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SHORT COMMUNICATION

Implantation of fresh and thawed–warmed embryos in single embryo transfer cycles: interpreting the initial beta-HCG

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Abstract Little is known about the effects of human embryo cryopreservation on developmental potential. Initial beta-HCG, indicating embryo implantation, was measured in 322 single embryo transfer cycles (246 fresh and 76 thawed–warmed). Median initial beta-HCG was higher for fresh compared with thawed–warmed transfers (126 versus 100 mIU/ml; $P = 0.04$). Blastocyst slow cooling resulted in a lower initial beta-HCG compared with vitrification ($P = 0.01$). Live birth rates were lower for blastocyst slow cooling (25%) compared with vitrification (71%) and fresh transfer (70%). We conclude that cryopreservation may impair an embryo's ability to produce beta-HCG, but that vitrification does not impair developmental potential.

KEYWORDS: blastocyst, cryopreservation, embryos, single embryo transfer, vitrification, βhCG

Introduction

Cryopreservation of embryos with subsequent warming and transfer has become a widespread and successful mechanism for achieving pregnancy. In 2012, the Society for Assisted Reproductive Technology reported 33,035 autologous embryo transfer cycles using cryopreserved embryos in the USA, with a live birth rate of about 38% when an average of 1.8 embryos were transferred. Slow cooling methods for embryo cryopreservation have been used in the past, although vitrification methods for blastocysts have been favoured by some recently (Herrero et al., 2011). Little, however, is known about the effects of cryopreservation on an embryo's ability to produce beta-HCG and its developmental potential compared with fresh cycle transfers. Compared with fresh cycles, a lower initial beta-HCG level in thawed–warmed cycles may indicate a

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compromised trophoblast. Our objective was to determine the effect of cryopreservation on the embryo's ability to produce beta-HCG and its developmental potential.

Materials and methods

The study was approved by Baystate Medical Center Institutional Review Board on 12 March 2014 (Reference: BH-11-139). We interrogated our assisted reproduction techniques database at Baystate Reproductive Medicine for all single embryo fresh and thawed–warmed transfer cycles carried out between January 1, 2007 and December 31, 2012. We chose to study only single embryo transfer cycles to eliminate the confusion about interpretation of the initial beta-HCG if multiple embryos implanted. A total of 374 cycles were identified in which the initial serum beta-HCG was greater than 10 mIU/ml (285 fresh and 89 thawed–warmed). Only patients having blood drawn between 07:00 and 10:00 for the initial beta-HCG level 9 days after blastocyst transfer or 11 days after cleavage stage transfer were included in this study (n = 32 fresh and 13 thawed–warmed cycles excluded). Patients pregnant with twins after single-embryo transfer were also excluded (seven fresh cycles), leaving 322 cycles available for analysis (246 fresh and 76 thawed–warmed).

Immunoassays during this entire study using the sandwich principle for beta-HCG were carried out at the Baystate Medical Center Hospital Chemistry Laboratory, and were standardized against the fourth international standard for chorionic gonadotropin (Roche Diagnostics USA, Indianapolis, IN, USA). Inter and intra-assay coefficients of variation for beta-HCG were less than 10%.

During the study period, embryos not transferred during a fresh cycle were cryopreserved at the cleavage stage or cultured to the blastocyst stage and cryopreserved. Between 2007 and 2012, our laboratory used different methods for embryo cryopreservation as the technology evolved. From 1 January 2007 to 1 July 2009, slow cooling cryopreservation and thawing of cleaved embryos (n = 6) and blastocysts (n = 32) were carried out using commercially available solutions following the manufacturer's instructions (Irvine Scientific, Santa Ana, CA, USA and Sage Biopharma, Bedminster, NJ, USA) and programmable controlled rate freezing. Blastocyst vitrification and warming were carried out using commercially available solutions and manufacturer's instructions (Innovative Cryo Enterprises, Rockaway, NJ, USA). Blastocysts were vitrified either in Cry Bio System straws (original vitrification: n = 17, 2 July 2009 to 31 August 2010) or in a stripper tip (microsecure vitrification: n = 21, 1 September 2010 to 31 December 2012). All cryopreserved, thawed–warmed embryos were held in embryo transfer media until transferred into the uterus. During the study period, no other substantial changes were made in the laboratory or in clinical stimulation protocols.

