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Original Article

Vitrified/warmed single blastocyst transfer in preimplantation genetic diagnosis/preimplantation genetic screening cycles

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Abstract: Objective: To investigate the single blastocyst transfer in preimplantation genetic diagnosis (PGD)/preimplantation genetic screening (PGS) cycles. Methods: 80 PGD/PGS cycles undergoing blastocyst biopsy were studied. There were 88 warming cycles during the study period. Only one warmed blastocyst was transferred per cycle. The outcomes were followed up to the infants were born. Results: The embryo implantation rate was 54.55% (48/88). The clinical pregnancy rate was 54.55% (48/88) per transfer cycle and 60% (48/80) per initial PGD/PGS cycle. There was no multi-pregnant in this study. The live birth rate was 42.05% (37/88) per transfer cycle and 46.25% (37/80) per initial PGD/PGS cycle. Conclusion: In PGD/PGS cycles, single blastocyst transfer reduces the multiple pregnancy rate without affecting the clinical outcomes.

Keywords: Preimplantation genetic diagnosis/preimplantation genetic screening, single blastocyst transfer, multiple births, vitrification

Introduction

Multiple pregnancies have become one of the most common complications of modern assisted reproductive technology (ART). To alleviate the adverse effect of multiple pregnancies on the outcome of pregnancy, researchers have proposed the strategy of elective single blastocyst transfer [1-3]. Although multifetal pregnancy reduction can reduce fetal number, it may result in the loss of all fetuses, and it is not an acceptable option for many couples. Transfer of single blastocyst with good developmental potential can not only improve embryo implantation rate, but also avoid or reduce maternal and neonatal complications caused by multiple pregnancies.

In preimplantation genetic diagnosis (PGD)/preimplantation genetic screening (PGS) cycles, the embryo to be transferred should meet both of the following criteria: i. Specific morphological criteria for transfer. ii. The genetic diagnosis is OK. The available embryos in PGD/PGS are limited. To ensure or improve the outcome of pregnancy in PGD/PGS cycles, the number of embryos that are transferred is not the focus to be considered in the majority of these cycles.

However, in recent years, trophectoderm-cell-biopsy and thawed blastocyst transfer regimen has been used for an increasing number of PGD/PGS cycles, which makes it possible to apply single blastocyst transfer in PGD/PGS cycles. This study retrospectively analyzed clinical data of PGD/PGS cycles with vitrified/warmed single blastocyst transfer in our center.

Materials and methods

This study was approved by the institutional review board of Peking University Third Hospital. We obtained written informed consent from every participant before the treatment.

A total of 162 PGD/PGS cycles underwent trophectoderm-cell-biopsy in Peking University

Table 1. The results of the single warmed blastocyst transfer in PGD/PGS cycles

Observation items	Results
Number of PGD/PGS cycles	80
Number of warming cycles	88
Mean age	30.24±4.076
Survival rate of the thawed blastocysts	96.70% (88/91)
Embryo implantation rate	54.55% (48/88)
Clinical pregnancy rate per transfer cycle	54.55% (48/88)
Clinical pregnancy rate per initial PGD/PGS cycle	60% (48/80)
Live birth rate per transfer cycle	42.05% (37/88)
Live birth rate per initial PGD/PGS cycle	46.25% (37/80)
Pregnancy duration (wk)	39.16±1.75
Premature delivery (<37 wk)	8.11% (3/37)
34-37 wk	2
<34 wk	1
Weight (g)	3406.94±557.10
Live-birth length (cm)	50.09±2.43

Third Hospital during January to October, 2013. The indication of the PGD cycles was chromosome translocation or invertion, the indication of the PGS cycles was recurrent miscarriage. After genetic examination, there were available blastocyst(s) to transfer in 127 cycles, and the available blastocysts were warmed and transferred in 80 cycles during this period. In this study, we mainly analyze the data of these 80 PGD/PGS cycles.

The ovarian stimulation protocols are the same as our previous described [4]. Ovarian activity was monitored by regular ultrasound scans and serum sex hormone assays. A dose of 10,000 U of hCG was administered when the leading cohort follicles reached a diameter of 18 to 20 mm. Oocyte retrieval was performed through vaginal puncture under ultrasound guidance 36 hours after hCG.

In all of these cycles, fertilization was performed by intracytoplasmic sperm injection (ICSI) on the day of oocyte retrieval. Zona pellucida drilling was conducted on day 3 of ICSI, followed by blastocyst culture. All of the cycles were subjected to trophectoderm-cell-biopsy by laser. The biopsied blastocysts were vitrificated individually. The morphological criterion for a blastocyst biopsy was a score above 5 BC according to Gardner's criterion [5]. After the genetic results were obtained, vitrified blastocysts were thawed and single blastocyst was transferred per cycle. The vitrification and warming protocol are the same as the previous described [6].

