—Mini Review—

Blastocyst Transfer: Means of Overcoming Disadvantages Focused on Embryo Selection

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Cleavage-stage embryos have been transferred to the uterus since the first successful *in vitro* fertilization-embryo transfer (IVF-ET). Physiologically the embryos transferred to the uterus should be at the blastocyst stage, but cultivation to the blastocyst stage was difficult because of a poor culture environment. At the first attempt reported by Bolton *et al.* in 1989 [1] and 1991 [2] only 17% of embryos reached the expanded blastocyst stage and the implantation rate for blastocyst transfer (BT) was 7%. Subsequently, Menezo *et al.* [3] introduced a coculture system with Vero cells, with which 55–60% of embryos reached the blastocyst stage. With the sequential medium, it is now possible to culture embryos to the blastocyst stage without feeder cells [4]. Gardner *et al.* [5] reported that the proportion of embryos reaching the blastocyst stage was 46.5% and the implantation rate for BT was 50.5%. Furthermore, their comparison study with conventional embryo transfer at the cleavage stage (ET) showed that the implantation rate for BT was much higher than that for ET [5].

Advantages of BT

BT offers two major advantages. Cultivation to the blastocyst stage naturally selects viable embryos (embryo selection) and physiologically synchronizes the developmental stage of embryos with the uterus environment (physiological synchronization). The higher implantation rates of BT are the result of these two advantages. Cultivation to the blastocyst stage also has diagnostic value in confirming embryonic gene expression. Furthermore, BT makes it possible to transfer single embryos without a decrease in implantation rates, and avoids multiple pregnancies.

Disadvantages of BT

Although in cultivation to the blastocyst stage viable embryos are selected, its selection to avoid chromosome abnormalities is not sufficient. Sandalinas *et al.* [6] reported that extensive mosaicism was detected in blastocysts, and that trisomic embryos reached the blastocyst stage with a frequency of 37%. Extended culture is not a reliable tool for selecting to avoid chromosomal abnormalities such as trisomies.

Extended culture has promoted improvement of the general culture environment, including such factors as the quality of the medium, cleanliness of the air in the laboratory and the culture equipment, etc. Nevertheless, the *in vitro* environment will not equal the *in vivo* one. Extended culture *in vitro* might increase stress and damage embryos.

The possibility of a correlation between blastocyst transfer and the occurrence of monozygotic twins was suggested by Peramo *et al.* [7]. The implication of this correlation is quite important because of the high obstetrical risk involved. Although the cause of monozygotic twins is unclear, it is possible that *in vitro* culture loosens cell-to-cell adhesion in the inner cell mass (ICM) [8].

Extended culture also imposes burdens on

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Table 1. Clinical outcomes of day 2 and day 5 embryo transfer

	ET on day 2	BT on day 5
OR	204	187
2PN	106	104
Embryos available for ET on day 2 (a)	105	99
Blastocysts on day 5	–	55
Cycles with ET	105	55
ET/OR	105/204 (51.5)	55/187 (29.4)
Clinical pregnancy (b)	25	22
PR per ET	25/105 (23.8)*	22/55 (40.0)*
PR calculated per ET on day 2 (b/a)	25/105 (23.8)	22/99 (22.2)
PR per OR	25/204 (12.2)	22/187 (11.7)

*P<0.05. (Vlaisavljevic *et al.* 2001)

embryologists and the laboratory. Incubator space must be twice as large, and the workload of embryologists is increased. Careless operations directly result in failure of the culture. Extended culture is therefore a disadvantage in terms of laboratory workload.

Pitfall of BT

An interesting comparative study of ET versus BT was reported by Vlaisavljevic *et al.* [9] (Table 1). They compared the pregnancy rates of single embryos transferred on day 2 with those transferred on day 5 in non-stimulated cycles. This study completely eliminated the influence of embryo selection. In the 2-day cultivation group, 105 embryos were transferred on day 2, and 25 pregnancies were established. In the 5-day cultivation group, 99 embryos were available for transfer on day 2 (but not transferred on day 2), and 55 reached the blastocyst stage and were transferred on day 5, and 22 pregnancies were established. The pregnancy rates per transfer in the 2-day and 5-day cultivation group were 23.8% (25/105) and 40.0% (22/55), respectively, but the pregnancy rates per embryo available for ET on day 2 in the 2-day and 5-day cultivation group were 23.8% (25/105) and 22.2% (22/99), respectively. These findings showed that the proportion of viable embryos in the two groups were equivalent, and that viable embryos could achieve pregnancy regardless of ET or BT.

