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Blastocyst cultures: Today and Tomorrow

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ABSTRACT

Recently, multiple pregnancies have been the major problem for both the mother and fetuses. The best advantage of blastocyst transfer in normal responders is a single birth due to a single good-quality blastocyst transfer. The establishment of the vitrification methods for blastocysts makes it possible to elevate the singleton delivery rate without Ovarian Hyperstimulation Syndrome(OHSS). However, evidence is lacking as to whether blastocyst culture is effective in poor responders. In fact, only a few centers have adopted blastocyst transfer for all patients. Blastocyst development rate is around 50-60%. Several kinds of commercial sequential media are currently available and almost all Assisted Reproductive Technology (ART) units use these for treatment of patients. However, the period in which these media can be used is limited to 26-70 days according to the instructions provided by manufacturers. The half-life of pyruvate and antibiotics is supposedly short, and vitamins and hormones are unstable. Ideally, centers would make and use their own media within 72 h. In the near future, optimal ovarian stimulation, preparations of sperm and eggs, culture conditions, embryo transfer and luteal support should enable the possibility of providing healthy pregnancy following single blastocyst transfer in almost all patients.

Key words: *blastocyst; sequential media; multiple pregnancy; single birth; vitrification*

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INTRODUCTION

The development of sequential media to promote embryonic growth through genome activation, blastocele development and embryonic expansion has allowed for selection of those embryos with the greatest implantation potential [1]. However, according to the Cochrane Review [2], meta-analysis showed no significant differences in pregnancy rates between day-2/3 and day-5/6 embryo transfer (ET; day 2/3, 39.6% vs. day 5/6, 42.0%). At Ladies Clinic Kyono (LCK), blastocyst culture was initiated for repeated-failure patients in 1998. The percentage of blastocyst cultures has increased gradually since then and was around 60% in 2004. Clinical pregnancy rate has remained around 35% over the last 5 years (Fig. 1).

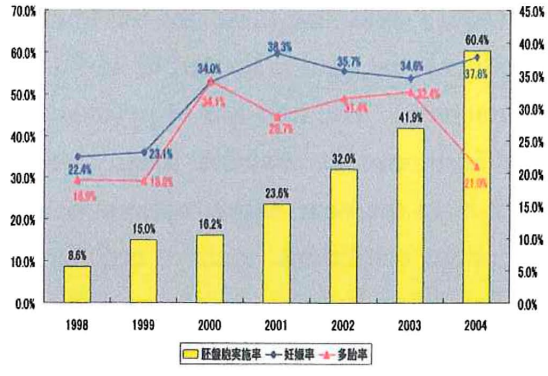

Fig.1 Percentage of blastocyst transfer, pregnancy rate and multiple pregnancy rate from 1998 to 2004

Blastocyst culture thus does not seem to have improved pregnancy rates. However, the establishment of vitrification methods for blastocysts have increased implantation rates and reduced multiple pregnancy rates due to single blastocyst transfer. The present study examined current blastocyst development procedures and problems, and clarified key determinants leading to viable good-quality blastocysts.

MATERIALS AND METHODS

This retrospective study made use of 3 periods: Stage I, September 1998 to February 2000 [3]; Stage II, January 2002 to December 2002 [4]; and Stage III, January 2003 to June 2004. Ovarian stimulation was performed using human menopausal gonadotropine (hMG) (Humegon; Organon, Japan) with a Gonadotropine releasing hormone (GnRH) agonist (Spurecur; Aventis, Japan) long protocol for good responders and a GnRH agonist short protocol for poor responders. Human chorionic gonadotropine (HCG) 10,000 IU (Profaci; Serono, Switzerland) was administrated when dominant follicular diameter reached 20 mm. Transvaginal oocyte retrieval was performed 35 h after HCG injection. Oocytes were fertilized either conventionally or using intracytoplasmic sperm injection (ICSI). The sequential media approach was used for extended culture of embryos to the blastocyst stage under 37 °C in a 6% CO_2, 5% O_2, 89% N_2 humidified environment.

