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ARTICLE

Comparison of gender-specific human embryo development characteristics by time-lapse technology

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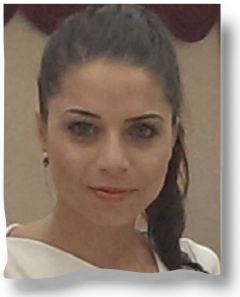

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Abstract Numerous studies indicate that there might be differences in embryo growth dynamics between male and female embryos. However, current data in humans are scarce and the results are inconclusive or conflicting. This study asks whether there exist gender-specific embryo development kinetics or parameters between human male and female embryos that can be observed by time-lapse technology. Study included data from 139 consecutive cycles (177 embryos transferred, 179 sacs analysed) with positive pregnancy that resulted in 100% implantation. Single- or double-embryo transfers were performed. Cases were analysed for parameters including cleavage time points and duration in each cleavage from two cells to hatching blastocyst stages and time interval between cleavages. Morphokinetic parameters of 78 female and 60 male embryos from a total of 119 cycles (139 sacs were examined after transfer of 138 embryos) were processed for data analysis according to the gender group. A detailed analysis of the data regarding each time point or interval between consecutive events according to these groups showed them to be similar in cell division kinetics, from the early cleavage through their development to blastocyst stage. However, female embryos showed earlier cavitation than male embryos, but the results did not reach statistical significance.

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Introduction

The sex of the preimplantation embryo is primarily determined at fertilization by the spermatozoon which carries either X or Y chromosome (Alomar et al., 2008; Setti et al., 2012). However, due to numerous confounding factors such as the weight of the mother, age of the parents, family size, stress, geographic and climatic conditions and environmental toxins,

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the sex ratio of the offspring at birth can be slightly skewed to either male or female (James, 2006a, 2006b).

Several animal studies have analysed the kinetics of male and female embryo development and it has been reported that the gender has an effect on the kinetics and metabolism of early embryo development (Alomar et al., 2008; Bermejo-Alvarez et al., 2011; Holm et al., 1998; Kochhar et al., 2001). In some cases, it was found that embryo development rate is higher in male embryos, whereas other studies have shown that female embryos cleave significantly faster (Avery et al., 1991; Bernardi and Delouis, 1996; Valdivia et al., 1993). Also, there are reports indicating that no differences exist in terms of the pace of embryo development between both sexes (Holm et al., 1998; King et al., 1991; Totey et al., 1996; Yadav et al., 1993).

Mammalian studies, including humans, have also tried to establish a similar correlation between embryo development characteristics and gender. Several studies have shown that male embryos develop significantly faster than female embryos (Alfarawati et al., 2011; Hentemann et al., 2009; Menezo et al., 1999; Tarin et al., 1995). But so far, other published studies have not reported such a shift, and no relationship between male and female sex ratio has been found (Fanchin et al., 1998; Ng et al., 1995; Weston et al., 2009). Until now, the results in both animals and humans are inconclusive.

When study designs of these reports are analysed, the relationship between the pace of embryo development and sex ratios were mainly investigated by observational studies utilizing static embryo culture conditions and scoring criteria (Kochhar et al., 2001; Richter et al., 2006). Nowadays, the recent development of time-lapse technology has allowed monitoring of embryo development and early morphokinetic changes in real time (Chen et al., 2013; Wong et al., 2013). Effective use of time-lapse technology in human IVF in delineating the developmental clues regarding the implantation potential of developing human preimplantation embryos has already produced promising results (Basile et al., 2013; Campbell et al., 2013; Cruz et al., 2011; Dal Canto et al., 2012; Holm et al., 1998; Kirkegaard et al., 2012a; Meseguer et al., 2011, 2012; Montag et al., 2011; Wong et al., 2010). As far as is known, there is no study investigating sex-associated differences in human preimplantation embryos that have been analysed by using time-lapse image technology. The aim of the present study was to determine whether there is any relationship between gender-specific embryo development kinetics and parameters from the early cleavage stages of development up to hatching blastocyst stage in human male and female embryos by time-lapse technology.

Materials and methods

The present research is a retrospective observational study, which was conducted at Bahceci Umut and Fulya assisted reproduction technology centres. The study was approved by the Bahceci Health Group Ethics Committee (reference no. 22, approved 1 April 2013). Cycles performed between March 2011 and March 2013 were included in this analysis. In all cycles, ejaculated spermatozoa were used and embryos were cultured in a special tri-gas incubator with built-in time-lapse monitoring system. A total of 139 consecutive cycles (177 embryos) were evaluated. The gender of the offsprings was first assessed through ultrasonography performed at 14–16 weeks of gestation and then confirmed with live birth outcome. Embryos with <100% implantation or 100% implanted twins with two different genders (female/male) were excluded from the analysis. In addition, patients, who had preimplantation genetic diagnosis or testicular sperm extraction during treatment or endometrial factor were excluded.

Ovarian stimulation

Ovarian stimulation was performed as described previously by this study group (Ulug et al., 2007). When at least two follicles reached 18 mm in diameter, human chorionic gonadotrophin (HCG) injection (5000 IU) (Ovidrelle; Merck Serono, UK) was administered in order to induce ovulation.

Oocyte recruitment, denudation, ICSI and embryo culture

Oocyte aspiration was performed 35–36 h after HCG administration under ultrasound guidance. Oocytes were washed and collected into 50-μl drops of modified human tubal medium (mHTF; Irvine Scientific, CA, USA) in 10% synthetic serum substitute (SSS; Irvine Scientific), which was kept under humidified and heated conditions (2 h at 37°C, 6% CO_2 and 5% O_2) in a mini-incubator placed in a laminar flow cabinet for 2 h until denudation. O_2O_2Enzymic removal of granulosa cells was performed using hyaluronidase (Irvine Scientific). Patients' semen samples were processed using discontinuous colloidal silica gel gradient (PureSperm; Nidacon, Sweden) and sperm pellets were washed twice with sperm-washing medium (Irvine Scientific). Following denudation, intracytoplasmic sperm injection (ICSI) was performed in mHTF containing HEPES for 1 h and microinjected oocytes were cultured individually in a special pre-equilibrated culture dish (EmbryoSlide, Unisense FertiliTech, Aarhus, Denmark) until the day of embryo transfer. On day 3 of embryo development, embryos were transferred into a new EmbryoSlide dish that was pre-equilibrated with single-step media (SSM; Irvine Scientific) and 10% SSS as a laboratory procedure; in this study, only SSM supplemented with 10% SSS was used for embryo culture. EmbryoSlide wells were filled with 25–30 μl SSM and covered with 1.4 ml mineral oil (Irvine Scientific) to prevent evaporation and equilibrated overnight before use. All oocytes/embryos were cultured in a time-lapse incubator (EmbryoScope, UniSense Fertilitech) at 37°C, 6% CO_2 and 5% O_2, which is a tri-gas incubator with a built-in microscope that captures images automatically for up to 72 individual embryos every 10 min from seven different focal planes during embryo development.

Analysis of morphokinetic parameters and embryo morphology

Time-lapse morphokinetic assessment was performed as described by Meseguer et al. (2011). Analysed parameters were cleavage times and duration for each cleavage from 2 cells

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