Although physiological synchronization is definitely an advantage of BT, recently the hypothesis that the hish implantation rates of BT can be explained purely in terms of embryo selection has been proposed [10]. Several comparison studies have supported this hypothesis [11, 12]. A pitfall in BT we often encounter is overestimation of physiological synchronization. IVF cases in which only one or two embryos are available for transfer on day 2–3 are typical. In such cases, extended culture has only diagnostic value in confirming embryonic gene expression because we have only one or two embryos for choice to transfer. Embryos which fail implantation with transfer on day 2 are unlikely to succeed with transfer on day 5. Considering the disadvantages of BT, such as the stress of *in vitro* culture on embryos, the risk of monozygotic twins and laboratory workload, it might be better to transfer embryos on day 2–3.

Means of Overcoming Disadvantages (1)

In order to overcome the above disadvantages without a decrease in implantation rates, we focus on embryo selection. Cultivation to the blastocyst stage is definitely efficient in the selection of viable embryos without invasion. If an alternative method for selecting viable embryos without extended culture were established, implantation rates comparable to those of BT would be realized by transferring embryos at the cleavage stage.

Although morphological evaluation at the cleavage stage has improved the pregnancy rate in IVF-ET, efficiency in selecting viable embryos is not sufficient. A candidate alternative method is pronuclear evaluation at the zygotic stage. Scott *et al.* [13] evaluated zygotes at 16–18 and 22 hours post-insemination, and transfer at 24–26 hours post-insemination. The embryos were scored for alignment of pronuclei and nucleoli and the appearance of the cytoplasm at 16–18 hours and pronuclear membrane breakdown at 22 hours post-insemination. The implantation rate of high-scoring embryos was 28%. They insisted that there was a

Fig. 1. The ideal morphology of pronuclei. The two pronuclei are equal in size, and are attached tightly to each other with a line produced at the pronuclear junction. The nucleoli in two pronuclei are equal in number and size, and align along the pronuclear junction.

strong correlation between embryo scores and implantation and pregnancy rates. Tesarik *et al.* [14] also reported that embryos judged normal at the zygotic stage had 44.8% pregnancy and 30.2% implantation rates. Scott *et al.* [15] reported that the combined use of a revised scoring system at the zygotic stage (Z-score) and embryo evaluation at the cleavage or blastocyst stage improved the implantation rate. In our laboratory, zygotes were evaluated by alignment of pronuclei and nucleoli, and the embryos judged to have ideal morphology at the zygotic stage (Fig. 1) had a 58% pregnancy rate. Although the effectiveness of pronuclear evaluation is still unclear, it could be an alternative method of extended culture for the selection of viable embryos.

Means of Overcoming Disadvantages (2)

In order to overcome the disadvantage of BT with regard to chromosomal abnormalities, preimplantation genetic diagnosis (PGD) could be an additional method on BT. Although the initial purpose of PGD was to avoid inheritance of single-gene disorders, recently PGD has been used for genetic screening purposes. PGD is typically applied to embryos at the 4–8 cell stage. Chromosomal abnormality of embryos is diagnosed by fluorescence *in situ* hybridization (FISH) with biopsied blastomeres. We reported PGD by means of two-step FISH for chromosome-X, Y, 13, 16, 18, 21 and 22 [16]. Gianaroli *et al.* [17] reported that screening for chromosome-X, Y, 13, 14, 15, 16, 18, 21 and 22 has an immediate impact on implantation rate.

The combination of PGD and BT yielded sufficient time for diagnosis. Previously biopsy was performed in the morning on day 3 and then embryo transfer was done in the evening on the same day. Therefore, only 4–6 hours was available for diagnosis. Now biopsy is performed in the morning on day 3 and embryo transfer on day 5. Two days are made available for diagnosis. The sufficient time for diagnosis makes it possible to attempt to obtain information on all chromosomes, such as visualization of metaphase plates or *in vitro* culture of single biopsied blastomeres [18]. Although PGD to screen chromosomal abnormalities on embryos is invasive and is not permitted in Japan, the combination of PGD and BT will make it possible to transfer blastocysts without chromosomal abnormalities.

Conclusion

BT offers two major advantages, embryo selection and physiological synchronization. Embryo selection mainly contributes to the hish implantation rate of BT. In IVF cases in which only one or two embryos are available for transfer on days 2–3, we often overestimate physiological synchronization and extend cultivation period. Considering the disadvantages of BT, such as the stress of in vitro culture on embryos, the risk of monozygotic twins and laboratory workload, it might be better to transfer embryos on days 2–3.

In this review, we focus on embryo selection and two possible means of overcoming the disadvantages of BT are mentioned. One is pronuclear evaluation to select viable embryos without extended culture. Its effectiveness is still unclear and further studies of it are expected. The second is PGD to select embryos without chromosome abnormality. The combination of PGD and BT will make it possible to transfer blastocysts without chromosome abnormalities.

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