

—Mini Review—

Blastocyst Transfer **— Its Efficacy and Problems**

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In vitro fertilization-embryo transfer (IVF-ET) is not yet one of the most effective treatment for infertile couples. The changes in embryo culture techniques and implantation rates since the first successes with IVF which were reported in 1976 [1] have not been so marked, however. In recent years, the blastocyst transfer (BT) technique using embryos that have been cultured *in vitro* for about 5 to 6 days has been gaining acceptance throughout the world as a substitute for the conventional Day 2 or Day 3 embryo transfer (early cleavage embryo transfer; D2/3 ET). The primary reason is that BT synchronizes the endometrial implantation window with the embryo and is therefore closer to the physiological state, so that it has the potential to increase implantation and pregnancy rates [2, 3]. Improved pregnancy rates by virtue of making the IVF-ET environment closer to the physiological state were anticipated. Nevertheless, in its early stages BT did not yield significantly higher implantation and pregnancy rates, compared with D2/3 ET [4]. The exponential growth in the clinical application of BT in recent years, with improved implantation and pregnancy rates, is considered to be due to the recent advances in the culture system based on clarification of important nutrients required for early human embryogenesis [5]. As a result of the progressive changes in nutrient metabolites with advancing embryonal cleavage, the use of a sequential culture system with sequential culture media, grossly classified into media for early-stage embryos and those for late-stage embryos, has been reported in culture up to blastocysts [6]. This article deals with the efficacy and advantages of blastocyst transfer as well as with current and anticipated problems.

Efficacy of BT

Synchronization of intrauterine environment with embryo

Normally, after fertilization with sperm in the ampulla of the fallopian tubes, a human embryo migrates through the oviduct into the uterine cavity where it is subsequently implanted in the endometrium within about 7 days. This stage represents morulation and ensuing events [7], so D2/3 ET exposes the embryo to the intrauterine environment at the early cleavage stage, and problems arise in the events included by interaction between the embryos and the endometrium. BT is therefore considered to provide a more natural intrauterine environment for embryonic implantation [8]. The endometrium at 5–6 days after aspiration of oocytes may be said to constitute a suitable environment for embryo transfer because it has less peristaltic movement and the uterus has diminished contractility and cervical mucous secretion [7].

Selection of good quality embryos

Cultivation of the human embryo *in vitro* for 2 days longer than the early cleavage embryo transfer make it possible to obtain good quality embryos, including genetical selection of the embryo. In D2/3 ET, the embryo morphology is currently evaluated, e.g. with Veeck's classification [9], and many researchers have discussed the evaluation by this classification to achieve good clinical results. In the D2/3 ET, morphologic diagnosis alone is insufficient to accurately predict whether embryogenesis will be arrested. This process may also be affected by the culture medium environment. The greater the number of blastomeres there are 72 hrs after insemination, the higher the rate of achieving blastocyst stage embryo increase. This parameter was reported as an index for evaluating embryo quality [10]. Embryos having achieved the

Received: December 20, 2002

Accepted: February 20, 2003

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blastocyst stage morphologically are thought to have a satisfactory growth potential. Genetic evaluation of an embryo does not necessarily coincide with the morphologic intactness of the embryo. A report documented chromosomal aberrations in approximately 80% of Day ≤ 3 embryos [11]. It has been reported that genetic activation occurs with consequent initiation and accumulation of RNA transcription at Day ≤ 3 , and embryo development is controlled by that information [12]. Early interruption of an embryo culture results in the arrest of control and may affect the subsequent process of embryogenesis. It would therefore follow that the quality of an embryo, grown into a blastocyst by *in vitro* culture till Day 5, is more likely to be genetically satisfactory [13].

Reduction in number of transferred embryos - Prevention of multiple pregnancy

There is an inextricable link between the occurrence of multiple pregnancy and improved pregnancy rates. In multiple pregnancy, especially with three or more fetuses in utero, the possibility of serious complications during perinatal management exists. Depending on the circumstances, the outcome of the pregnancy ends in the tragic loss of the fetuses despite the gleam of hope promised by the pregnancy. The number of embryos transferred should be decreased to reduce the incidence of multiple pregnancy without lowering the implantation rate. This number varies around the world and unfortunately there are some institutions that permit an increased number of transferred embryos to obtain high implantation and pregnancy rates. In Japan, the Japan Society of Obstetrics and Gynecology stipulates that the number of embryos to be transferred should not exceed 3 in D2/3 ET, to limit multiple pregnancies [14]. Several articles have compared the rate of multiple pregnancies in D2/3 ET and BT. According to a report by Elster *et al.* there was no statistically significant difference between the two transfer techniques [15]. Nevertheless, it has been reported that the latest embryo culture system affords a strong probability of obtaining good blastocysts without failure by culturing for 5 or 6 days, so that BT is feasible to lower the rate of multiple pregnancies by placing a single good-quality embryo into the uterus [16]. With further refinement of the embryonal culture environment it will become practicable to transfer only a single blastocyst.

Satisfactory environment for embryo transfer

Conventional D2/3 ET is undertaken at the institutions where guaranteeing embryo cultures up to the

blastocyst stage is problematic. In particular, BT is frequently not chosen in institutions where the number of oocytes retrieved is small. At institutions with an established embryo culture system up to the blastocyst stage, improvement of the pregnancy rate may be anticipated in cases of repeated failure with D2/3 ET.