Embryos were initially cultured in 20 μl early stage culture media until day 3. On day 3, embryos were transferred for further development into 20 μl late-stage culture media. As sequential culture media, K-SICM-K-SIBM (Cook, Australia), G1.2-G2.2 (Scandinavian IVF Science, Sweden) and P1-Blastocyst medium (Irvine, USA) were used in stage I and II. BAS1-BAS2 (Medicult, Denmark) and QAFM (Sage Biopharma, USA) - Blastocyst Medium (Irvine, USA) were used in stage III.

RESULTS

The percentage of ETs performed using blastocyst culture has gradually increased since 1998, and was around 60% in LCK for 2004. Clinical pregnancy rate has remained around 35% over the last 5 years. Blastocyst transfer (BT) thus does not seem to have improved pregnancy rates. However, the establishment of vitrification methods for blastocysts has increased implantation rates, while limits to the number of transferred embryos (<3) have reduced multiple pregnancies (Fig. 1). BT was performed in patients after repeated failures from September 1998 to February 2000 (Stage I). Pregnancy rate did not differ between day-3 and day-5 ET. Miscarriage rates tended to be higher for day-5 ET than for day-3 ET (Table 1). BT has been performed in LCK on the request of patients since January 2003 (stage III).

Previous cycles were significantly decreased in stage III compared to stages I and II (January, 2001-December, 2002). Implantation and pregnancy rates were significantly higher, and miscarriage and multiple pregnancy rates tended to be lower in stage III compared to stages I and II (Table 2).

Table1. Comparison of outcome between day 3 and day 5 ET Cycles

	D3	D5	P
Cycles	63	117	
Age	36.2(28-42)	34.2(25-44)	NS
Previous Cycles	3.6	4.1	NS
No. of retrieved oocytes	6.0	8.0	NS
Fertilization rate(%)	61.3	74.5	NS
Cleavage rate(%)	95.2	97.3	NS
Blastulation rate(%)	29.3		
No. of Transferred embryos	2.5	2.7	NS
Implantation rate(%)	15.3	13.2	NS
Pregnancy rate(%)	29.6	28.4	NS
Miscarriage rate(%)	12.5	34.5	NS
Cancellation rate(%)	14.3	12.3	NS
Multiple pregnancy rate(%)	31.3	28.5	NS

Table2. Comparison of outcome among three stages

variables	stage I	stage II	stage III
Cycles	117	105	144
Age	34.2	35.5	34.7
Previous cycles	4.1[a]	4.0[a]	1.4[b]
No. of retrieved oocyte	8.0	11.2	12.1
Fertilization rate(%)	74.5	76.7	74.7
Cleavage rate(%)	97.3	99.6	99.0
Blastulation rate(%)	29.3	56.6	53.2
Implantation rate(%)	13.2	15.9[a]	27.9[b]
Pregnancy rate(%)	28.4[a]	29.9	42.4[b]
Miscarriage rate(%)	34.5	34.6	21.3
Multiple pregnancy rate(%)	28.5	30.8	24.6
Cancellation rate(%)	12.3	17.1	14.7

※a vs b: P<0.01
Stage I From September,1998 to February,2000
Stage II From January 2001 to December 2001
Stage III From January,2002 to June,2004

In conclusion, BT is effective in good-response patients due to the elevation of implantation rate and the

reduction in multiple pregnancy rate. However, BT is ineffective in patients with repeated failure, including poor-response patients.

DISCUSSION

Theoretically, the human blastocyst should display intrinsically higher viability than cleavage embryos. The 60% implantation rate obtained for in-vivo developed blastocysts transferred to recipients as reported by Buster et al. certainly reinforces this proposition [5].

The advantages of blastocyst culture are the best selection of culturing embryos past the maternal/embryonic genome transition, synchronizing embryonic stage with the female tract, decreases in uterine contraction and cervical mucus, and establishment of vitrification methods [6,7]. From a different perspective, blastocyst culture and transfer may be useful for diagnosing causes of infertility. If a couple in which cause of infertility is unknown achieves pregnancy at BT after failure on day-3 ET, an intra-uterine condition that is hostile to early-stage embryos could be diagnosed. If development of early-stage embryo is arrested, cause of infertility would be suspected as due to abnormal oocytes.

