ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Obstetric and neonatal outcomes in blastocyst-stage biopsy with frozen embryo transfer and cleavage-stage biopsy with fresh embryo transfer after preimplantation genetic diagnosis/screening

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Objective: To study whether embryo biopsy for preimplantation genetic diagnosis/preimplantation genetic screening (PGD/PGS) can influence pregnancy complications and neonatal outcomes.
Design: Retrospective analysis.
Setting: University-affiliated center.
Patient(s): This study included data from women and their neonates born after PGD/PGS (n = 317).
Main Outcome Measure(s): Questionnaires were designed to obtain information relating to pregnancy complications and neonatal outcomes.
Intervention(s): Two major strategies for PGD/PGS were evaluated. Blastocyst-stage biopsy and frozen embryo transfer (BB-FET) was carried out in 166 patients, and cleavage-stage biopsy and fresh embryo transfer (CB-ET) was carried out in 129 patients.
Result(s): The incidence of gestational hypertension was significantly higher in BB-FET compared with in CB-ET (9.0% vs. 2.3%, adjusted odds ratio [OR] and 95% confidence interval [CI], 4.85 [1.34, 17.56]). In twins, the birthweight (median [range], 2.70 kg [1.55–3.60 kg] vs. 2.50 kg [1.23–3.75 kg]) was higher in BB-FET than in CB-ET and the gestational age was longer in BB-FET than in CB-ET (median [range], 36.71 weeks [31.14–39.29 weeks] vs. 35.57 weeks [30.57–38.43 weeks]). There was no difference in the incidence of singleton births between the two groups except in the incidence of preterm births (28–37 weeks; 5.3% vs. 16.5% in CB-ET and BB-FET). No significant differences were detected in the incidence of perinatal deaths, birth defects, gender of neonates, and large for gestational age in both singletons and twins, although the numbers of some events were small.
Conclusion(s): BB-FET is associated with a higher incidence of gestational hypertension but better neonatal outcomes compared with CB-ET, especially in twins. (Fertil Steril® 2016;■: ■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Preimplantation genetic diagnosis, blastocyst-stage biopsy, cleavage-stage biopsy, neonatal outcomes, pregnancy complications, frozen embryo transfer

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Received October 20, 2015; revised and accepted March 4, 2016.
S.J. has nothing to disclose. K.L. has nothing to disclose. H.H. has nothing to disclose. C.L. has nothing to disclose. S.Z. has nothing to disclose. Y.T. has nothing to disclose. F.G. has nothing to disclose. G.L. has nothing to disclose. G.L. has nothing to disclose.
Supported by grants from the Major State Basic Research Development Program of China (no. 2012CB944901), the National Science Foundation of China (no. 81222007), the Program for New Century Excellent Talents in University, and the Research Fund of National Health and Family Planning Commission of China (201402004).
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Fertility and Sterility® Vol. ■, No. ■, ■ 2016 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2016.03.010

Preimplantation genetic diagnosis (PGD) is an evolving technique that could end the transmission of genetic diseases or chromosome abnormalities from at-risk couples to offspring before implantation (1). The technology has also been applied as preimplantation genetic screening (PGS) to improve the effectiveness of assisted reproduction technology treatment in patients at high risk of embryonic chromosomal abnormalities, such as advanced maternal age, recurrent miscarriage, and repeated implantation failure. PGD/PGS techniques have been widely applied in clinics in recent years (2–5). According to the European Society for Human Reproduction and Embryology PGD consortium, as of 2010, a total of 45,163 retrieval cycles have been conducted worldwide, resulting in 9,499 clinical pregnancies and 8,694 newborns, and the numbers of newborns and cycles are increasing (6–8).