Pregnancy and obstetric outcomes were followed up until the infants were born. We recorded the survival rate of blastocysts, the implantation rate, the clinical pregnancy rate, and the live-birth rate. Moreover, gestational age, live-birth weight and live-birth length were recorded, too.

The genetic diagnosis was performed by 24 chromosomes analysis with array comparative genomic hybridization (CGH) or array single nucleotide polymorphism (SNP). Array CGH was performed on 24 sure-plus chips (Bluegenome). The Sure Plex DNA amplification system was used for whole genome amplification (WGA). Samples and control DNA were labeled with Cy3 and Cy5 fluorophores, then hybridized overnight. After laser scanning, Blue Fuse software was used to analyze microarray data concerning chromatin loss/gain across all 24 chromosomes [7]. SNP array was performed on Human CytoSNP-12V2.1 chips (Illumina). The biopsied cells were WGA using phi 29 polymerase to generate template DNA. Each DNA product underwent enzymatic end-point fragmentation, and the resuspended DNA samples were dispensed onto Human CytoSNP-12 DNA analysis bead chips (Illumnia) and allowed to hybridize for 12 hours. The bead chips were scanned using an Illumina iScan Bead Array Reader. Raw data analysis was accomplished using Illumina Genome Studio software [8].

Results

A total of 80 PGD/PGS couples who transferred their available blastocyste in the thawing cycles during the study period. The mean female age was 30.24±4.076 years old. 72 couples were transferred only once, while 8 couples were transferred twice. That is, there were 88 warming cycles during the study period. Only one blastocyst was transferred per warming cycle.

91 blastocysts were thawed in the study and 88 blastocysts were survival. The survival rate of blastocysts was 96.70%. 48 blastocysts

were implanted, the embryo implantation rate was 54.55% (48/88) (**Table 1**).

In 88 warming cycles, 48 cycles were pregnant. The clinical pregnancy rate was 54.55% (48/88) per transfer cycle and 60% (48/80) per initial PGD/PGS cycle. There was no multi-pregnant in this study.

Further analysis of the data of this study, 43 couples had clinical pregnancies after the first embryo transfer, accounting for a clinical pregnancy rate of 53.75% (43/80). Another eight couples underwent the second transfer cycle, of which five had a clinical pregnancy. At this stage, the cumulative clinical pregnancy rate of the 80 couples was 60% (48/80). Among the 32 non-pregnant couples, 23 couples still had available blastocysts to be thawed and transferred. That is, the 23 non-pregnant couples also may have a chance to be pregnant.

Among the 80 couples, 28 couples had only one available blastocyst to transfer and there were 15 cases of clinical pregnancy after transfer, accounting for a pregnancy rate of 53.57% (15/28). Additionally, 52 couples had ≥ two available blastocysts to transfer and there were 33 cases of clinical pregnancy after transfer, accounting for a pregnancy rate of 63.46% (33/52).

37 healthy babies were born in this study. All the karyotypes of the infants are consistent with the PGD/PGS results by amniocentesis. The live birth rate was 42.05% (37/88) per transfer cycle and 46.25% (37/80) per initial PGD/PGS cycle. The pregnancy duration was 39.16±1.75 weeks. The mean newborn weight was 3406.94±557.10 grams and the mean newborn length was 50.09±2.43 centimetres.

There are 3 premature deliveries in the study, whose pregnancy duration are 33 wk, 35 wk and 36 wk, respectively. The gravida whose pregnancy duration is 33 wk delivered a 1600 g healthy boy. No newborn malformation was found in this study.

Discussion

Although ART has been beneficial for many infertile families, it has also greatly increased the proportion of multiple pregnancies in humans. Over the past 30 years, the twin pregnancy rate in the total population has increased by 50%-70% and the triplet pregnancy rate has increased by 400% [9, 10]. Multiple pregnancies severely threaten maternal and newborn safety, which has increased maternal and neonatal complications (e.g., gestational heart disease, amniotic embolism, preeclampsia, gestational diabetes mellitus, and postpartum hemorrhage) by 3-7 fold and neonatal mortality and morbidity (e.g., premature birth, low birth weight of newborns, and cerebral palsy) by 4-10 fold [10, 11].

In 2002, the European Society of Human Reproduction and Embryology proposed and clearly defined the criterion for measuring treatment effects of ART: A single healthy birth [12]. Thereafter, elective single embryo transfer has been recommended in European countries and was gradually introduced to the United States, Japan, and Australia. In 2001, single embryo transfer accounted for 12% of total embryo transfer cycles in all of the European countries, and this rate increased to 20.8% by 2006. Despite the decreased number of embryos transferred, the overall pregnancy rate was not affected and even slightly increased [13]. In recent years, increasing emphasis has been placed on ART-related multiple pregnancies, and many medical centers have begun to use single embryo transfer in the high-risk group of multiple pregnancies and have achieved good pregnancy outcomes [14-16].

