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ARTICLE

Extended culture of vitrified–warmed embryos in day-3 embryo transfer cycles: a randomized controlled pilot study

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Abstract Synchronization between embryonic stage and endometrium is vital to achieve a successful pregnancy. The objective of this study was to assess the implantation, clinical pregnancy and live birth rates of cryopreserved embryo transfer cycles using embryos after extended culture for 16 h. A prospective randomized controlled pilot study was performed on women who underwent vitrified–warmed embryo transfer. Of the 540 women assessed for eligibility, 479 were randomly allocated to either extended culture for 16–18 h (EC group, $n = 242$) or conventional culture for 2 h (control group, $n = 237$). Endometrial preparation was the same in both groups. No significant differences were found between the extended culture and control groups respectively in clinical pregnancy rate per embryo transfer (42.48% versus 40.95%), implantation rate (21.79% versus 20.82%) or live birth rate per embryo transfer (37.61% versus 34.05%); however, the spontaneous reduction rate was lower in the extended culture group (10.04% versus 20.80%; $P = 0.032$) In conclusion, extended culture of day-3 cleavage embryos for 16 h would not influence the pregnancy outcome of day-3 cryopreserved embryo transfer cycles.

KEYWORDS: cryopreserved embryo transfer, endometrium, extended culture, implantation, pregnancy outcome, synchronization

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Introduction

Selective embryo transfer, in combination with cryopreservation of surplus high-quality embryos (Grady et al., 2012), significantly reduces multiple pregnancy risks and cryopreserved embryos can subsequently be used for later transfer, thus improving cumulative pregnancy rate of a single IVF cycle. Also, in cases of ovarian hyperstimulation syndrome (Griesinger et al., 2007; Griesinger et al., 2011) and premature luteinization (Bosch et al., 2003; Van Vaerenbergh et al., 2011), the retrieved oocytes and generated embryos can be vitrified and transferred into a more favourable uterine environment (Krikun et al., 2005; Liu et al., 2008).

For a successful implantation to be achieved in a cryopreserved embryo transfer cycle, it is crucial to transfer embryos in the implantation window, which is limited to 4–8 days after endogenous or exogenous progesterone stimulation. Using ultrasound, endometrial thickness (Kehila et al., 2010), endometrial morphological aspects (Bourgain and Devroey, 2007) and endometrial and sub-endometrial blood flow (Wang et al., 2010) are useful indicators to predict endometrial receptivity to embryonic implantation. However, although most precautions are undertaken, approximately two-thirds of embryo transfers fail to result in implantation, possibly due to inadequate endometrial receptivity or defects of the embryo–endometrium cross-talk (Altmae et al., 2012; Simon et al., 1998). An alternative hypothesis is that the endometrium only plays a generally permissive role; the decisive step is the actual synchronization between the viable embryo and the receptive endometrium.

Previous studies on endometrial receptivity show that the out-of-phase endometrium is detrimental to embryo viability and implantation, but to maintain the synchronization is difficult in practice. It has been suggested that the impairment of synchronization is due to the high concentration of steroids resulting from ovarian stimulation affecting the endometrium (Krikun et al., 2005; Liu et al., 2008; Wilcox et al., 1999), suboptimal culture conditions (Waldenstrom et al., 2009) and media (Gruber and Klein, 2011) which affect embryo quality. Asynchronization, influenced by a delay in development of the embryo or endometrial advancement, induces implantation failure.

A degree of asynchronization between the endometrium and embryo is acceptable within a certain range (Imbar and Hurwitz, 2004). On the other hand, many kinds of drugs such as human chorionic gonadotrophin (HCG) (Papanikolaou et al., 2010), progesterone (Kumar et al., 1998) and oestradiol (Ma et al., 2003) disturb endometrial development. If endometrial advancement is less than 3 days, a satisfactory outcome can be achieved (Papanikolaou et al., 2010). Knowledge of the degree of synchronization may be useful in improving implantation rates of cryopreserved embryo transfers. In addition, because early implantation helps to improve pregnancy outcome (Wilcox et al., 1999), patients may benefit from prewarming of their cryopreserved embryos. This study is a prospective randomized controlled trial, where the effects of 2 h versus 16 h culture of vitrified embryos and transfer into the same phase of endometrium, were compared.

Materials and methods

The study was performed from January 2010 to February 2011. According to their previous indication, the regimen was selected. In brief, in patients with ovulation disorders, cryopreserved embryo transfer was carried out either in a stimulated or hormone-replacement cycle. In patients with spontaneous ovulation and a normal menstrual cycle, transfers were performed in a natural cycle, a mild stimulation cycle or an artificial cycle. All patients undergoing cryopreserved embryo transfer were allocated with random assignment provided by the Research Randomizer (http://www.randomizer.org/) into the extended culture (EC) or the control group. Embryos were warmed and cultured overnight for 16 h in the EC group and for 2 h in the control group. All cryopreserved embryos were transferred to a well-prepared endometrium on day 3 of progesterone supplementation (**Figure 1**).

The inclusion criteria were: (i) age <38 years; (ii) vitrification performed on day 3; and (iii) insemination complete within 2–6 h on day of oocyte retrieval. The exclusion criteria were: (i) repeated IVF failure; and (ii) disorder of uterine

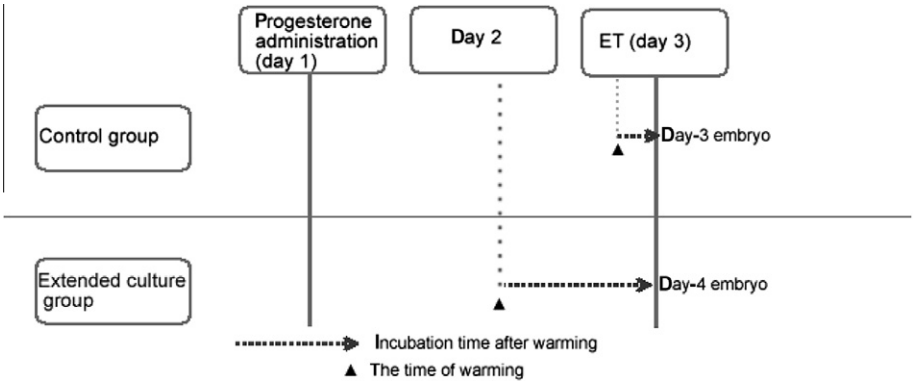

Figure 1 Schematic representation of the trial protocol. All embryos were cryopreserved on day 3 of the fresh cycles. In the extended culture group, embryos were warmed on day 2 of progesterone supplementation, cultured overnight and then transferred (at day 4 of preimplantation development). In the control group, embryos were warmed on day 3 of progesterone supplementation and transferred within 2 h of warming (at day 3 of preimplantation development). ET = embryo transfer.