CLINICAL ASSISTED REPRODUCTION

Routine Blastocyst Culture and Transfer: 201 Patients' Experience

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Purpose: The purpose was to compare the outcomes between day-5 blastocyst and day-2 embryo transfers.

Methods: Infertile women who accepted the ovarian hyperstimulation and oocyte retrieval were divided: Group 1, day 2 embryo transfer; group 2, cultured to day 5 in serum-free sequential culture medium and transfer. Early embryo quality and growth, blastocyst formation and quality, implantation rate (IR) and pregnancy rates (PR) were detected.

Results: Total blastocyst formation rate was 49.4%. Better early embryo quality (days 2, 3) and higher day 3 blastomere number possess higher blastocyst formation rate. The IR for day 2 and day 5 embryos were 10.8% and 22.2%, respectively. The PR in both groups were comparable (37.3% vs. 41.8%).

Conclusions: Blastocyst transfer has higher IR and comparable PR as those of day 2 embryo transfer. Early embryo qualities and day 3 blastomere number are useful in predicting the final blastocyst formation. Blastocyst formation rate is not related to maternal age, infertile causes, insemination methods, and early embryo number.

KEY WORDS: Blastocyst; embryo transfer; prolonged culture; sequential culture medium.

embryos and endometrium, better selection of viable embryos, improvement of the embryo implantation rate (IR), reduction in transferred embryo number, decreasing multiple pregnancy rate (PR), and availability of preimplantative genetic diagnosis (1). Gardner et al. (2) demonstrated the serum-free sequential culture medium (SFSCM) for prolonged embryo culture and observed the comparable PR of blastocyst transfer as that of day 2 embryo transfer. However, because of the lesser availability of embryos for transfer, few clinicians could adopt prolonged culture routinely. No investigators so far compared the day 2 and day 5 embryo transfer.

In this series, we divided our patients randomly and applied the prolonged culture routinely to detect the clinical differences between day 2 and day 5 embryo transfer. Furthermore, few investigators demonstrated the predictive values of early embryo quality in the subsequent blastocyst formation. No authors clearly demonstrated the roles of maternal age, infertile causes, insemination methods, early embryo number, and embryo growth rate in predicting the blastocyst formation. To our knowledge, this is the first random application in these aspects.

INTRODUCTION

Recently, prolonged embryo culture and transfer have been widely discussed. Advantages of prolonged embryo culture include synchronization between

MATERIALS AND METHODS

All infertile couples accepting the controlled ovarian hyperstimulation (COH) plus in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) at our unit between July 1998 and June 1999 were enrolled. All subjects fulfilled the following criterias: (1) age <40 years, (2) without azoospermia, and (3) ≥ 4 fertilized embryos [2-pronuclear (2PN) embryos].

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The protocol was approved by the Ethical Committee of the China Medical College Hospital. Informed consent was signed by all couples who were included.

The COH protocol included gonadotropin [follicle-stimulation hormone (FSH), Metrodin, Serono, Rome, Italy; human menopausal gonadotropin (hMG), Pergonal, Serono, Rome, Italy] administration and gonadotropin-releasing hormone-agonist (Leuprolide acetate depot, 1.875 mg subcutaneous single dosage, Takeda Chemical Industries, Ltd, Japan) down-regulation using the long protocol (3). Aspirin (100 mg per day, Bokey) was administered from menstrual day 1 to day 28. A dosage of 5000 IU human chorionic gonadotropin (hCG) (Profasi; Serono, Frenchs Forest, Australia) was administered when there were at least two follicles ≥ 17 mm. Oocyte retrieval was performed 34–36 hr after hCG injection. Semen preparation and IVF procedure were previously described (3). The ICSI was performed in cases with male infertility [World Health Organization (WHO) criteria] as described by Palermo et al. (4). In the following days, the embryo quality (grade, cell numbers) and growth were recorded everyday.

Patients were divided randomly into two groups according to day 2 or day 5 embryo transfer (day 0 = oocyte retrieval). In group 1, embryos were cultured solely in human tubal fluid (HTF) media till day 2 and transferred. In group 2, embryos were cultured in HTF media on day 0 and 1, in G1.2 medium on day 2 and 3, and in G2.2 medium on day 4 and 5 (1,2). On day 5, blastocyst morphology were assessed, including the presence of a fluid-filled cavity (blastocoele), differentiation of an outer trophectoderm layer, and an inner cell mass. All blastocysts were scored as follows: premature blastocyst (cavity <50% embryo volume) and mature blastocyst (cavity >50% embryo volume).

