Int J Clin Exp Med 2017;10(7):11001-11005
www.ijcem.com /ISSN:1940-5901/IJCEM0049288

Original Article

Frozen-thawed and fresh embryo mixed transfer cycles provide better pregnancy outcome in patients who experience repeated pregnancy failure

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Received December 7, 2016; Accepted May 5, 2017; Epub July 15, 2017; Published July 30, 2017

Abstract: Purpose: This study aims to investigate the outcomes of co-transfer of frozen-thawed and fresh embryos in in vitro fertilization-embryo transfer (IVF-ET) cycles to improve pregnancy outcome in patients who experience repeated pregnancy failure. Materials and Methods: Patients were divided into five groups based on the characteristics and number of embryos transferred. All embryos transferred were on cleavage stage (Day 3). Group A had 42 patients transferred with one fresh cleavage embryo and one frozen-thawed, group B had 84 patients transferred with one fresh cleavage embryo, group C had 84 patients transferred with two fresh cleavage embryos, group D had 84 patients transferred with one frozen-thawed cleavage embryo and group E had 84 patients transferred with two frozen-thawed cleavage embryo. Results: There were no significant differences in demographic characteristics of these groups ($P>0.05$). However, clinical pregnancy rate, implantation rate, abortion rate, heterotopic pregnancy rate, malformation rate and other indicators were different among these groups ($P<0.05$). Group A had higher implantation rate and clinical pregnancy rate. Conclusion: Frozen-thawed and fresh embryo mixed transfer provides better outcome for patients who experience repeated pregnancy failure.

Keywords: Mixed cycle, fresh cycle, frozen-thawed embryo transfer cycle, pregnancy

Introduction

Several important factors affect the pregnancy rate in vitro fertilization-embryo transfer (IVF-ET) cycles, including maternal age, the quantity and quality of transferred embryos, endometrial receptivity, hormone levels, sub-fertility types. The most important factor is maternal age [1]. For women over 35 years, ovarian reserve and quality of oocytes decrease significantly and pregnancy rate decreases noticeably [2]. Accordingly, the infertility incidence rate, ART success rate, abortion rate, recurrent implantation failure (RIF) rate, and birth defect rate also increase.

It is generally accepted that RIF indicates patients who experience failure of more than 3 IVF cycles or are transferred more than 10 available embryos [3]. The main reasons of RIF are uterine factors and poor quality embryo. Only small quantity of embryos is available in RIP patients over 35 years old, whose clinical pregnancy rate and take-home-baby rate are low. Improving those patients' pregnancy rate becomes an important problem.

This study aims to investigate the outcomes of co-transfer of frozen-thawed and fresh embryos in IVF-ET cycles to improve pregnancy outcome in RIF patients.

Materials and methods

Patients

This was a retrospective cohort study based on the medical records of patients undergoing IVF/ICSI treatment at Reproductive Medical Center of Henan Provincial People's Hospital, China, from January 2013 to January 2016. All embryos transferred were cleavage embryo on Day 3 after oocyte retrieval. This study was

approved by Ethics Committee of Henan Provincial People's Hospital. Informed consent was submitted by all subjects when patients were enrolled. Inclusion criteria were: the patients' partners had normal chromosome karyotype, all patients were treated controlled ovarian hyperstimulation with a gonadotropin releasing hormone (GnRH) agonist protocol, one fresh and one frozen embryos on day 3 were co-transferred in those specific IVF/ICSI cycle, all transferred embryos were good quality embryos (Embryos had 4 cells on Day 2 and/or 7 or 8 cells on Day 3, contained <20% nucleate fragments, and exhibited no apparent morphological abnormalities) [4]. Patients with severe genetic disorder, mental illness, acute infection in genitourinary system and sexually transmitted diseases (STD), and patients who received donor sperm and/or oocytes were excluded. The studied patients were divided into five groups. Group A (42 cases) had transfer of one Day 3 fresh and one Day 3 frozen embryos, group B (84 cases) had transfer of one Day 3 fresh embryo, group C (84 cases) had transfer of two Day 3 fresh embryos, group D (84 cases) had transfer of one Day 3 frozen embryo, group E (84 cases) had transfer of two Day 3 frozen embryos.

All patients underwent controlled ovarian hyperstimulation with a GnRH agonist protocol as described previously [5]. Ovarian follicle development was monitored based on serum estradiol (E_2) levels and transvaginal ultrasound. When one or more follicles reached an average diameter of 18 mm and blood E_2 concentration exceeded 500 pg/ml, 4,000-10,000 units of urinary human chorionic gonadotropin (hCG, Serono, Aubonne, Switzerland) were administered at night. 35.5 to 37 hours later, the oocytes were retrieved using a transvaginal ultrasound-guided suction.