Disadvantages of blastocyst culture include an increase in the percentage of patients not having an embryo transfer, risk of monozygotic twinning [8,9], and increased laboratory workload.

Interestingly, a lack of good quality embryos on day 3 resulted in no pregnancies for day-5 ET versus a 33% pregnancy rate for day-3 Et. These results strongly suggest that some suboptimal embryos may be rescued in the uterine environment and that extended culture might cause arrest of further development in such embryos [10,11]. Day-3 ET thus seems recommendable for poor responders. I would like to recommend key determinants to improve outcomes as based on experiences in this institution and recent reports. Dezortsev et al. reported that the ability of human oocytes to develop into viable embryos increases with the interval between hCG administration and ICSI, peaking at 37-39 h. after hCG administration, then gradually declining [12]. The rate of chromosomal aberrations in human oocytes increases from 3 h. after oocyte retrieval [13]. Optimal time for insemination occurs 37-39 h. after hCG administration and 2-3 h. after oocyte retrieval in conventional In Vitro Fertilization (IVF) and ICSI. Schuster et al.reported that the microfluidic device provides a novel method for isolating motile, morphologically normal spermatozoa from semen samples without centrifugation.Further studies will need to assess if motile spermatozoa isolated by microfluidics have improved DNA integrity compared with the original

samples[14]. Otuki et al. reported that pronucleus-sized translucent vacuoles, or smooth endoplasmic reticulum clusters (sERC) on transmission electron microscopy (TEM), are associated with lower chances of successful pregnancy. High estradiol levels could represent one cause of sERC formation. The sERC positive oocytes are best avoided for use in treatment [15].

Scott and Smith reported that oocyte quality and pronuclear embryo morphology (alignment of pronuclei and nucleoli, cytoplasmic halo, embryo score) are related to implantation rate and that pronuclear embryos can be successfully selected for ET [12].

Only 13.8% (67/484) of embryos with <7 cells and 27.5% (25/91) of those with >9 cells on day 3 formed blastocysts with apparently normal morphology, compared to 41.9% (252/602) of embryos with 7-9 cells on day 3 ($P<0.001$). Only 15.9% (22/1389) of embryos with ≥1 multinucleate cells on day 2 and/or 3 formed normal blastocysts, compared with 31.9% (335/1051; $p<0.001$) for cells without multinucleation [17]. Selection based on close pronuclei, aligned nucleoli, cytoplasmic halo, and early cleavage on day 1, multinucleate cells on day 2 and appropriate embryo development on day 2/3 appears recommendable.

Several kinds of commercial sequential media are currently available, and almost all ART units used these for patient treatment. At LCK, the percentage of blastocyst development in vitro using commercial sequential media is around 50-60% on days 5 and 6 [4]. Although delivery of a healthy baby has been reported after day-7 BT [18,19], implantation rate following day-5 viable BT appears optimal for humans. The detailed composition of commercial media has not been clarified to users. Comparisons must therefore be made to identify the best media for forming quality blastocysts and providing the best outcome. Media use is limited to 26-70 days according to the instructions from manufacturers, due to a short half-life for pyruvate and antibiotics, and instability of vitamins and hormones. Ideally, ART centers would probably be better off making and using their own media within 72 h. However, this is currently difficult in most ART units. High-quality blastocysts can be produced in vitro using sequential culture methods with commercially supplied perfect media under a low oxygen atmosphere phase in a multi-gas incubator.

In our data, blastulation rate was low in the mature oocytes derived from In Vitro Maturation(IVM) of immature oocytes, and from thawing mature oocytes after cryoperservation[20,21]. Further studies including the cytoplasmic maturation and the damage of spindles and chromosomes are needed.

The combination of preimplantation

genetic screening (PGS) and BT will enable BT without chromosome abnormalities [22]. In the near future, optimal ovarian stimulation [23], preparations of sperm and oocytes, culture conditions, ET and luteal support seem likely to allow the possibility of providing a healthy pregnancy following single BT in almost all patients.

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