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ARTICLE

Oocyte versus embryo vitrification for delayed embryo transfer: an observational study

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Abstract The aim of this observational prospective study was to compare multiple embryological and pregnancy outcomes for vitrified oocytes against the same outcomes using vitrified embryos in patients at risk for ovarian hyperstimulation syndrome. Ninety-six patients were included and allocated to vitrification of oocytes (Group 1) or embryos (Group 2). No statistical differences in baseline characteristics between groups were detected. Implantation rate was 30.6% versus 33.1%, and clinical pregnancy rate was 41.9% versus 7.1% in groups 1 and 2, respectively. A higher clinical spontaneous abortion rate occurred in group 2 (9.7% versus 21.9% for groups 1 and 2, respectively), but the same cumulative clinical pregnancy rate was observed after three embryo transfers (62.0% in group 1 and 69.6% in group 2). The ongoing pregnancy rate per patient was similar in both groups (56.0% and 54.3% in groups 1 and 2, respectively). Also, live birth rate per stimulation was similar (72.0% and 69.6% in groups 1 and 2, respectively). No differences were observed in outcomes according to vitrification timing. Oocyte vitrification achieved the same live birth rate as embryo vitrification.

KEYWORDS: embryo, ICSI, oocyte, ovarian hyperstimulation syndrome, pregnancy, vitrification

Introduction

With the availability and reproducibility of vitrification in the IVF laboratory, oocytes and embryos can be consistently cryopreserved and warmed to yield pregnancy outcomes similar to those achieved after the use of fresh oocytes and embryos (Cobo et al., 2010, 2012a, 2012b; Herrero et al., 2011a; Mukaida and Oka, 2012; Rienzi et al., 2010).

Vitrification combines ultra-rapid cryopreservation, minimum volume and a high concentration of cryoprotectants

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(Kuwayama et al., 2005). It has dramatically increased the efficiency and security of many cryopreservation programmes (Balaban et al., 2008; Kuwayama, 2007; Martinez-Burgos et al., 2011). To date, however, little is known about the optimal vitrification time needed for embryo development, so the question about vitrification of oocytes or embryos remains open.

In the present study, an important advantage of vitrifying oocytes instead of vitrifying embryos was identified. It is known that high-response patients are at risk of developing ovarian hyperstimulation syndrome (OHSS), and also, as a result of their high oocyte yield, may generate too many embryos for cryopreservation. Circumventing the problem of generating more cryopreserved embryos than the patient may ever use would be attractive to patients and desirable to many IVF units. Nonetheless, the aim of the study was to assess whether the results of cryopreserved embryo transfers were better than those achieved after the use of vitrified oocytes. Therefore, the aim of the present investigation was to prospectively compare two methods: IVF or intracytoplasmic sperm injection (ICSI) with vitrification of embryos directly, or continued vitrification of oocytes in patients at high risk of OHSS.

Materials and methods

Study population

A total of 96 patients at risk of OHSS were included in the study between January 2011 and June 2012. According to our institutional criteria (Isaza et al., 2002), if 4000 pg/mL or more serum oestradiol, 20 or more mature follicles (over 14 mm), or both, were observed, the patient was considered to have a high response to ovarian stimulation. Patients were prospectively assigned to group 1 (oocyte vitrification plus rewarming of the oocytes and embryo transfer in a later cycle) or group 2 (ICSI and embryo vitrification, plus cryopreserved embryo transfer in a later cycle) according to their clinical record number (even numbers were assigned prospectively to group 1 and odd numbers were assigned to group 2). Patients were informed by the clinicians about the aim of the study, and asked to voluntarily participate; all study participants provided informed consent, and Institutional Review Board approval from our institution was obtained before starting the study (November 15, 2010; MADLH-11–2010-01).

Ovarian stimulation

Participants underwent a daily antagonist protocol (Orgalutran; Schering-Plough, Madrid, Spain; Cetrotide; Merck-Serono, Madrid, Spain). Ovarian stimulation was carried out as described previously (Garcia-Velasco et al., 2001), with 225 IU of recombinant FSH (Gonal F, Madrid, Spain; Puregon, MSD, Madrid, Spain) as a starting dose, followed by individual dose adjustment as required according to the patient's ovarian response. As soon as two leading follicles were 18 mm or wider in mean diameter, triptorelin (0.2 mg Decapeptyl; IpsenPharma, Madrid, Spain) was administered and ovum retrieval carried out 36 h later.

Endometrial preparation for cryopreserved embryo transfer

The protocol for endometrial preparation has been described elsewhere (Soares et al., 2008). After menses, all participants received oral oestradiol valerate (Progynova; Schering-Plough, Madrid, Spain), starting with a daily dose of 2 mg that increased to 6 mg; about 10–15 days after starting oestradiol valerate, serum oestradiol levels and endometrial thickness were determined. Vaginal administration of micronized progesterone 800 mg/day (Progeffik; Effik Laboratories, Madrid, Spain) was started 3–5 days before the transfer of vitrified embryos, depending on embryo stage at cryopreservation.

The embryos were warmed and then evaluated, embryos with more than 50% of cells intact were considered viable. The embryos were incubated for at least 3 h before embryo transfer. In the case of blastocyst warming, a second check was made 2 h after warming to evaluate the appropriate re-expansion of trophoectoderm before embryo transfers.

Vitrification and warming

All materials required for vitrification were obtained from Kitazato (Tokyo, Japan). The cryotop method was used for oocyte vitrification as described by Kuwayama et al. (2005), with minimal modifications. Oocytes were equilibrated in three steps at room temperature for 15 min, and then placed in vitrification solution. After 1 min in this solution, oocytes were placed on the cryotop strip, and immediately submerged in liquid nitrogen. No more than four oocytes per cryotop were loaded. For warming, the cryotop was removed from the liquid nitrogen and instantly placed in various diluent solutions for 10 min at room temperature.

The vitrification protocol for embryos and blastocysts was the same as that described for oocytes, except that equilibration was performed in one step for 12–15 min. Oocytes were incubated in fertilization medium for 2 h before ICSI.

In the oocyte vitrification cases, all cryopreserved metaphase II (MII) oocytes were warmed immediately, and if more embryos than those selected for transfer developed correctly, they were re-vitrified. In the cryopreserved embryo transfers, only the number of embryos that had been intended for transfer were re-warmed, beginning with the best quality embryos. During the study period, no patient underwent more than three embryo transfers.

Embryo transfer

Embryo transfer was carried out with a Cook catheter (K-soft, J-SPPE; Cook Ob/Gyn, Spencer, IN, USA) using a standard technique under ultrasound guidance. In the case of warmed embryos, they were incubated for at least 3 h between warming and embryo transfer. In group 1, all viable embryos not transferred were vitrified.

The clinic's definition of a good-quality day 3 embryo is between seven and nine cells with no more than 15% fragmentation index. A blastocyst is defined as when the inner