In group 1, three to six embryos were transferred; in group 2, one to four blastocysts were transferred. Transferred embryo number was determined by the embryo availability and quality and patient's age, and previous history. We increased the transferred embryo number in patients with poor embryo quality and who were >35-year-old or repeated IVF failure (5–6 embryos in group 1; 3–4 blastocysts in group 2). Excess blastocysts were cryopreserved using the ultrarapid method (5). All patients received the luteal support with hCG (2500 IU/day) on days 2, 5, and 8 and progesterone (15 mg/day) from day 10 to day 16. Both groups were compared in their personal data (maternal age, infertility causes, previous assisted reproductive technology history) and clinical outcomes (transferred embryo numbers, IR, PR).

In group 2, the roles of maternal age, infertile causes, insemination methods (IVF or ICSI), and early embryo number on the blastocyst formation were analyzed. Predictive values of the day 2 and day 3 embryo grade (grade 1, 0% fragmentation; grade 2, 0–10% fragmentation or unequal blastomeres; grade 3, 10–50% fragmentation; grade 4; >50% fragmentation) and day 3 blastomere numbers on the final blastocyst formation and qualities were evaluated. The SAS system with *t*-test and liner regression were used for statistical analyses. Percentage data were compared using Fisher's exact test. A *P*-value of <.05 was regarded as statistically significant.

RESULTS

A total of 158 and 201 women were included in groups 1 and 2, respectively. All personal data between both groups were comparable (Table I). The IR of day 5 embryos was twofold higher than that of day 2 embryos (22.2% vs. 10.8%). The PRs in both groups were similar (group 1, 37.3%; group 2, 41.5%). In group 2, overall blastocyst formation rate was 49.4%, which included premature blastocyst (39.5%) and mature blastocyst (9.9%) (Table II). Maternal age ≥ 37 years had lower but nonsignificant blastocyst formation rate than age <37 years. The infertile causes, insemination method (IVF or ICSI), and early embryo number play no role upon the final blastocyst formation rates. Better embryo quality (days 2, 3) and higher blastomere number (day 3) possess statistically higher blastocyst formation rates and better embryo quality.

For the quality of day 2 embryos, grade 1–2 embryos had higher blastocyst formation rates than grade 3–4 embryos (52.6% vs. 36.1%) (Table II). Grade 1–2 embryos had 52.6% blastocyst formation rate, which

Table I. Clinical Outcomes between Day 2 and Day 5 Embryo Transfers

	Day 2 ^a	Day 5 ^a
Patients No.	158	201
Age (years) ^b	32.9 $\pm$ 3.1	32.5 $\pm$ 2.6
No. of fertilized oocytes ^b	12.3 $\pm$ 5.7	12.6 $\pm$ 5.2
Fertilization rate ^b	88.7%	89.2%
No. of embryos transferred ^c	3.7 $\pm$ 0.6	2.1 $\pm$ 0.7
Implantation rate ^c	10.8%	22.2%
Pregnancy rate ^b	37.3%	41.8%
Ongoing pregnancy rate ^b	29.7%	32.3%

^a Values are means $\pm$ SD.

^b Nonsignificant between both groups.

^c *P* value < 0.05.

Table II. Effect of Maternal Age, Infertile Causes, Different Insemination Methods, and Early Embryo Number upon Blastocyst Formation and Qualities

Variable	Patient No.	2-Pronuclear embryo No.	Day 5 (%) blastocyst No.	Premature blastocyst	Mature blastocyst
Age (years)					
<37 ^a	183	2414	1202 (49.8%)	961 (39.8%)	241 (10.0%)
≥37 ^a	18	109	44 (40.4%)	35 (32.1%)	9 (8.3%)
Infertility causes					
Tubal factor ^a	47	617	311 (50.4%)	242 (39.3%)	69 (11.1%)
Endometriosis ^a	51	579	285 (49.2%)	230 (39.7%)	55 (9.5%)
Polycystic ovaries ^a	19	287	135 (47.0%)	116 (40.4%)	19 (6.6%)
Male factor ^a	62	756	371 (49.1%)	297 (39.3%)	74 (9.8%)
Idiopathic ^a	22	284	144 (50.7%)	111 (39.1%)	33 (11.6%)
Insemination methods					
IVF ^a	85	1052	497 (47.2%)	384 (36.5%)	113 (10.7%)
ICSI ^a	116	1471	749 (50.9%)	612 (41.6%)	137 (9.3%)
2-pronuclear embryo No.					
<8 ^a	34	196	94 (48.0%)	79 (40.3%)	15 (7.7%)
≥8 ^a	167	2327	1152 (49.5%)	917 (39.4%)	235 (10.1%)
Total	201	2523	1246 (49.4%)	996 (39.5%)	250 (9.9%)

^a Nonsignificant differences.

included 41.5% premature and 11.1% mature blastocysts. Grade 3–4 embryos had 36.1% blastocyst formation rate, which included 31.1% premature and 5.0% mature blastocysts. For the quality of day 3 embryos, grade 1–2 embryos also had higher blastocyst formation rates than grade 3–4 embryos (53.2% vs. 35.7%). For embryo growth rates, day 3 embryos with 6–16 cells had significantly higher blastocyst formation rates than day 3 embryos with 2PN-5 cells (60.2% vs. 6.3%).