Laboratory protocols

IVF and ICSI were performed according to the laboratory's routine insemination procedures on the day of oocyte retrieval. The normally fertilized zygotes were then cultured in G-1™ PLUS (Vitrolife, Sweden) until day 3 in incubators at 37°C and 6% CO_2. Pronuclei were checked on the next morning after IVF/ICSI (Day 1, 16 to 20 hours after insemination). Cleavage stage embryo was assessed on Day 3 (62-66 hours after insemination) by cell number, fragmentation and symmetry as described previously [6]. One or two embryos were selected for transfer based on patients' age, medical history, number of assisted reproductive technique (ART) cycles and embryo quality. The other available embryos were cryopreserved by vitrification in fresh cycles. Vitrification and warming were performed according to the instructions with Vitrification Media Pack (Kitazzto, Japan) and Thawing Media Pack (Kitazzto, Japan). The embryos were thawed before embryo transfer, and embryos with a minimum of 50% living blastomeres were considered surviving.

Endometrial preparation for frozen-thawed cycles included natural cycle and hormone replacement cycles. In hormone replacement cycles with estradiol hemihydrate (6-8 mg per day) was administered from the first day of the menstrual cycle, and the dosage was modified according to endometrial thickness and morphology. When the endometrial thickness reached 8 mm, about menstrual cycle Day 15, micronized natural vaginal progesterone tablets (Utrogestan, Laboratoires Besins International SA, France) were added at a dosage of 600 mg per day. One or two embryos were transferred under ultrasound guidance. In all those embryo transfer cycles, the luteal phase support was continued for at least 8 weeks or stopped if blood β-hCG test was negative 14 days after embryo transfer. Clinical pregnancy was determined by transvaginal ultrasound after 4-6 weeks of embryo transfer and defined as the presence of the gestational sac in the uterus. Birth outcomes were evaluated by early miscarriage (<12 weeks of gestation), miscarriage (<28 weeks of gestation), birth defects and birth sex ratio.

Statistics analysis

All statistical analyses were performed with SPSS software (v17.0 Armonk, NY, USA). Quantitative variables were described as means ± SD. For data in normal distribution, one way of ANOVA was used for comparison among groups. Rank test was used for data without normal distribution. X^2 or Fishers exact test was used for comparison among groups. Statistical significance was established at $P< 0.05$.

Table 1. The general characteristics of five groups

Characteristic	Group A	Group B	Group C	Group D	Group E
Maternal age (year)	36.94±5.23	37.70±4.07	36.71±4.46	37.68±4.41	35.85±4.27
Paternal age (year)	35.50±6.38	37.61±5.63	35.68±3.94	38.14±6.01	36.76±6.26
Primary/secondary infertility	20/22	24/60	26/58	24/60	28/56
Duration of infertility (year)	4.69±3.94	5.91±4.20	5.08±3.61	4.89±3.97	4.82±3.27
BMI (kg/m^2)	21.92±2.04	22.86±3.41	26.70±19.27	23.48±2.93	23.50±3.00
Thickness of endometrium (mm)	9.94±2.31	10.37±2.24	11.69±2.34	9.08±1.88	9.59±1.65
Number of cycles	4.24±2.79$^{\#,※}$	2.10±1.86$^{*,\#,※}$	1.31±0.78$^{*,※}$	2.71±1.22$^{*,\#,※}$	2.62±1.13$^{*,\#}$

$^{*}P$<0.05, compared with group A, $^{\#}P$<0.05, compared with group C, $^{※}P$<0.05, compared with group E.

Table 2. Comparison of clinical outcomes of five groups

Item	Group A	Group B	Group C	Group D	Group E
Clinical pregnancy rate (%)	57.14 (24/42)	28.57 (24/84)$^{*,\#,※}$	61.91 (52/84)	33.33 (28/84)$^{\#,※}$	57.14 (48/84)
Implantation rate (%)	66.67 (56/84)	28.57 (24/84)*	44.05 (74/168)*	33.33 (28/84)*	30.95 (52/168)*
Multiple pregnancy rate (%)	16.67 (4/24)	0$^{\#}$	46.15 (24/52)$^{※}$	7.14 (2/28)$^{\#}$	16.67 (8/48)$^{\#}$
Miscarriage rate (%)	41.67 (10/24)	12.50 (3/24)*	15.39 (8/52)	14.29 (4/28)	33.33 (16/48)
Early abortion rate (%)	25.00 (6/24)	8.33 (2/24)	11.54 (6/52)	14.29 (4/28)	25.00 (12/48)
Ectopic pregnancy rate (%)	0	0	3.85 (2/52)*	0	8.33 (4/48)*
Birth defect rate (%)	0	0	0	0	7.14 (2/28)*
Sex ratio (%)	8/6	11/10	34/24	14/10	22/16

$^{*}P$<0.05, compared with group A, $^{\#}P$<0.05, compared with group C, $^{※}P$<0.05, compared with group E.