Patient treatment protocols for fresh IVF cycles included pituitary down-regulation with gonadotrophin-releasing hormone (GnRH) agonists, diluted GnRH agonist administered after oral contraceptives, or oestradiol patch administered before gonadotrophins with GnRH antagonist pituitary down regulation. When leading follicles reached 18–20 mm diameter, HCG was administered, and egg retrieval was carried out 36 h later. Fertilization resulted from conventional insemination or intracytoplasmic injection *in vitro*, and embryos were cultured in protein supplemented Quinn's Advantage® sequential culture media system (Sage Biopharma, Bedminster, NJ, USA) until reaching the blastocyst stage. Starting on the day after egg retrieval, patients initiated luteal phase support consisting of two oestradiol patches (Vivelle Dot 0.1 mg, Novartis Pharmaceuticals Corporation, USA) and vaginal progesterone three times daily (Prometrium 200 mg, AbbVie Products, LLC, Abbott Park, IL, USA), and continued these until 6 weeks gestational age if pregnant for fresh cycle embryo transfers.

For cryopreserved embryo transfer cycles, patients began oestradiol patches (Vivelle Dot 0.1 mg) on the first day of menses, and increased up to four patches daily on day 12. Vaginal progesterone (Prometrium 200 mg) was initiated on day 14. Cleavage stage embryos were transferred after 5 days, and blastocysts were transferred after 7 days of progesterone. Oestradiol patches were continued until 8 weeks, and vaginal progesterone was continued until 12 weeks gestational age for thawed–warmed transfers.

Mean and median patient ages were compared, and the initial beta-HCG values for fresh versus thawed–warmed embryo transfers using the unpaired Student's t-test and the Kruskal–Wallis rank sum test, respectively, with significance for differences between groups accepted at $P < 0.05$.

Results

The mean age of patients at the time of embryo transfer for fresh cycles and at the time of retrieval for thawed–warmed cycles did not differ significantly (32.3 versus 32.0 years). Median ± interquartile ranges of the initial beta-HCG values by type of embryo transfer are shown in **Figure 1**. No differences were observed in median initial beta-HCG values between fresh cleavage stage and fresh blastocyst transfers (132.8 mIU/ml versus 141.9 mIU/ml). Embryo cryopreservation, collectively, however, resulted in a significantly lower initial serum beta-HCG level compared with fresh transfer cycles (median cryopreserved 100 mIU/ml versus median fresh 126 mIU/ml; $P = 0.04$). In addition, a significant difference was found between cryopreserved transfer cycle methods with respect to initial median beta-HCG, with slow cooling blastocysts having an initial beta-HCG of 88.9 mIU/ml compared with blastocyst original vitrification beta-HCG pf 182.6 mIU/ml ($P = 0.01$).

In our cycles, with an initial beta-HCG greater than 10 mIU/ml, the overall clinical pregnancy rate per transfer was 244 out of 322 (75.8%), with clinical pregnancy rates of 81.0% (17/21), 76.5% (13/17), 100% (6/6), 34.4% (11/32), 78.8% (67/85), and 80.7% (130/161) for microsecure blastocyst vitrification, original blastocyst vitrification, slow cooling cleavage, slow cooling blastocyst, fresh cleavage, and fresh blastocyst transfers, respectively. In these cycles, our overall live birth rate was 213 out of 322 (66.1%), with live birth rates of 71.4% (15/21), 70.6% (12/17), 100% (6/6), 25.0% (8/32), 65.9% (56/85), and 72.0% (116/161), respectively. In cases in which a live birth occurred, the median initial beta-HCG was higher than in cases in which a live birth did not occur (149 versus 66 mIU/ml; $P < 0.001$).