DISCUSSION

A coculture system has been demonstrated to improve the prolonged embryo culture (6,7). However,

the coculture system has the inevitable disadvantages of inconvenience, being time consuming, and increasing risk of virus infection. Development of sequential media offers a fast, simple, and safer alternative in such situation. Recently, the knowledge of the embryo's developmental requirements at different stages has increased considerably. Human embryos can be cultured to the blastocyst stage in SFSCM (1,2,8).

About 30% of human 4-cell embryos have chromosome abnormalities (9), which means that less than 70% zygotes could develop to viable fetuses (10). Gardner et al. (1,2) and Jones et al. (8) have demonstrated that 50–60% embryos can develop to blastocyst in G 1.2 and G 2.2 media. In this study, around half the zygotes could transform to blastocyst stage, which

Table III. Effect of Early Embryo Grade and Day 3 Blastomere Number upon Blastocyst Formation and Qualities

Variable	2-Pronuclear embryo No.	Day 5 Blastocyst No. (%)	Premature blastocyst (%)	Mature blastocyst (%)
Day 2 embryo grade ^a				
Grade 1,2 ^b	2038	1071 (52.6%)	845 (41.5%)	226 (11.1%)
Grade 3,4 ^b	485	175 (36.1%)	151 (31.1%)	24 (5.0%)
Day 3 embryo grade ^a				
Grade 1,2 ^b	1971	1049 (53.2%)	831 (42.2%)	218 (11.0%)
Grade 3,4 ^b	552	197 (35.7%)	165 (29.9%)	32 (5.8%)
Day 3 blastomere No.				
2 Pronuclei-5 cells ^b	506	32 (6.3%)	31 (6.1%)	1 (0.2%)
6–16 cells ^b	2017	1214 (60.2%)	965 (47.8%)	249 (12.4%)
Total	2523	1246 (49.4%)	996 (39.5%)	250 (9.9%)

^a Grade 1: 0% fragmentation, Grade 2: 0–10% fragmentation or unequal blastomeres, Grade 3: 10–50% fragmentation, Grade 4: >50% fragmentation.

^b Significant difference (*P* value <0.05) in the blastocyst formation rate and blastocyst quality.

further confirms that the SFSCM is optimized for prolonged embryo culture. We also observed that the IR of blastocysts was twice that of day 2 embryos. Our IR and PR were compatible with those of previous reports (1,2,8).

Scholters and Zeilmaker (11) demonstrated that the biological ovarian age, rather than chronological age, determines the oocyte and blastocyst numbers. In this series, we also observed that the older women had fewer oocytes and blastocysts. However, the blastocyst formation rate might not be related to the maternal age or oocyte number. It has been demonstrated that the blastocyst formation rate was not affected by the maternal age and 2PN embryo number, as well as infertility causes (2,8). In this series, we also observed that there is no relation between these factors (maternal age, early embryo number, infertile reasons) and the blastocyst formation rates.

Janny and Menezo (12) reported that severe male infertility had a significant negative effect on the blastocyst development rate under the coculture media with Vero cells. Jones et al. (8) also demonstrated that the poor semen parameter compromised the blastocyst development rate. In contrast, Gardner et al. (2) reported that the effect of ICSI on the blastocyst formation rate is nonsignificant. In this series, we observed the comparable embryo formation rates after IVF or ICSI insemination procedure. Few investigators clearly demonstrated the importance of embryo quality or growth in predicting the blastocyst formation. Rijnders and Jansen (13) demonstrated the limited predictive value of early embryo morphology on the final blastocyst formation. In contrast, we observed that the day 2 and day 3 embryo quality are useful in predicting the subsequent blastocyst formation. We also observed that the embryo growth (day 3 blastomere number) provides the higher predictive value than embryo quality in the final blastocyst formation.

CONCLUSIONS

In conclusion, blastocyst transfer has higher IR and comparable PR as those of day 2 embryo transfer. Around half the blastocysts can be obtained after 5 day embryo culture in SFSCM. Blastocyst formation

rate is not related to maternal age, infertile causes, insemination method, or early embryo number. Early embryo quality is useful in predicting the blastocyst formation rate. Embryo growth (day 3 blastomere number) during prolonged culture provided the highest predictive value in the final blastocyst formation.

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