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ARTICLE

No adverse effects were identified on the perinatal outcomes after laser-assisted hatching treatment

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Abstract The aim of this study was to evaluate the safety of laser-assisted hatching (LAH) by comparing obstetric and neonatal outcomes between assisted hatching and control groups in cryopreserved embryo transfer cycles. A retrospective cohort analysis was carried out. A total of 699 women with 392 infants delivered were included. Laser- assisted hatching was carried out on D-3 thawed and warmed embryos before transfer in 480 cryopreserved embryos transfer cycles. Obstetric outcomes, neonatal outcomes, and congenital birth defects were recorded. A total of 815 cryopreserved embryo transfer cycles (480 in LAH group and 335 in control group) in 699 patients were analysed. Statistically significantly higher implantation (31.85% versus 16.95%), clinical pregnancy (53.96% versus 33.43%) and live delivery (44.58% versus 23.88%) rates were observed in the LAH group (all $P < 0.001$). For either singleton or multiple gestations, no statistically significant differences were found in mean gestational age, mean birth weight and mean Apgar score. Four major malformations occurred in the assisted hatching group and three malformations (one major and two minor) in the control group. This study did not identify any harmful effect of LAH on neonates, which suggested that LAH may be a safe treatment in cryopreserved embryo transfer cycles.

KEYWORDS: frozen-thawed embryo transfer, laser assisted hatching, live birth rate, malformation, vitrification

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Introduction

Hatching of the blastocyst is a critical step before implantation into the endometrium of the uterus. Failure to hatch is thought to be one of the factors limiting further embryo development (Cohen et al., 1990, 1992; Hammadeh et al., 2011). Assisted hatching artificially disrupting the zona pellucida has been proposed as a method for improving the capacity of the embryo to implant after IVF (Practice Committee, 2004, 2008). Although the available published evidence does not support its routine application in all IVF cycles at this time, assisted hatching is used as a strategy to improve clinical pregnancy rates, especially in cryopreserved embryo transfer cycles (Basak et al., 2006; Martins et al., 2011). A variety of techniques have been used for assisted hatching, including acid tyrodes, proteinases, piezon vibrator manipulators and lasers. Laser-assisted hatching (LAH) is used widely because it is easier to control and is more precise (Hsieh et al., 2002; Makrakis et al., 2006; Sagoskin et al., 2007).

The main question about the use of LAH, however, is whether thermal damage can affect embryos adversely (Cohen, 1991; Cohen et al., 1992; Makrakis et al., 2006). Despite the many trials already published, and the systematic reviews and meta-analyses conducted (Hammadeh et al., 2011; Martins et al., 2011), no proper conclusions can be drawn about live birth, spontaneous abortion and risk of malformation. Because of the small sample sizes in the prospective studies, no difference has been detected (Hammadeh et al., 2011; Martins et al., 2011; Practice Committee, 2008). Moreover, only a few studies have reported perinatal and neonatal outcomes (Hagemann et al., 2010; Kanyo and Konc, 2003; Sai et al., 2006). In view of insufficient clinical evidence on live births and neonatal outcomes, a retrospective cohort study was carried out to investigate the differences in perinatal and neonatal outcomes between an LAH group and a control group. Although this was a retrospective cohort study, it included a large sample size reporting the follow-up of children born after LAH treatment (the largest reported sample size as far as is known).

Materials and methods

Patients

The Assisted Reproduction Centre is an outpatient clinic in Shaanxi Province, China. In this study, data from the centre's IVF database were retrospectively analysed. The study was approved by the Institutional Review Board of the Maternal and Child Health Care Hospital of Shaanxi Province. A total of 843 frozen–thaw cycles were carried out between January 2008 and August 2010. Sixteen cycles had no surviving embryos for transfer, and blastocysts were transferred in 12 cycles. A total of 815 embryo transfer cycles with day 3 cryopreserved embryo transfer in 699 patients were analysed in this study. Assisted hatching was gradually carried out on all day 3 embryos in cryopreserved embryo transfer cycles from June 2009. Not all cycles were allocated to the assisted hatching group between June and August 2009. As a new technology when LAH was introduced, patients were invited to undergo this treatment if they had previous failed cycles (≥1), were more than 35 years of age, and zona pellucida abnormalities were observed. After confirming no adverse results (unpublished observations), after August 2009, assisted hatching was routinely used in all cryopreservation cycles. The trial was approved by the Ethics Committee of Maternal and Child Health Care Hospital of Shaanxi Province (5 April 2009). All patients undergoing LAH treatment voluntarily gave their informed consent from the initial introduction of the treatment. No LAH treatment was carried out if patients refused it. The LAH group constituted all day 3 cryopreserved embryo transfer cycles with LAH treatment, and the control group constituted all the day 3 cryopreserved embryo transfer cycles without LAH treatment. Therefore, the study is limited by the fact that patients in each group were not matched, and had their treatments at different times. Other procedures were similar between two groups.

Clinical procedures

The clinical procedure was carried out according to standard protocols, as previously described (Shi et al., 2012, 2013). For the cryopreserved embryo transfer cycles, endometrial preparation was carried out in both spontaneous natural and artificial cycles. Exogenous oestrogen and progestogen were administered to prime the endometrium in artificial cycles but not natural cycles. A total of 60 mg per day of progesterone (injection) was added 1 day before the transfer in both natural and artificial endometria preparation. Embryos were incubated in media (G-Series™, Vitrolife AB, Göteborg, Sweden). Embryos were cryopreserved when patients had surplus embryos available after fresh embryo transfer or when they were at risk of ovarian hyperstimulation syndrome (OHSS). Embryos were also cryopreserved in patients who presented with fluid in the endometrial cavity, hydrosalpinx, abnormal endometrium and acute marital problems. Both slow-freezing and vitrification methods of cryopreservation were used. Tools and solutions required for slow-freezing were obtained from Vitrolive (FreezeKit/ThawKit Cleave™, Vitrolife AB, Göteborg, Sweden). For vitrification, they were from Kitazato (Kitazato BioPharma Co.). All procedures were carried out in accordance with standard protocols, as previously described (Shi et al., 2012, 2013).

Assisted-hatching procedures

Assisted hatching was carried out on cleavage-stage (day 3) embryos in cryopreserved embryo transfer cycles with a laser treatment (ZILOS-tk; Hamilton Thorne Instruments Biosciences, Beverly, MA01915, USA). The point in the circle of the laser beam was focused where the perivitelline space was widest. With a setting of 50–100% power and 500 μs plus duration, the zona pellucida was thinned to more than two-thirds of its initial thickness and a distance of 30–40 mm. To avoid causing thermal radiation damage to the blastomeres, the point of assisted hatching on the zona pellucida was selected where the blastomere membrane was furthest from its inner edge.

Outcome measures and statistical analysis

The obstetric outcome measures were implantation, clinical pregnancy, spontaneous abortion, preterm delivery