Discussion

The analysis of single-embryo transfer cycles and initial beta-HCG values drawn at standardized times after embryo transfer

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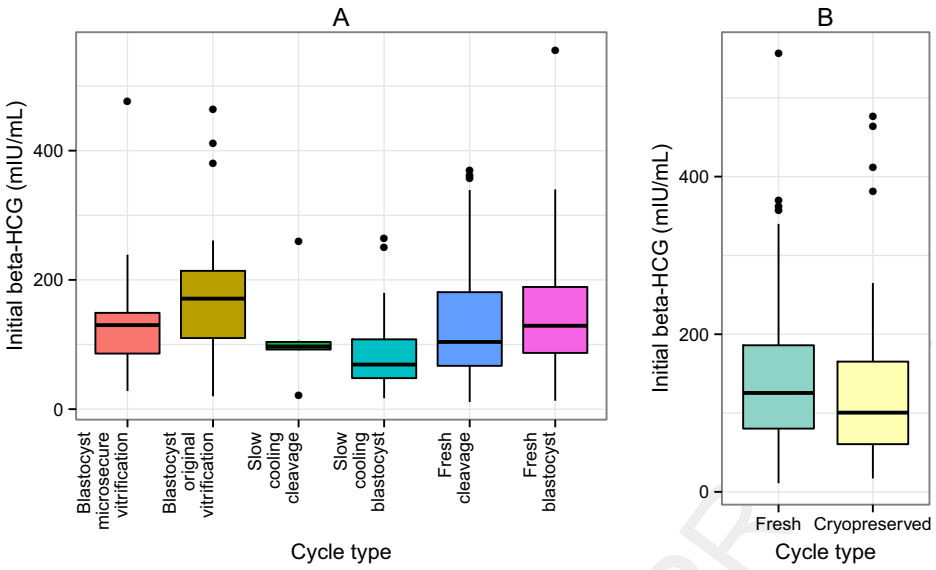

Figure 1 Initial beta-HCG value by type of single-embryo transfer. (A) The initial beta-HCG after transfer of cryopreserved slow cooling blastocysts was significantly lower than that of blastocysts transferred after the original vitrification method ($P = 0.01$); (B) the initial beta-HCG after transfer of cryopreserved embryos was lower than that after transfer of fresh embryos ($P = 0.04$). Values are median ± interquartile ranges.

gives insight into the effect of embryo cryopreservation on the ability of an embryo to produce beta-HCG and its developmental potential. Lower initial beta-HCG levels were found after embryo transfers with cryopreserved embryos compared with fresh embryos. This may be due to apoptotic damage to blastocysts as a result of the cryopreservation, warming processes, or both (Li et al., 2012). Live birth rates were similar between fresh transfers and most methods of embryo cryopreservation, other than following blastocyst slow cooling, suggesting that most methods of cryopreservation do not affect an embryo's developmental potential. To the best of our knowledge, this is the first comparison of fresh and thawed–warmed single-embryo transfers in relation to initial beta-HCG and developmental potential.

Similar initial beta-HCG levels were found in patients having fresh cleavage compared with fresh blastocyst single embryo transfers. These findings differ from those of Zhang et al. (2003), who found lower initial beta-HCG concentrations in pregnancies resulting from blastocyst compared with cleavage stage transfers, and to those of Kathiresan et al. (2011), who found higher initial beta-HCG levels after blastocyst compared with cleavage stage transfers. Limitations of both studies include the transfer of multiple embryos. Our results include only single-embryo transfers, and thus reflect implantation of only one embryo and the effect of cryopreservation on its trophoblast and developmental potential.

The effect of various cleavage-stage embryo cryopreservation methods on the initial beta-HCG has been reported recently (Xue et al., 2014). Median initial beta-HCG after transfer of vitrified-warmed cleavage stage embryos was lower than that after transfer of slow cooled–thawed cleavage-stage embryos. Multiple embryos were transferred in this study, limiting the interpretation of results. The present study is unique in comparing fresh and various cryopreserved thawed–warmed single embryo transfers, including blastocyst transfers and developmental potential.

This retrospective study is limited because various methods of embryo cryopreservation were used over time, with small numbers of embryos in some groups. Our analysis of 322 single-embryo transfers, however, including fresh and cryoprserserved thawed–warmed, with standardized initial beta-HCG testing, is unique and helpful in interpreting the effect of cryopreservation on the trophoblast and viability of embryos.

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