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Original Article

Pregnancy outcomes of single cleavage-stage embryo transfer in the ultra-short protocol

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Abstract: The aim of this study was to assess the pregnancy outcomes with only single cleavage-stage embryo transfer (SET) in Day 3 and investigate the factors resulted in only one cleavage-stage embryo transfer. Methods: The patients were categorized into three study groups depending on different grade of embryo transferred. We observed that the pregnancy (32.43% and 23.81% and 24.27%; *P*=0.921) and abortion (16.67% and 13.30% and 8.00%; *P*=0.191) rate showed no significant difference in SET groups with different embryo grades. The total clinical pregnancy rate in SET group was significantly lower compared with double cleavage-stage embryo transfer (DET) group (25.62% and 39.14%; *P*=0.001). Between the two groups, there was no significant difference in female's age, total gonadotrophin dose, mean length of stimulation, basal serum FSH, endometrial thickness or infertile time (*P*>0.05). The number of retrieved oocytes (4.23 and 6.03, *P*=0.002) and normal fertilization (48.36% and 65.01%, *P*<0.001) rate were significantly lower in SET group than in DET group. When only single embryo transferred, the normal fertilization rate (48.36% and 47.74%, *P*=0.860) exhibited no significant difference between IVF (in vitro fertilization) and ICSI (intracytoplasmic sperm injection) group. The clinical pregnancy rate (25.62% and 10.14%, P=0.007) was significantly lower in ICSI group than in IVF group. These results suggest that the patients with single cleavage-stage embryo transfer have a lower clinical pregnancy probability. If not pregnant, in next cycles, increasing oocyte retrieval and normal fertilization rate needed to be a prior consideration rather than replacing IVF to ICSI.

Keywords: Single embryo transfer, fertilization, pregnancy

Introduction

The delivery of a single healthy child is increasingly perceived as the better outcome for assistant reproductive technology (ART). However, in three decades the twin pregnancy rate of overall population increased almost 50%-70% [1]. As advances in ART improved the pregnancy rate of IVF, multiple-gestation pregnancies increasingly became a problem and embryo selection (ES) gradually significantly affected routine IVF with introduction of blastocyst stage embryo transfer (BSET), when investigators reported that implantation and clinical pregnancy rates after BSET appeared superior to cleavage-stage embryo transfers (CSET) [2-4].

Although the utilization of BSET has made as more IVF centers switched from day-3 cleavage stage to routine day-5/6 transfers of blastocyst-stage embryos, there were a majority of patients who have no opportunity to try blastocyst transfer [5]. As in ultra-short ovarian stimulation protocol, the patients demonstrated diminished ovarian reserve and less number of retrieved oocytes. After conventional fertilization, in a part of cycles only single available embryo could be gained in the end. For longer embryo culture in vitro embryologist could favorably select best embryos, while poorer quality embryos will arrest on the way to culture days-5/6 [6, 7]. There was no doubt that the pregnancy rate of BSET was higher than CSET, so the pregnancy probability for the patients with only single embryo was less likely. For these patients, if one more viable embryo could be gained, the pregnancy probability would be higher. So we should attach great importance to this part of patients and make more transferable embryos for them.

With this in mind, the aim of this study was to assess the clinical outcomes with only single cleavage-stage embryo transfer and investi-

Table 1. Comparison of Characteristic and clinical outcomes in groups with different embryo grades

Parameter	Grade I	Grade II	Grade III	*P* value
No. Single embryo transfer cycles (n)	37	63	103	-
Female's age (y)	33.96±5.13	34.52±4.88	34.27±4.96	*P*=0.562
Total gonadotrophin dose (ampule)	40.20±12.99	43.48±13.64	42.19±13.38	*P*=0.484
Mean length of stimulation (d)	10.88±1.98	10.26±2.26	10.44±2.44	*P*=0.293
Basal Serum FSH (mIU/ml)	8.86±2.89	8.53±3.12	8.22±3.06	*P*=0.652
Endometrial thickness (cm)	10.92±2.12	10.26±2.36	10.54±2.04	*P*=0.421
Infertile time (yr)	4.22±3.18	4.53±3.36	4.68±3.12	*P*=0.494
Oocytes retrieved (n)	4.96±2.53	4.36±2.18	4.08±1.94	*P*=0.662
Clinical pregnancy rate (n, %)	12 (32.43)	15 (23.81)	25 (24.27)	*P*=0.921
Early abortion rate (n, %)	2 (16.67)	2 (13.30)	2 (8.00)	*P*=0.191

gate the factors resulted in only one cleavage-stage embryo transfer. We also investigate whether ICSI should be adopted for improving this situation for these patients with low number of retrieved oocytes in the ultra-short protocol.

