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CASE REPORT

# Two singleton live births after the transfer of cryopreserved—thawed day-3 embryos following an unstimulated in-vitro oocyte maturation cycle

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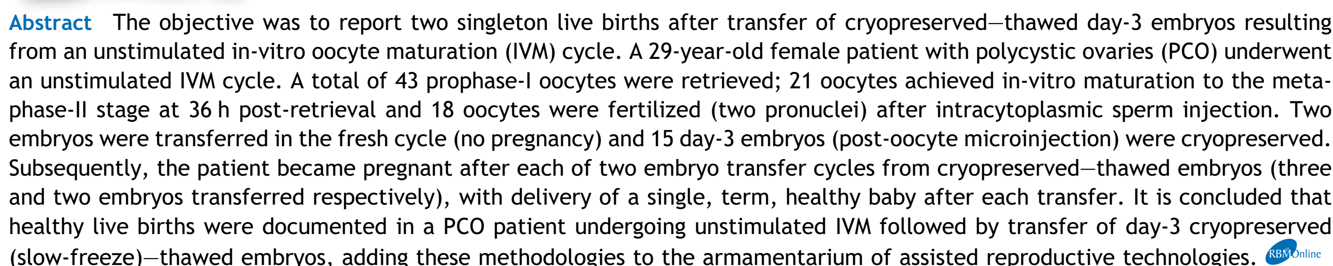

**Abstract** The objective was to report two singleton live births after transfer of cryopreserved—thawed day-3 embryos resulting from an unstimulated in-vitro oocyte maturation (IVM) cycle. A 29-year-old female patient with polycystic ovaries (PCO) underwent an unstimulated IVM cycle. A total of 43 prophase-I oocytes were retrieved; 21 oocytes achieved in-vitro maturation to the metaphase-II stage at 36 h post-retrieval and 18 oocytes were fertilized (two pronuclei) after intracytoplasmic sperm injection. Two embryos were transferred in the fresh cycle (no pregnancy) and 15 day-3 embryos (post-oocyte microinjection) were cryopreserved. Subsequently, the patient became pregnant after each of two embryo transfer cycles from cryopreserved—thawed embryos (three and two embryos transferred respectively), with delivery of a single, term, healthy baby after each transfer. It is concluded that healthy live births were documented in a PCO patient undergoing unstimulated IVM followed by transfer of day-3 cryopreserved (slow-freeze)—thawed embryos, adding these methodologies to the armamentarium of assisted reproductive technologies. RBMOnline
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**KEYWORDS:** embryo cryopreservation, in-vitro maturation, IVF, live birth, PCO

## Introduction

In-vitro maturation (IVM) represents an additional tool to treat patients with polycystic ovaries (PCO), including women with typical polycystic ovary syndrome (PCOS) and with PCO-like ovaries that require the use of assisted reproductive technologies (Le Du et al., 2005; Rao and Tan, 2005). It has been claimed that IVM offers a simpler, more economical, safer and less wasteful IVF procedure (Edwards, 2007). Moreover it has been suggested that IVM can be extended to

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cryopreservation in cancer patients and in egg donation programmes (Hashimoto, 2009; Rao and Tan, 2005).

Although successful pregnancies from unstimulated IVM fresh cycles have been reported worldwide, the developmental competence of oocytes, the overall quality of embryos and pregnancy rate derived from IVM appear to be lower than conventional IVF (Gunby and Daya, 2007; Le Du et al., 2005). In addition, a recent study showed that there was a higher frequency of abnormal meiotic spindle and chromosomal alignment with IVM raising some concerns about the safety of this procedure (Li et al., 2006). On the other hand, the results of obstetric outcome and followup of children born after IVM appear to be reassuring; however, only a limited number of pregnancies have been followed up (Buckett et al., 2007; Chian et al., 2009; Söderström-Anttila et al., 2006).

It has been speculated that endometrial thickness during an unstimulated IVM cycle may not be optimal and can cause lower implantation rates (Holzer et al., 2007). Some studies have therefore added the short-term use of low dose gonadotrophins to IVM protocols; others recommended micronized 17β-oestradiol to be initiated several days before retrieval of oocytes (Chian et al., 2004; Elizur et al., 2008; Godin et al., 2003).

Success with the use of cryopreserved–thawed embryos resulting from IVM cycles has been very limited so far. In fact, there have only been a few reports of live births following embryos generated from an IVM programme that were frozen at the 2-pronuclei (2PN) stage with slow-freezing (Chian et al., 2001; Kyono et al., 2002). Perhaps more promisingly, in recent years, several successful pregnancies have been reported after cryopreservation using vitrification at 2PN (Hashimoto et al., 2007) or blastocyst stage after IVM cycles (Chian et al., 2009; Lee et al., 2007; Son et al., 2002, 2005).