Results

General characteristics of the patients

This study consisted of 378 patients, which were divided into five groups as previously described, and their main characteristics are shown in **Table 1**. There were no significant differences in such characteristics as maternal age, paternal age, body mass index (BMI), infertility causes (primary or secondary infertility), duration of infertility, maternal body mass index (BMI) and thickness of endometrium among groups. The number of IVF cycles showed no significant difference between group D and E, but showed significant differences among the other groups, the number of IVF cycles in group A was the highest.

Outcomes of the patients after embryo transfer

The birth outcomes among five groups are shown in **Table 2**. There were no significant differences in early abortion rate, sex ratio at birth (boy/girl). The clinical pregnancy rate of group B and group D was significantly lower than that of group C and group E. The embryo implantation rate of group A was the highest. The multiple pregnancy rate of group C was the highest. Only two monozygotic twins occurred in group D. The micarriage rate of group A was the highest. No ectopic pregnancy occurred in group A, group B and group D. The ectopic pregnancy rate of group C and group E were significantly higher than group A. Only one birth defect case occurred in group E, the birth defect rate was significantly higher than group A.

Discussion

It is still controversial which is the most optimal number of embryo transfer for RIF patients. Since the rapid development of ART, the clinical pregnancy rate has improved continuously. Reducing the incidence of complication has been a main concern in reproductive medicine. Elective single-embryo transfer (eSET) has been widely accepted as a strategy to reduce the morbidity and healthcare costs associated with multiple birth [7, 8]. In 2013 the Practice Committee of the Society for Assisted Reproductive Technology and Practice Committee of the American Society for Reproductive Medicine (ASRM) recommended that young patients (under 35 years of age) should be transferred

one or two embryos. For old patients (above 35 years of age) who have a better prognosis, more than two embryos are suggested to be transferred. All patients must be counseled regarding their permanent medical record and the risks of multiple pregnancy [9]. Fujimoto reported that eSET led to increased CLBR in women under 37 years old but not in older women. So the significance of eSET in older women is limited [10]. In this study, the average age of each group was above 35, and those groups transferred two embryos (including fresh, frozen and mixed cycles) had significantly higher clinical pregnancy rate than single fresh embryo transfer and single frozen embryo transfer group (P<0.05). The embryo implantation rates of those two embryos transferred groups were also higher than two single embryo transfer groups, but no significant difference was observed (P>0.05). The average number of IVF cycles in the mixed cycles group was 4.24±2.79, significantly higher than the other groups (P<0.05). The data suggested that most of mixed cycles group were aged RIF patients. Those patients always had very limited number of available embryos, so combined fresh and frozen embryo transfer may be a good option for them.

In addition, there was no significant difference in clinical pregnancy rate among the three groups with two embryos transferred (P>0.05). However, the embryo implantation rate of mixed cycles group (66.67%) was significantly higher than transferred two fresh embryos group (44.05%) and transferred two frozen embryos group (30.95%) (P<0.05), and had better pregnancy outcomes. Meanwhile, compared with frozen cycles, mixed cycles cost less time and money.

This study also reveals that the multiple pregnancy rate of transferred two fresh embryos group was significant higher than transferred single fresh embryos group (P<0.05) and significant lower than transferred two frozen embryos group (P<0.05). The multiple pregnancy rate of mixed cycle group was lower than the other transferred two embryos group (P> 0.05). Therefore, mixed cycle strategy may not increase the risk of multiple births.

Ectopic pregnancy (EP) as a serious pregnancy complication accounts for 1-2% of all pregnancies, which usually causes hemorrhage and can be life threatening [11, 12]. Compared with natural conception, ART has increased incidence of EP, approximately 2.5 to 5 fold higher after IVF-ET [13]. The risk factors for EP include fallopian tube disease, previous EP, history of pelvic inflammatory disease, endometriosis, the age, smoking, use of intrauterine devices (IUDs), female sterilization and ART [14-16]. In our study, the EP rate of mixed cycles (0%) was significant lower than transferred two fresh embryos group (3.85%) and transferred two frozen embryos group (8.33%) (P<0.05). Considering the small sample size of this study, large scale studies are required to confirm our conclusion.

The miscarriage rate of mixed cycle group was the highest in five groups, and significantly higher than the group with one fresh embryo transferred (P<0.05). Early abortion rate, birth defect rate and sex ratio were similar among five groups (P>0.05). A multi-center large-scale study showed that live birth sex ratio in ART babies was influenced by the use of ICSI and blastocyst transfer [17]. Our study showed the imbalance of birth gender ratio, but birth gender ratio had no significant difference among different groups, which may be due to the small sample size.

In conclusion, clinical pregnancy rate and embryo implantation rate of mixed cycles were much higher. Thus we recommend mixing fresh and frozen embryos for patients who already have one frozen embryo. Mixed cycle would be a good choice for advanced RIF patients [18]. Large population-based studies are needed to confirm our conclusion.

Disclosure of conflict of interest

None.

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