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ARTICLE

Sibling embryo blastocyst development as a prognostic factor for the outcome of day-3 embryo transfer

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Abstract This study assessed the development of sibling embryos to blastocyst as a prognostic factor for patients undergoing embryo transfer at day 3. A retrospective analysis of a clinical and embryology database including 353 patients who underwent 393 cycles of intracytoplasmic sperm injection with day-3 embryo transfer and excess embryos, maintained in culture until day 5, was performed. Cycles were divided into group A and group B (with and without blastocyst formation, respectively). Age and basal FSH were similar in both groups. Statistically significant differences in clinical pregnancy rates (55.8% versus 40.6%; $P = 0.0031$), live birth rates (50.0% versus 37.2%; $P = 0.012$) and implantation rates (34.2% versus 23.7%; $P = 0.0035$) were observed in groups A and B, respectively. Odds ratios showed women from group A had 1.85- and 1.68-times the odds of patients from group B of achieving clinical pregnancy and a live birth, respectively. Cumulative live birth rate for group A, after one cycle of vitrified–warmed blastocyst transfer, was 66.4%. The development of sibling embryos to blastocyst is a prognostic factor for the outcome of the cycle in which transfer is performed at day 3 and provides valuable information about the prognosis of subsequent cycles.

KEYWORDS: blastocysts, cumulative live birth rate, cumulative pregnancy rate, sibling embryos

Introduction

Multiple factors influence the outcome of assisted reproduction treatment, the most relevant being female age, duration of infertility, ovarian reserve and embryo quality (Hathaway et al., 1999; van Loendersloot et al., 2010). For many years these elements have been considered as prognostic factors for becoming pregnant or having a live birth in a particular treatment cycle (Minaretzis et al., 1998). However, in the last decade the focus has moved towards predicting the probability of singleton or twin pregnancies when two cleaving embryos could be transferred

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(Hunault et al., 2007), in order to transfer them or to perform an elective single-embryo transfer (SET) of one selected cleavage-stage embryo and avoid a multiple pregnancy (De Sutter et al., 2003; Practice Committee of Society for Assisted Reproductive and and Practice Committee of American Society for Reproductive, 2012). Furthermore, when enough good-quality embryos are available at day 3, an extended culture until day 5 is advisable, in order to improve embryo selection and pregnancy rates (Papanikolaou et al., 2005, 2008). But patients without four or five good-quality embryos at day 3 do not have the minimum requirements for extended embryo culture until day 5 and must undergo cleavage-stage embryo transfer (Papanikolaou et al., 2005; Dessolle et al., 2010), because of the risk of cycle cancellation due to a lack of blastocysts.

Many embryo selection criteria for cleavage-stage embryos have been developed in order to choose the best embryos and reduce the number of them to be transferred at day 3 (Geraedts and Gianaroli, 2012), although, embryo selection at this stage has some limitations. Indeed, Rijnders and Jansen (1998) reported that only 51% of embryos that were transferred on day 5 had been preselected for transfer on day 3. Therefore, it is difficult to predict the result of assisted reproduction treatments based on the quality of embryos transferred at day 3, and other criteria have to be introduced to establish the prognosis of the treatment cycle. These could be previously known clinical criteria of patients (van Loendersloot et al., 2010) or data coming from the embryology laboratory in the same cycle in which the transfer is performed. This information can also be considered to define patients that are eligible for elective SET or single-blastocyst transfer in future cycles.

The main objective of this study is to assess the development of sibling embryos to blastocyst stage as a prognostic factor for patients undergoing embryo transfer at day 3.

Materials and methods

This is a retrospective analysis of the embryology database from January 2006 to December 2011 at the Unit of Reproductive Medicine of the department of obstetrics and gynaecology at Clinica Las Condes, Chile. All patients signed institutional review board approved consent forms before initiating and any patient specific identifiers were removed from database prior to the analysis. Retrospective approval for this study was granted by the ethical committee of Clinica Las Condes on 30 January 2013.

