilization (IVF).

Blastocyst transfer has been associated with a significant increase in the implantation rate,

resulting in a reduction of the number of embryos transferred to attain acceptable pregnancy rates,^{1,2} Certainly, in younger patients, and those with good ovarian reserve, blastocyst transfer appears to be a more effective treatment and looks like the most promising stage, to replace in the uterus when one considers the move to single embryo transfer. Conventionally, human embryos conceived through IVF are transferred after fertilization on the second or third day of development while at the 4- to 8-cell stage. With the introduction of more physiological culture conditions over recent years, it has become possible to culture human embryos to the blastocyst stage as a matter of routine. Therefore, there is now a healthy debate regarding the optimum stage to transfer the human embryo to the uterus after IVF. Therefore we aimed to carry out a Comparative Study of Pregnancy Outcome in Day 2 Embryo Transfer (ET) Versus Day 5 Transfer

Methods

A randomized controlled study of 100 cases undergoing IVF-ET was conducted at the Assisted Reproductive Technology Centre of Tertiary Care Teaching Hospital. The total patients were divided into 2 groups of 50 patients each. The first study group of 50 patients would undergo embryo transfer on day 2 (D2) whereas the second study group would undergo embryo transfer on day 5 (D5). The patients selected were either of unexplained infertility or infertility due to tubal pathology. The Exclusion criteria were patients with Endometriosis, Tuberculosis, Poly Cystic Ovarian Syndrome (PCOS), previous IVF cycle failure, women requiring Intra Cytoplasmic Sperm Injection (ICSI) and difficult embryo transfer.

Stimulation Protocol

The inclusion criteria for ovulation stimulation were: patient's FSH concentration of <10 IU/ml and E2 concentration of < 50 pg/ml. All subjects were started with oral contraceptives (OCs) containing 0.05 mg ethinyl estradiol and 0.5 mg norgestrel (Mala -N) on day 5 of menstrual cycle.

On day 21 of the cycle, patients were started with daily subcutaneous injection of GnRH agonist (GnRH-a) leuprolide acetate (Lupride, Intas Pharmaceuticals, 4mg/4ml) at a dose of 1ml/day which was reduced to 0.5 ml/day after the onset of periods.

Stimulation with gonadotropins began at least 7 days after the last OC pill. A transvaginal scan was done to rule out any cysts. FSH concentration of <10 IU/ml and E2 concentration of < 50 pg/ml was also confirmed before starting gonadotropins. Highly purified FSH (Gonal F, Serono, Geneva, Switzerland) was injected sub-cutaneously in a dosage of 225 IU of FSH daily for the first 4 days. This was followed by the first folliculometry wherein a careful note of the number of follicle and their respective size was made. The endometrial thickness was also noted in every ultrasound. After this further stimulation of the ovaries by HMG (Human Menopausal Gonadotropin) 300 IU containing 150 IU of FSH and 150 IU of LH, was carried out. Trans vaginal folliculometry was done at every visit. Any patient who lagged behind in the endometrial growth was excluded from the study. Serial folliculometry was done till the mean diameter of the dominant follicle had reached 18 mm. At this juncture, Human Chorionic Gonadotropin (hCG) was administered in the dosage of 10,000 IU intra-muscularly. Follicular puncture followed 36–37 hours later.

Oocyte Retrieval and Insemination

MediCult media (MediCult, Jyllinge, Denmark) was used for the culture of oocytes, zygotes, and embryos, as well as for semen preparation. All media paraffin oil overlay and then incubated at 37°C for 24 hours with 5% CO₂ and 95% relative humidity. The cumulus–oocyte complexes (COC), found in the aspirates of the follicles, were collected in a flushing medium in 35-mm Falcon dishes (BD, NJ). The COCs intended for the IVF procedure were transferred into 0.5 mL of the medium in four-well dishes (Nunc, Roskilde, Denmark). Up to day 2, culture was done in MediCult universal IVF medium. The oocytes

were inseminated with 150,000 to 250,000 motile spermatozoa.

Any oocytes which would require fertilization by ICSI due to poor sperm count was excluded from the study.

Semen Preparation

Semen preparation was done with double layer of 1 mL of 55% over 1 mL of 80% SupraSpem (MediCult) and then 1ml semen was topped over this gradient. The prepared tubes were then rotated for 13 minutes at 300 X g. The sediment was resuspended in 10 mL of Sperm Preparation Medium (MediCult) and processed by centrifuge for 5 minutes at 200 X g. After throwing the supernatant the sediment was covered with 0.5 ml of Universal IVF Medium (Medicult) and then incubated for 30 – 60 min at 37°C, 95% relative humidity and 5% CO₂. The upper layer of the medium with motile spermatozoa was then transferred to a fresh tube.

Fertilization, Embryo Culture

The embryos cultivated in Universal IVF medium were denuded and transferred to ISM 1 (Medicult) 0.5 mL of medium in Nunc's four-well dishes for Day 2 Culture. For Day 5 culture the embryos were transferred to Blast Assist Medium on Day 2 evening where they remained till day 5.

Embryo Grading

Embryo quality was evaluated on day 2 with grades from G1 to G4, depending on the fragmentation of the embryo (G1: fragments ≤ 10%; G2: fragments 10% – 20%; G3: fragments 20% – 50%; G4: fragments > 50%). For analysis, G1 and G2 embryos combined into non-fragmented embryos group while G3 and G4 into fragmented embryo group.