To obtain the material for analysis, embryos are usually biopsied at either the cleavage stage or the blastocyst stage. In the former case, one or two blastomeres are biopsied from day 3 embryos, and in the latter case, five to10 trophectoderm cells are biopsied from expanded blastocysts (9). In fact, almost 84% of all reported PGD cases have been performed at the cleavage stage, and <1% of the cases were biopsied at the blastocyst stage before 2010 (8). However, evidence from many studies has suggested that cleavage-stage biopsy might impact the developmental potential of embryos and impair the pregnancy and implantation rates compared with nonbiopsied embryos (10, 11). At this stage, only a small amount of material is available for genetic analysis, and the fact that a high mosaicism rate of chromosomal abnormalities exists in cleavage-stage embryos may also lead to increased inaccuracy of diagnosis for aneuploidy (12–14). In addition, many researchers have concerns about the safety of cleavage-stage biopsy. Several animal studies have demonstrated that the offspring of mouse embryos that underwent cleavage-stage biopsy were at potentially high risk of neurodegenerative disorders, mitochondrial diseases, aberrant epigenetic modification, reduced female ovarian function, and decreased birth weight (15–20). In humans, cleavage-stage biopsy was not associated with significantly increased risk, although a lower birthweight was found in twins compared with in babies born after intracytoplasmic sperm injection (ICSI), and the overall perinatal death rate was higher (21–26). Considering these disadvantages of cleavage-stage biopsy, several studies have recommended the use of blastocyst-stage biopsy instead (9, 27).

Blastocyst-stage biopsy can obtain more material for genetic analysis, and the incidence of mosaicism after blastocyst-stage biopsy is also reduced compared with after cleavage-stage biopsy (27–29). Furthermore, numerous reports have suggested that trophectoderm biopsy increases the implantation, pregnancy, and delivery rates (9, 27). However, owing to the limitations of genetic analysis, most of the biopsied blastocysts need to be cryopreserved by vitrification, and blastocysts with normal results would be transferred in the next frozen cycle. In the cleavage-stage biopsy and fresh embryo transfer (CB-ET) strategy, embryos were biopsied at day 3 and cultured in vitro to the blastocyst stage; there were 2 days between the biopsy and transfer. In the blastocyst-stage biopsy and frozez embryo transfer (BB-FET) strategy, however, the time available for analysis of blastocyst biopsy was reduced to a maximum of 24 hours. Thus, in the biopsy at day 5, freezing of the embryos cannot be delayed, which may be the biggest flaw in the BB-FET strategy. This may increase the risk in puerperae and offspring. Nevertheless, very few studies have investigated the safety of puerperae and offspring after blastocyst-stage biopsy. It remains to be determined whether blastocyst-stage biopsy and vitrification is safer than blastomere biopsy. Therefore, in this study we compared the safety of puerperae and offspring between the CB-ET and BB-FET strategies.

MATERIALS AND METHODS

Patients

This was a retrospective study conducted using the data of infertile women who underwent CB-ET/CB-FET between March 2008 and November 2012 or BB-FET between January 2012 and August 2013 at the Reproductive and Genetic Hospital of CITIC-Xiangya. The Ethical Committee of CITIC-Xiangya approved the study protocol (no. 31171379). Women who were over 28 weeks pregnant were included in this study, including those with late abortions and induced labor. Women were categorized according to age, body mass index (BMI), parity (nulliparity vs. multiparity), causes of infertility, methods of genetic testing, and genetic categories according to availability of transferable embryos after PGD (Robertsonian translocations, reciprocal translocations, other chromosomal abnormalities, single-genetic disorders, and PGS).

In all, 318 PGD/PGS cycles met our criteria. Of these, there was only one triplet, in the CE-ET group, so it was not included; thus, a total of 317 PGD cycles were included in our study. The patients were divided into three groups depending on the biopsy and ET approach: BB-FET (n = 166), CB-ET (n = 129), and CB-FET (n = 22).

IVF

Protocols of controlled ovarian hyperstimulation were carried out according to the ovarian reserve of patients as described elsewhere (30). Oocytes were collected 34–36 hours later after hCG administration through transvaginal ultrasound. All oocytes were fertilized by ICSI 4–6 hours after oocyte retrieval, and 16–18 hours after injection the oocytes were checked for normal fertilization. For further manipulation, all the embryos were cultured in sequential media (G1 and G2; Vitrolife) to blastocyst stage under 6% CO_2, 5% O_2, and 89% N_2 in a mini-incubator (Cook).

Embryo Biopsy and Diagnosis

Cleavage-stage biopsy was executed on day 3, and only one blastomere was biopsied. Briefly, one blastomere was collected by aspiration with a biopsy pipette (internal diameter, 40 μm) from a hole drilled by laser on the zona pellucida. Removal of a cell without lysis, rendering it available for fixation and subsequent analysis, was considered a successful biopsy (31). The blastomeres were used for fluorescence in