Conversely, results of cryopreserved–thawed day-3 cleaving embryos obtained from IVM patients have been disappointing. Son et al. (2009) reported a significantly lower survival rate for slow-freeze day-3 embryos when compared with vitrification (61.8% versus 85.5%; $P = 0.0007$). In addition, no pregnancies were reported for slow-freeze day-3 embryos by Son et al. (2009). Godin et al. (2003) reported on a twin pregnancy obtained from day-3 frozen embryos but in a stimulated IVM cycle using mild human menopausal gonadotrophin stimulation. Here, live births are reported from day-3 cryopreserved–thawed embryos using the slow-freeze method in a PCO patient undergoing unstimulated IVM.

## Materials and methods

### Patient details

A 29-year-old nulligravida patient sought consultation with a previous medical history of irregular menstrual cycles, with oligo-ovulation, without evidence of clinical hyperandrogenism. She had ultrasonographic PCO-like appearance, with a typical cortical multifollicular presence and hyperechogenic stroma. The patient had a serum FSH:LH reversal, normal TSH and prolactin concentrations and normal testosterone and dehydroepiandrosterone, haemoglobin A1C and fasting glucose/insulin concentrations. The patient had failed to conceive after 2 years of unprotected intercourse and use of clomiphene citrate in four consecutive cycles with documented ovulation. Hysterosalpingography revealed a normal uterine cavity and bilaterally tubal spillage with normal rugae. She was on metformin 500 mg twice daily orally. The male partner's semen analysis was normal with a volume of 3 ml, sperm concentration of 52 million/ml, 70% progressive motility and normal morphology 11% (strict criteria). The patient consented to participate in an unstimulated IVM clinical trial study instead of undergoing the standard treatment of ovulation induction with gonadotrophins. The study was approved by the institutional review board of Eastern Virginia Medical School.

### In-vitro maturation and embryo cryopreservation–thawing

In the fresh IVM cycle, a baseline profile of the patient on cycle day 3 was obtained with ultrasonographic evidence of bilateral multiple follicles (approximately 15 follicles per ovary, 4–7 mm in diameter) and measurement of serum hormone concentrations, including FSH (6 mIU/ml), LH (12 mIU/ml) and oestradiol (39 pg/ml). On unstimulated cycle day 8, her serum values were oestradiol 74 pg/ml, LH 17 mIU/ml and progesterone 0.7 ng/ml, with no follicle greater than 9 mm in diameter. The endometrial lining had a trilayer appearance with a thickness of 5 mm. On that night the patient received 10,000 IU human chorionic gonadotrophin (Novarel, i.m.; Ferring Pharmaceuticals, Parsippany, NJ, USA) and oocyte retrieval took place 35 h thereafter using a transvaginal ultrasound-guided approach with a 17-gauge single lumen needle (Cook, Spencer, IN, USA) with an aspiration pressure of 100 mmHg. Oestrogen therapy (17β-oestradiol, 6 mg four times daily orally) was initiated on the night of oocyte retrieval (Estrace; Mead Johnson, Evansville, IN, USA) and progesterone supplementation was started on the morning after oocyte retrieval (Prometrium, 200 mg three times daily vaginally; Solvay Pharmaceuticals, Marietta, GA, USA).

At retrieval, follicles were flushed with HEPES-Ham's F10 (Gibco, Carlsbad, CA, USA) supplemented with Heparin (20 IU) (Baxter, Round Lake, Illinois, USA). The recovered immature oocytes were cultured in M199 medium (Irvine Scientific, Irvine, CA, USA) supplemented with 18.25 IU of human menopausal gonadotrophin (Pergonal; Serono, Rockland, MA, USA), 1 μg/ml of oestradiol (Sigma, St. Louis, MO, USA) and 10% heat-inactivated maternal serum. The oocytes were cultured for 36 h, stripped of cumulus cells and assessed for maturation status by confirmation of the presence of a first polar body. Metaphase-II oocytes were injected by ICSI following standard procedures and cultured in P1 medium (Irvine) supplemented with 10% synthetic serum substitute (Irvine) in open-well organ culture dishes (Falcon 3037, Franklyn Lakes, NJ, USA).

Fertilization assessment was performed at 12 h post injection. On day 3 after oocyte microinjection (day 4 post-retrieval), embryos were transferred to the uterine cavity using trans-abdominal ultrasound guidance (Mirkin et al., 2003). Embryo scoring followed the criteria established by Veeck (1991), where grade 1 is best morphology and grade 5 is poorest morphology. Cleaving embryos of good quality remaining after transfer were cryopreserved with slow-freeze, slow-thaw protocol using a programmed cell freezer (Planer Kryo 10–1.7; TS Scientific, Perkasie,

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