In clinical settings it is crucial to maintain a favorable *in vitro* environment for embryo culture. Environmental homeostasis is mandatory. This must be strictly maintained in the blastocyst culture system, and success in embryonal cultivation up to the blastocyst stage is linked to the strict quality control of ART Laboratory. Under good laboratory conditions, we can clearly attribute a failure in achieving pregnancy after BT to an inadequate uterine environment. Maintaining the condition of the laboratory may prove to be the key to success in IVF-ET.

Drawbacks and Points at Issue

Indications for blastocyst transfer

There are as yet no clearly defined indications for blastocyst transfer. Some reports have shown higher pregnancy and implantation rates with BT than with D2/3 ET [2, 3, 17, 18], but no statistically significant difference has been established. Certainly the implantation rate in BT increases per ET, but the pregnancy rate is not high, because some embryos cannot reach the blastocyst stage, so the cancellation of ET is increased. The important indication for BT is, first of all, prevention for high-order multiple gestations. Secondary, it is for failure cases. We are able to know the true reason for the patient's infertility. Unless the cancellation is dissolved, we cannot decide to use BT for recurrent cases.

Prolongation and complexity of in vitro embryo manipulation

Sequential culture medium are now readily available, but using a sequential medium unflinchingly increases embryo manipulations *in vitro* and entails a problem in the selection of the optimal medium from among the several available. Many things should be clarified in terms of the structural and functional impacts on the embryo of long-term culture *in vitro*.

Increased incidence of monozygotic twins

The incidence of monozygotic twins is reportedly increased with BT [19] whereas BT has been reported to be effective in preventing multiple pregnancies. The prognosis for monozygotic twins is poorer than for

normal twin pregnancies and such pregnancies frequently require meticulous perinatal management. This might be due to the structural impact of prolonged *in vitro* culture upon the embryo.

Evaluation of blastocyst

The criteria reported by Gardner *et al.* are commonly applied to the evaluation of blastocysts [20]. They include observation of the blastocyst with respect to:

(1) Grade

1. An early blastocyst with a blastocoel that is less than half the volume of the embryo.
2. A blastocyst with a blastocoel that is half of or greater than half the volume of the embryo.
3. A full blastocyst with a blastocoel completely filling the embryo.
4. An expanded blastocyst with a blastocyst volume larger than that of the early embryo.
5. A hatching blastocyst with a trophectoderm starting to herniate through the zona.
6. A hatched blastocyst, in which the blastocyst has completely escaped from the zona.

(2) Inner cell mass (ICM)

For blastocysts grade 3–6 (i.e., full blastocysts onward).

- A. Tightly packed, many cells.
- B. Loosely grouped, several cells.
- C. Very few cells.

(3) The trophectoderm.

- A. Many cells forming a cohesive epithelium.
- B. Few cells forming a loose epithelium.
- C. Very few large cells.

These are ultimately only a morphologic evaluation and sometimes do not pertain to the evaluation of embryo quality [21]. Further additional evaluation criteria would help to increase the pregnancy rate.

Cancellation of embryo transfer and excess cryopreserved embryos

According to the Cochrane Study, a significantly higher rate of cancellation of embryo transfer was reported for BT, compared to D2/3 ET [22]. Although the rate reported includes cases of cancellation in the early period after BT introduction and does not necessarily reflect the present status it is probably due to the fact that the proportion of embryos reaching the blastocyst stage is by no means high. The current use of improved culture media is considered to have led to a decreased cancellation rate. As technology advances, surplus blastocysts will be achieved after BT. With regard to embryo cryopreservation, Mukaida *et al.*

reported cases in which pregnancy occurred and resulted in delivery, accomplished by vitrification with a cryoloop [23]. This technique may be more commonly applied in the future.

Optimal culture environment for early embryos

Several types of sequential culture media are currently commercially available. There are many compositional variations but the compositions of media commonly mimic the composition of secretion in the environment where embryos are placed. For early embryos, the composition mimics the intra-oviduct environment, while that for late-stage embryos mimics the intrauterine environment. Generally, the media for early cleavage embryos have a low glucose concentration (0.5 mM) and hyposmolality (260 nOsm), whereas those for late cleavage embryos (blastocysts) provide a high-glucose environment (2.0–3.0 mM) [24]. In order to obtain better quality blastocysts it is essential to consider the optimal culture environments for more refined embryogenetic stages. The oxygen concentration prescribed for the culture environment affects early-stage (D2/3) embryos. Whilst there was reportedly no significant difference between 5% and 20% oxygen atmospheres in terms of the proportion reaching the blastocyst stage, there is a report documenting a high rate of blastocyst formation at a low oxygen concentration (5% O₂) [25], so that many institutions employ low oxygen concentrations in view of the toxicity of oxygen. This issue requires future investigation.

Conclusions

We have discussed the advantages and problems with BT. According to the views presented in the Cochrane Study, there are significant differences in terms of the increase in the transfer cancellation rate and decrease in the embryo cryopreservation rate for BT, compared to the conventional D2/3 ET, whereas no significant differences are noted in the implantation, pregnancy, abortion or multiple pregnancy rates. The use of hyaluronan and recombinant human albumin for achieving good quality and confidence in the embryo and improving the blastocyst cryopreservation was reported by Gardner [26]. The Cochrane Study states the possibility that BT may prevent multiple pregnancies and lead to high implantation and pregnancy rates. Important problems remain to be solved, for example, optimizing the culture environment and evaluating the influence of culture environments on embryos. Further

improvement and assessments of these are expected in order to realize more natural embryo transfer.

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