PGD/PGS involves genetic test of gametes or preimplantation embryos, and selects embryos without genetic material abnormalities for transfer into the uterus, thereby reducing or avoiding pregnancy with fetal genetic diseases [17]. PGD/PGS is established on the basis of in vitro fertilization-embryo transfer. The embryo that is eventually transferred in PGD/PGS cycles should not only comply with the morphological criterion, but also pass the genetic diagnosis. Therefore, available embryos of PGD/PGS are particularly valuable. To achieve a good pregnancy rate, the number of embryo(s) transferred is not the focus of consideration in PGD/PGS cycles.

In 2007, a study reported that single embryo transfer did not decrease the pregnancy rate in PGD patients in women aged less than 36 years, but it avoided maternal and neonatal

complications caused by multiple pregnancies [18]. More recently, Schoolcraft et al. [19] carried out chromosomal screening of embryos in elderly women in 2013 and a satisfactory pregnancy rate was achieved using the strategy of single thawed embryo transfer. Additionally, Forman et al. [20] randomly divided couples undergoing IVF into two groups: one group was transferred with a blastocyst after aneuploidy screening, and the other was transferred with two embryos without screening in accordance with the conventional regimen. They showed that there was no significant difference in the parturition rate between these two groups, but the double-embryo-transfer group had a significantly greater multiple pregnancy rate, preterm birth rate, proportion of low-birth-weight newborns, and hospitalization period of newborns in the neonatal intensive care unit than the single-blastocyst-transfer group.

We got the similar results with the previous studies. Our results show that only one embryo was transferred in each thawed cycle. The embryo implantation rate was 54.55% (48/88). There was no multi-pregnant in this study. The clinical pregnancy rate was 54.55% per transfer cycle and 60% per initial PGD/PGS cycle. The live birth rate was 42.05% (37/88) per transfer cycle and 46.25% (37/80) per initial PGD/PGS cycle. Of course, we want to compare these data to the data of the appropriate control group. But, from the beginning of blastocyst biopsy PGD/PGS in our reproductive center, we executed the regime of a single warmed blastocyst transfer. So, we don't have a matched "two blastocysts transfer PGD/PGS" group to be compared. However, the ordinary vitrification blastocysts' implantation rate was 42.9% in our IVF lab, and the clinical pregnancy rate and live birth rate was 47.6% and 39.8%, respectively [6]. The data of the present study was a little higher than that of the ordinary vitrification blastocysts. Single blastocyst transfer reduces the multiple pregnancy rate without affecting the clinical outcomes.

In this study, 43 couples got clinical pregnancy after the first transfer cycle (53.75%, 43/80) and 5 couples got clinical pregnancy after the second transfer cycle. During this period, the cumulative clinical pregnancy rate of the 80 couples was 60% (48/80). 23 out of the 32 non-pregnant couples still had available blastocysts to be thawed and transferred.

There was no multiple pregnant in our study group. The premature delivery rate was 8.11% (3/37), and there was no premature delivery less than 32 weeks. The pregnancy duration was 39.16±1.75 weeks. The mean newborn weight was 3406.94±557.10 grams and the mean newborn length was 50.09±2.43 centimetres. There was only one gestational diabetes mellitus in this study.

These years, more and more scholars pay attention to the safety of the embryo biopsy. In 2010, the Reproductive Center at the University of Ziekenhuis (Brussels, Belgium) reported on a cohort of 581 children born after blastomere biopsy (1992-2005). Their results showed that embryo biopsy does not increase the risk of single births but the higher mortality of multiple pregnancies in the perinatal period deserves further investigation [21]. Recently, trophectoderm biopsy is more popular [22]. In our study, we took the trophectoderm biopsy. The blastocyst implantation rate was 54.55%, higher than that of the ordinary vitrification blastocysts [6]. This result means two things, one is that the aneuploidy screening increase the implantation rate, the other is that the trophectoderm biopsy probably didn't reduce the ability of implantation.

Of course, there are some other limitations in our study. The mean female age in the study was 30.24±4.076 years old. The patients are relatively young, and the results couldn't stand for the results of the advanced maternal age. Moreover, the indication of the PGD/PGS in this study is chromosome aberration and recurrent abortion.

In summary, we retrospectively analyzed clinical data of PGD/PGS cycles with single warmed blastocyst transfer in our center. Application of single warmed blastocyst transfer does not reduce the clinical pregnancy rate in PGD/PGS cycles but decreases the multiple pregnancy rate. Therefore, applying a single warmed blastocyst transfer in PGD/PGS cycles is feasible.

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Disclosure of conflict of interest

None.

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