Embryo Transfer

An ET catheter (Cook Soft pass embryo transfer catheter) was loaded with 30µL of Blast Assist medium (Medicult) and then embryos were loaded and then transferred under sonographic guidance 1 cm below the uterine fundus.

Evaluation of Pregnancy and Implantation

The pregnancy was tested 16 days after retrieval of oocytes with the method of quantitative measurement of beta HCG in the serum. Ultrasonographic evaluation of the number of gestational sacs was done 3 – 4 days after positive serum β-hCG. Evaluation of cardiac activity was done 7 days later.

Results

The study was completed in 2 years after the delivery of the pregnancies resulting from both the study groups. The results of the study is depicted in table 1

Outcome in day 2 versus day 5 embryo transfer (Table 1)

Study parameters	Day 2 Embryo Transfer (n = 50)		Day 5 Embryo Transfer (n = 50)	
	Number	%	Number	%
Pregnancy Rate	18	36	19	38
Abortion Rate	5	10	5	10
Take Home Baby Rate	13	26	14	28

As it can be observed from the table that both day 2 and day 5 embryo transfers have similar pregnancy rates with 36% in day2 compared to 38% at the blastocyst stage. The abortion rates and the take home baby rates were also found to be similar.

Day 5 embryo transfer entails a more tedious protocol and requires stricter monitoring of the embryos. It also requires a separate incubator for the storage of the embryos as there is an increase in the number of embryos handled. The amount of man hours per case is also significantly higher. Day 2 Embryo Transfer on the other hand is a relatively simple method to achieve results in IVF. The need of trained man-power is also significantly lesser than that involved in Day 5 Embryo Transfer. Despite the additional efforts in terms of man-hours and the additional expenditure incurred, both the methodologies have similar results.

Discussion

Various studies have shown that extended exposure of embryos to in vitro conditions is not advisable, if the embryos had developed from non-optimal cycles. Racowsky et al.³ and Coskun et al.⁴ had warned against prolonged culture if none of the embryos reaches the eight-cell stage on day 3 or if all embryos have a poor morphology on day 3.

Neither the equipment and the techniques, nor the staff have changed during the course of 2 years the study was conducted. The same team has maintained a constantly high fertilization, pregnancy, and implantation rate. The comparison of group characteristics shows that we had analyzed equal groups of embryos derived from patients having similar stimulation protocol cycles and similar embryo transfer techniques.

The oxygen concentration in the atmosphere of the incubator is important for increased blastulation.⁵ In our study the CO₂ incubators had 21% O₂ concentration in its milieu and 51.3% of embryos blastulated on day 5. This observation is comparable with other studies. The blastulation rates in these studies were between 28% and 66%.^{6,7,8,9,10} The pregnancy rates were between 36% vs. 38% per cycle and did not differ between day 2 and day 5 transfer groups.

In some patients with extended culture because of absence of blastulation and non-availability of blastocyst the embryo transfer was not performed. The number of embryos were transferred in the blastocyst group was half as compared to Day2 group

The implantation rate did not improve after day 5 transfer either, even though the endometrial receptivity physiologically is expected to be better at later stage. That extended culture does not influence implantation and further development is finally confirmed by the finding of similar percentages in compared groups of born babies.

Embryo selection for D5 transfer group included all the embryos post compaction stage to expanded blastocyst on D5. Embryos that failed to compact were discarded on D5. Pregnancy was

often noted to result from the transfer of compact morulae or early blastulation on D5.

The embryo morphology on D2 has good predictive value for blastulation on D5 in sequential culture media of ISM and BlastAssist for culture. Seventy-one percent of non-fragmented, normal-cleaving embryos reached this stage, representing 78% of all morulae and blastocysts. It was observed in this study that as per their physiologic characteristics, the embryos develop better in the BlastAssist media than in ISM Media after the first 2 days, even though they had identical the total pregnancy rate and the take-home baby rate. The extended culture using BlastAssist Media with constant laboratory condition had no effect on the implantation ability of the embryo.

Conclusion

Implantation rate is one of the determining factors, if not the determining factor, in human in vitro fertilization (IVF). Factors that can affect implantation rate include oocyte quality (which in turn depends on the patient's etiology, dietary status, and stimulation regimen), laboratory conditions, stage of embryonic development at the time of transfer, embryo transfer medium, and the embryo transfer procedure itself.

With increasing implantation rates, we have been able to reduce the number of embryos transferred to achieve an acceptable pregnancy rate. Unfortunately, the term "acceptable pregnancy rate" is not the same within the global IVF community. These perceived differences in acceptable rates are due to the socioeconomic nature of the country or state in which IVF is being performed. However, despite such differences, there is a growing global movement to reduce the number of embryos transferred to alleviate the problems encountered when a multiple gestation is conceived.

The best-case scenario for patients and society alike is the transfer of a single embryo. Then, with the rare exception of twinning, the result will be a singleton birth, thereby reducing the risks to both

child and mother. Furthermore, when a single embryo is transferred, pregnancy rates will directly reflect implantation rates. Implantation rate is among the most suitable ways to compare clinics. In other words, implantation rates for specific groups of patients can be used for benchmarking the success of IVF.

Both Day 2 Embryo Transfer and Day 5 Embryo Transfer are accepted methods in the successful conduct of IVF protocol.

Even though Day 5 Embryo Transfer entails more man-hours, more clinical embryology support, more equipment; the results are not significantly different from those achieved by a Day 2 Embryo Transfer.

Day 5 Embryo Transfer could be the norm of the future as we are moving towards a single embryo transfer (SET).

Declarations

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Conflict of interest: NA

Ethical approval: *Institutional*

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