Methods

Patients and setting

The study was performed at Northwest Women's and Children's Hospital. 203 fresh single embryos were contained in this retrospective comparative study between January 2013 and November 2014. Our inclusion criteria: (1) Female ages ≤40 years old. (2) First fresh transfer cycles. (3) Ultra-short ovarian stimulation protocols. For ICSI patients in this study, TESA and 100% teratozoospermia patients were not included. All the semen samples were collected by masturbation

Stimulation protocol for IVF

All the studied patients used ultra-short protocols with GnRH agonist (GnRH-a, Decapeptyl Germany) and recombinant FSH (GONAL-f, Merck Serono Italy; Puregon, Organon Netherlands) for controlled ovarian hyperstimulation (COH). Ultrasound measurements of follicular growth and serum E2 level every 1-3 days, starting on the fifth or sixth day of stimulation, and the dosages of follicle-stimulating hormone (FSH) and human menopausal gonadotropin (hMG) were adjusted accordingly. 10,000 units of human chorionic gonadotrophin (hCG) was administered when >3 follicles were >18 mm. Oocyte retrieval was performed 36 h later by transvaginal ultrasonography-guided aspiration [8].

Evaluation and selection for embryo transfer

A morphologic score was given for day-3 embryo according to the number of blastomeres, homogeneous degree of blastomeres and degree of cytoplasmic fragmentation: grade I (8-10 blastomeres, even homogeneous blastomeres <10% cytoplasmic fragmentation), grade II (6-7 or >10 blastomeres with even homogeneous blastomeres of no cytoplasmic fragmentation, 8-10 blastomeres, even homogeneous blastomeres with 10%-20% cytoplasmic fragmentation), grade III (uneven and non-homogeneous blastomeres with 20-50% cytoplasmic fragmentation), and grade IV (uneven and non-homogeneous blastomeres with >50% cytoplasmic fragmentation). In double embryos transfer group of our study, the embryos of grade I group contained grade I + grade I, grade I + grade II or grade I + grade III; grade II group contained grade II + grade II or grade II + grade III; grade III group contained grade III + grade III [9]. Clinical pregnancy was confirmed by the presence of a gestational sac.

Statistical analysis

Data were analyzed using the SPSS 17.0 software. When comparing means between different groups, the paired student's t-test was applied. Differences between proportions were computed by using the χ^2 test or the Fisher exact test, as appropriate. Differences were considered statistically significant at $P<0.05$. When $P<0.05$ in three groups, comparison should be carried out with single factor analysis of variance between two groups.

Results

In single cleavage-stage embryo transfer cycles, our data demonstrated that the preg-

Table 2. Comparison of Characteristics and clinical pregnancy rate in groups with different number of transferred embryos

Parameter	Single embryo	Double embryos	*P* value
No. fresh transfer cycles (n)	203	654	-
Female's age (y)	34.20±5.00	33.61±5.06	*P*=0.622
Total gonadotrophin dose (ampule)	42.66±13.78	44.84±14.09	*P*=0.163
Mean length of stimulation (d)	10.65±2.32	11.00±2.12	*P*=0.438
Basal Serum FSH (mIU/ml)	8.83±3.24	8.76±3.17	*P*=0.751
Endometrial thickness (cm)	10.87±2.31	10.69±2.68	*P*=0.463
Infertile time (yr)	4.59±3.51	4.14±3.48	*P*=0.284
Oocytes retrieved (n)	4.23±2.95	6.03±3.03	*P*=0.002
Normal fertilization rate (%)	48.36	65.01	*P*<0.001
Clinical pregnancy rate (n, %)	52 (25.62)	255 (39.14)	*P*=0.001
Early abortion rate (n, %)	4 (7.69)	19 (7.45)	*P*=0.952
Transfer embryo grade			
Grade I (n)	37	282	
Pregnancy (n, %)	12 (32.43)	135 (47.87)	*P*=0.076
Grade II (n)	63	205	
Pregnancy (n, %)	15 (23.81)	75 (36.59)	*P*=0.060
Grade III (n)	103	167	
Pregnancy (n, %)	25 (24.27)	45 (26.95)	*P*=0.626

Table 3. Comparison of single embryo transfer in IVF and ICSI groups

	IVF group	ICSI group	*P* value
No. fresh transfer cycles (n)	203	69	/
Normal fertilization rate (%, n)	48.36 (414/856)	47.74 (127/266)	*P*=0.860
High quality embryo rate (%, n)	24.39 (100/410)	22.22 (28/126)	*P*=0.618
Transferrable embryo rate (%, n)	49.51 (203/410)	54.76 (69/126)	*P*=0.303
Implantation rate (%, n)	25.62 (52/203)	10.14 (7/69)	*P*=0.007
Clinical pregnancy rate (%, n)	25.62 (52/203)	10.14 (7/69)	*P*=0.007
Early abortion rate (%, n)	7.69 (4/52)	14.28 (1/7)	*P*=0.557

nancy rate in grade I group was higher than in grade II and grade III group, however, there was no significant difference (32.43% and 23.81% and 24.27%; *P*=0.921). The abortion rate was no significant difference among three groups (16.67% and 13.30% and 8.00%; *P*=0.191). No significant difference was observed in female's age, total gonadotrophin dose, mean length of stimulation, basal serum FSH, endometrial thickness, infertile time or the number of retrieved oocytes between two groups (**Table 1**).

