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Assisted Reproductive Technologies

Consecutive Transfer of Day 3 Embryos and of Day 5–6 Blastocysts Increases Overall Pregnancy Rates Associated with Blastocyst Culture

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***Purpose*:** To investigate whether the consecutive embryo transfer of day 3 embryos and of blastocyst protects against failure to reach embryo transfer and provides additional pregnancies.
***Methods*:** An embryo transfer was performed on day 3 following which all remaining embryos were cultured to the blastocyst stage for a possible second transfer.
***Results*:** One hundred and forty-two patients were selected for extended culture. Thirty-two of these patients did not develop blastocysts in culture, however, there were 12 pregnancies achieved in this group.
***Conclusions*:** The consecutive transfer of day 3 embryos and blastocysts can prevent the total loss of a cycle when embryos fail to develop to the blastocyst stage in culture and thereby provide additional pregnancies.

KEY WORDS: Blastocyst; embryo culture; embryo transfer; IVF.

INTRODUCTION

Improvements in embryo culture systems and the introduction of sequential media has permitted successful in vitro culture of embryos to the blastocyst stage (1). This has led to blastocyst transfer being implemented for many patients undergoing IVF cycles. Blastocyst transfer is associated with higher implantation rates and has allowed a reduction of the number of embryos transferred thereby reducing multiple pregnancy rates while maintaining overall pregnancy rates.

However, despite these improvements, there are still those patients whose embryos fail to develop in culture to the blastocyst stage. A number of these may be patients with an intrinsic problem in early embryonic development, hence explaining their inability to conceive. However, there may be patients whose embryos cannot reach blastocyst in vitro but who would have conceived following conventional day 3 embryo transfer. The reported proportion of patients whose embryos are left to culture to the blastocyst stage in vitro but fail to reach blastocyst and therefore do not have an embryo transfer in their IVF cycle ranges from 2 to 40% (1–4).

Ashkenazi J. *et al.* (5) reported consecutive transfer of cleaved embryos followed by the transfer of blastocysts 2 days later. Although they compared the results of those patients who had both day 3 cleaved embryos and a blastocyst against patients receiving only a day 3 embryo transfer, they excluded from their study those patients who failed to reach a second embryo transfer of blastocyst(s). It is exactly these patients for whom performing the consecutive transfer of embryos both on day 3 and at the blastocyst stage, may be of benefit.

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The objective of this study was, therefore, to determine whether consecutive transfer of day 3 embryos and of blastocysts will "rescue" the cycles of patients whose embryos fail to reach blastocyst in culture thereby improving the overall pregnancy rate.

METHODS AND MATERIALS

All patients undergoing IVF or IVF/ICSI cycles between January 2001 and July 2002 were eligible to be included in the study based on embryo parameters on day 3 following oocyte retrieval.

Patients were prepared for IVF as previously described (6). Ovarian stimulation was performed using the modified GnRH-a long protocol. An injection of HCG was administered when three follicles were over 18 mm. Transvaginal ultrasound guided oocyte retrieval was carried out 36 h after HCG administration. Luteal support was commenced following oocyte retrieval using progesterone suppositories (Prometrium, Schering Canada).

Oocyte cumulus complexes were collected into 14-mL tubes (Falcon, VWR Canlab) and then immediately transferred into IVF media (Vitrolife, Goteberg, Sweden) in organ culture dishes (Falcon F3037, VWR Canlab). As required follicle flushing was performed using heparinized saline (7). For standard IVF, insemination was performed 4 h following oocyte retrieval at a concentration of 5–7 M/mL motile sperm and oocytes were checked for the presence of two pronuclei the following morning. In severe male factor cases ICSI was used to achieve fertilization.

At fertilization check oocytes were moved into G1.2 medium (Vitrolife, Goteberg, Sweden) in droplets (Falcon F3801). On day 3 following oocyte retrieval, embryos were scored for cell number and morphological appearance. All patients who had at least 5 embryos with 6 cells or more and grade 2–3 or above were selected for possible consecutive embryo transfer (6). All other patients who did not meet these criteria were excluded from the study and underwent standard day 3 embryo transfer.

The first embryo transfer was performed on day 3 using a soft catheter (Wallace, Sims Portex Ltd., UK) and the embryos were placed 1.0 cm from the fundus of the uterus. The number of embryos transferred depended on the patient's age. In patients under 35 years of age a single day 3 embryo would be transferred. In patients between 35 and 40 years of age two day 3 embryos were transferred, and in patients over 40 years old two or three day 3 embryos were transferred depending on the patient's choice. Following embryo transfer, the remaining embryos were washed carefully and transferred into pre-equilibrated G2.2 medium (Vitrolife, Goteborg, Sweden) droplets under oil.

On day 5 following oocyte retrieval, embryos were scored for blastocyst formation. Blastocysts were graded according to the level of expansion, presence, and quality of inner cell mass, and quality of trophectoderm. If no blastocysts had formed on day 5, the embryos were washed through fresh G2.2 media, moved to a fresh G2.2 dish, and reassessed on day 6. The best quality blastocyst was selected for transfer to the patient and any additional good quality blastocysts were cryopreserved for future use using Blastocyst Freezing Kit (Vitrolife, Goteborg, Sweden). Blastocyst transfer was performed using the same soft catheter and the blastocyst was placed 2.0 cm from the fundus in order not to disturb the embryos transferred at the earlier embryo transfer. Only fully expanded blastocysts were transferred.