In total, 353 patients, who underwent assisted reproduction treatment in 393 cycles, had embryos transferred at day 3 and had excess embryos that remained in culture until day 5 were included in this study. The age of women was (mean ± SD) 34.5 ± 3.8 years and their basal FSH was 7.1 ± 2.7 mIU/ml.

Intracytoplasmic sperm injection was performed in all cases. The oocytes were stripped of their cumulus cells after incubation in hyaluronidase solution (80 IU/ml; Life Global, USA) for 60 s. Spermatozoa were selected by the swim-up procedure and sperm injection was performed with holding and injection micropipettes (Humagen, USA) under an Olympus inverted microscope and a Narishige micromanipulation system. Injected oocytes were incubated in global fertilization media (Life Global) in a 6% CO_2 incubator (Forma Scientific, USA). Eighteen hours after sperm injection, oocytes were inspected for fertilization and those fertilized were transferred to fresh global media (Life Global, USA). A total of 5020 zygotes from 393 oocyte retrievals were followed from day 1 to day 3. Embryo morphology was assessed at day 3 using criteria reported by Veeck (1999), which considers cell number, uniformity of blastomere size and extent of fragmentation. Each embryo was designated based on its cell number and grade of uniformity and fragmentation, as follows; grade 1, symmetric blastomeres without fragmentation; grade 2a, symmetric blastomeres and <20% fragmentation; grade 2b, symmetric blastomeres and 20–50% fragmentation; grade 3, uneven size of blastomeres, without fragmentation; and grade 4, uneven blastomeres with fragmentation.

A total of 960 embryos were transferred at day 3 with a mean of 2.44 ± 0.55 embryos per patient. Of these, 748 (77.9%) were classified as good-quality embryos (grades 1 and 2a). Embryo transfer was performed with an ultrasound-guided Frydman ultra-soft catheter (CCD, France). Fourteen days after oocyte retrieval the patients were tested for pregnancy (β-human chorionic gonadotrophin) and, if pregnant, an ultrasound was scheduled 1 and 2 weeks later to confirm a clinical pregnancy, register the number of gestational sacs and the presence of embryos with heartbeats. Pregnancies were followed until 24 weeks of amenorrhoea, to register those ending in miscarriages, and then until the delivery in all remaining cases.

A total of 4060 excess embryos remained in culture and were followed until day 5, 459 of them were classified as good quality or grade 1 and 2a (11.3%). Blastocyst formation was evaluated at day 5, using criteria reported by Veeck and Zaninovic (2003). A total of 350 embryos reached this stage and were vitrified using the Cryotop Kitazato method, initially described by Kuwayama et al. (2005). For warming, the Cryotop was removed from the liquid nitrogen and instantly placed in thawing solution at 37°C; after 1 min, blastocysts were placed in diluting solution at room temperature for 3 min and, finally, a 5-min wash followed by a 1-min wash was performed with washing solution at room temperature and processed as described by the manufacturer (Kitazato). Viability was assessed and transfer of vitrified–warmed blastocysts was performed in cycles with hormone replacement with oral oestradiol valerate and vaginal progesterone.

For the purposes of this study, the 393 sample cycles were divided into two groups: those who had at least one blastocyst at day 5 (group A) and those who did not have blastocysts at day 5 (group B). For statistical analysis Stata 12 software (Stata Corporation, College Station, Texas, USA) was used. The independent variable was blastocyst and primary outcomes were clinical pregnancy rate (transvaginal ultrasound confirmation of a gestational sac inside the uterine cavity) and live birth rate. Secondary outcomes were implantation rate (number of gestational sacs observed divided by the number of embryos transferred), multiple pregnancy rate and miscarriage rate (number of spontaneous clinical pregnancy losses divided by the number of clinical pregnancies). Continuous variables were described by mean ± standard deviation (normal distribution) and categorical variables by proportions and rates,