There was no significant difference in female's age, total gonadotrophin dose, mean length of stimulation, basal serum FSH, endometrial thickness and infertile time between two groups. Both the number of retrieved oocytes and normal fertilization rate were significantly higher in double embryos group than in single embryo transfer group (6.03 and 4.23, *P*=0.002; 65.01% and 48.36%, *P*<0.001). The total clinical pregnancy rate was significantly lower in single embryo transfer group than in double embryos transfer group (25.62% and 39.14%; *P*<0.001). The abortion rate was no significant difference between two groups (7.69% and 7.45%; *P*=0.952). When transferred embryo was grade I or grade II, the pregnancy rate was lower in single embryo transfer group than in double embryos transfer group with same level (32.43% and 47.87%, *P*=0.076; 23.81% and 36.59%; *P*=0.060). However, when the transferred embryo was grade III, the pregnancy rate was no significant difference between two groups (24.27% and 26.95%; *P*=0.626) (**Table 2**).

When transferred single embryo, the normal fertilization (48.36% and 47.74%, *P*=0.860), high quality embryo (24.39% and 22.22%, *P*=0.618), transferrable embryo (49.51% and 54.76%, *P*=0.303) and abortion (7.69% and 14.28%, *P*=0.557) rate were no significant difference between IVF and ICSI group. However, the implantation and clinical pregnancy rate in IVF group was significantly higher than in ICSI group (25.62% and 18.18%, *P*=0.023) (**Table 3**).

Discussion

In assisted reproductive technology (ART) cycles, ET at the blastocyst stage (on day 5/6 of culture) has become increasingly utilized due to several advantages over cleavage stage or day 3 ET [10, 11]. Blastocysts have been confirmed to have higher implantation rates than cleav-

age stage embryos [12-14]. However, for most patients in the ultra-short protocol, their high ages, weak ovarian reserve and low response for stimulation usually brought themselves a lower number of retrieved oocytes than in the long protocol. For this part of patients, they generally chose cleavage-stage embryo transfer rather than blastocyst transfer.

This study included a total of 857 fresh ET cycles. 203 cycles were single cleavage-stage embryo transfer and 654 cycles were double cleavage-stage embryos transfer. This result shows single cleavage-stage embryo transfer accounted for 23.69% in all fresh embryo transfer cycles and suggests nearly one in four patients have no choice of embryo for transfer. It is well-known that the choice of a long or short protocol was made by the treating physician on the basis of the patient's characteristics or her response during previous IVF cycles [15-17], however, in the ultra-short protocol most of the patients were older and their basic characteristics were weaker. For this part of patients, the likelihood of conception was lower compared to in the long protocol and one of main restriction factors was decreasing number of available embryos resulted from low number of retrieved oocytes. So it is relatively significant to improve the methods for patients with only single embryo transfer. Our study assessed the pregnancy outcomes of single cleavage-stage embryo transfer in the ultra-short protocol and showed that there were no significant differences in the group with different embryo grade. It might be caused by totally lower pregnancy rate in the ultra-short protocol and when single embryo transferred grade had a little effects on pregnancy rate.

We observed that the total pregnancy rate of single cleavage-stage embryo transfer was 25.62% significantly lower than in the double cleavage-stage embryo transfer group (39.14%). In SET group, the number of retrieved oocytes and normal fertilization rate were significantly lower than in DET group. When single cleavage-stage embryo transferred was a good-quality embryo, the pregnancy rate was lower than double embryos with at least one was good-quality embryo. Although our data showed that there were no significant differences, this might be mainly caused by inadequate cases of single cleavage-stage embryo transfer. Interestingly, when transferred one or two grade III embryos, the pregnancy rate showed no significant difference. Thus, if a patient employing SET in the previous cycle failed in pregnancy, much more attention should be paid in the next cycle. Flush the ova during picking up to increase the retrieving rate and precisely regulate the semen concentration to avoid polyspermy and in the end to reduce the loss of usable embryo.

In normal cycle, ICSI could effectively prevent polyspermy from happening and the fertilization rate is generally higher than IVF. We wonder whether the fertilization rate improved by ICSI could subsequentially increase transferable embryos [18]. We selected high quality semen and perform ICSI treatment with SET. Compared with IVF group, no significant difference in fertilization rate were observed but the pregnancy rate in ICSI group was only 10% which is significantly lower than IVF group indicating that ICSI treatment sabotage the pregnancy rate in ultra-short ovarian stimulation protocols. Since the oocyte in ultra-short ovarian stimulation protocols are generally weak, ICSI treatment would cause mechanical damage during the procedure hence affects the embryo development and interferes the implantation. Thus, we believe IVF should have the priority once the semen meets the criteria since ICSI would not improve but sabotage the pregnancy rate in ultra-short ovarian stimulation protocols.

In conclusion, our findings suggest that patients with single cleavage-stage embryo transfer have a lower clinical pregnancy probability. If not pregnant, in next cycles, increasing oocyte retrieval and normal fertilization rate needed to be a prior consideration rather than replacing IVF to ICSI.

Disclosure of conflict of interest

None.

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