Pregnancy was defined as elevated serum βHCG 16 days following oocyte retrieval. Clinical pregnancy was defined as evidence of pregnancy by ultrasound scan. Implantation rate was defined as the number of fetal sacs divided by the number of embryos transferred. This study was approved by the McGill Reproductive Centre Clinical Review Board.

Data were assessed for normality using the Shapiro–Wilk W test. Normally distributed data were reported by means and standard deviation and compared using the unpaired t test. Non-normally distributed data were reported by median and range and were compared using the Mann–Whitney U test. χ^2 was used for all categorical data. $P < 0.05$ was considered statistically significant.

RESULTS

Eight hundred and sixty-six cycles of IVF or IVF/ICSI were performed at our centre during the study period. One hundred and forty-two cycles met the day 3 embryo quality criteria for extended culture and consecutive transfer and were therefore included in the study. Of these, 110 (77.5%) developed blastocysts in culture and received two transfers. The remaining 32 (22.5%) did not develop blastocysts in culture and therefore only had the single embryo transfer on day 3.

There were 12 pregnancies amongst the 32 women whose embryos failed to reach the blastocyst stage in culture.

Table I. Comparison of the Two Cohorts of Patients

	Consecutive transfer (achieved blastocysts in culture) ($n = 110$)	Single transfer (did not achieve blastocysts in culture) ($n = 32$)
Mean age (SD)	34.7 (3.48)	35 (4.36)
Median attempt number (range)	2 (1–5)	2 (1–5)
Median number of embryos available day 3 (range)	11 (6–23)	10 (5–21)
Median total number embryos transferred (range)	3 (2–6)	2 (1–4)
Proportion of patients having ICSI (%)	30	41

Table II. Comparison of Outcome Measures

	Consecutive transfer (achieved blastocysts in culture) ($n = 110$)	Single transfer (did not achieve blastocysts in culture) ($n = 32$)
Pregnancies	56 (52.7%)	12 (37.5%)
Clinical pregnancies	47 (42.7%)	11 (34.4%)
Implantation rate	20.4%	25.0%
Miscarriages	8 (17.0%)	2 (18.2%)
Ectopic pregnancies	0	0
Ongoing pregnancies	39 (35.5%)	9 (28.1%)
Ongoing twin pregnancies	11 (28.2%)	4 (44.4%)
Ongoing high order multiple pregnancies	2 (4%)	0 (0%)

These two groups of patients did not differ in terms of age (35 vs. 35) or attempt number (2 vs. 2). The total number of embryos available on day 3 did not differ significantly between the two groups either (11 vs. 10). The number of embryos including blastocysts transferred in the two groups was obviously higher in the group that went on to have consecutive transfer (2.9 vs. 2, Table I).

Laser assisted hatching was performed on four patients in the single transfer group and five patients in the consecutive transfer group. No differences were observed in outcome amongst these patients. The pregnancy rate did not differ whether the second transfer took place on day 5 or day 6 (50 vs. 55%).

DISCUSSION

In this study, patients in the group, who developed blastocysts in culture, and had a combination of cleavage stage and blastocyst transferred, had an expectedly high pregnancy rate (52.7%). However, the group who failed to develop blastocysts in culture also had a high pregnancy rate (37.5%) and we believe that the transfer of cleavage stage embryos in these patients has resulted in a number of pregnancies that would not otherwise have occurred (Table II).

Despite great improvements in culture conditions and the introduction of sequential culture media there are some patients whose embryos do not develop to the blastocyst stage in culture. This is obviously very upsetting for the patients and means that a pregnancy cannot be obtained during that particular treatment cycle. The proportion of patients in this group varies from clinic to clinic and although the quality and developmental stage of embryos on day 3 is an indication of blastocyst potential, it is not absolutely predictive.

Although it must be conceded that some of the initially transferred day 3 embryos may have gone onto the blastocyst stage in culture and therefore possibly resulted in pregnancies, our overall blastocyst failure rate (22%) is comparable with that reported in the literature. (3,4,8).

There are many different approaches to predict the development of embryos to the blastocyst stage. It has been reported that the careful analysis of the pronuclear stage embryo including the pattern of pronuclei within the pronucleus may predict the embryos with the most developmental potential (9). The interaction of embryos with their culture environment, in terms of essential nutrient uptake, has also been proposed as a possible method for assessing embryo development potential (10). Of course, embryo quality on day 3 can be used as a guide to further embryo development but the best quality embryos on day 3 do not always develop to blastocyst while some day 3 embryos which may appear of poorer quality may do so. As such, there is currently no absolute method of predicting which embryos will continue developing to the blastocyst stage.

In summary, consecutive transfer of day 3 embryos and a blastocyst diminishes the risk to a patient of all the embryos failing to develop to the blastocyst stage in culture. It should be considered for women in whom blastocyst culture is being considered for the first time, or where the decision to proceed to blastocyst culture

is borderline in terms of the number of good quality embryos available on day 3. The initial transfer of day 3 embryos combined with the attempt to culture the remaining embryos on to the blastocyst stage results in high pregnancy rates whether or not the successful culture of blastocysts is obtained.

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