Case report

Birth of a baby conceived from frozen oocytes of a 40-year-old woman

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Abstract

The potential, limits and safety of oocyte freezing are still being explored. Female age may play a relevant role in treatment outcome. The present study is the first report of the birth and normal development of a baby conceived from frozen oocytes of a 40-year-old woman. IVF was carried out in an infertile 40-year-old woman, and seven metaphase II (MII) oocytes were obtained after ovarian stimulation. Three fresh oocytes were inseminated by intracytoplasmic sperm injection (ICSI), according to Italian law. Two embryos were transferred, but pregnancy did not occur. The four remaining MII oocytes were frozen (by slow freezing protocol) and ICSI was performed in the two oocytes surviving after thawing. Two embryos were obtained on day 2. Both embryos were transferred, resulting in a singleton pregnancy, and a healthy male baby was born. So far, the child (now 3 years old) has scored normally according to the WHO Child Growth Standards. The Denver Developmental Screening Test for psychomotor development was normal. This report demonstrates that conception and pregnancy from cryopreserved oocytes belonging to women up to 40 years of age is possible, and can yield normal children. This finding has implications for women who want to preserve their reproductive potential.

Keywords: age limit, birth, fertility preservation, 40-year-old woman, oocyte cryopreservation

Introduction

The potential, limits and safety of human oocyte cryopreservation are still unclear. Oocyte freezing represents an alternative to circumvent many of the ethical issues associated with embryo cryopreservation, and allows the preservation of female fertility. Furthermore, oocyte ‘cryo-banking’ could represent a more efficient approach in oocyte donor–recipient treatment. Oocyte cryopreservation is currently applied in numerous assisted reproduction centres, with two main methods being used: the slow-cooling computer-controlled protocol (Porcu et al., 2000; Winslow et al., 2001; Yang et al., 2002; Boldt et al., 2003; Fosas et al., 2003; Borini et al., 2004; Chen et al., 2005; Bianchi et al., 2007; Gook and Edgar, 2007; Parmegiani et al., 2008a) and the ultra-rapid cooling (vitrification) protocol (Kuwayama et al., 2005; Okimura et al., 2005; Antinori et al., 2007; Chian et al., 2008). The progressive increase in success rates suggests that oocyte cryopreservation could be used on a more routine basis and in ‘difficult’ cases; nevertheless, some authors maintain that this technique needs further studies on safety and efficiency (Jain and Paulson, 2006; Oktay et al., 2006). Optimizing the timing of freezing could be useful in improving the outcome of oocyte thawing cycles. It has been demonstrated that timely cryopreservation (≤2 h) can yield very encouraging clinical results when using a slow freezing/rapid thawing protocol with high sucrose concentration (Parmegiani et al., 2008a).

The verification of the biological competence maintained after several years of cryostorage represents another fundamental step towards the development of safe oocyte ‘cryo-banking’ programmes. For this reason, the first live birth after the transfer of a single blastocyst developed from a 5 year cryopreserved human oocyte was reported (Parmegiani et al., 2008b). Perhaps the last important factor that needs to be established is the age limit for oocyte freezing: the female age at cryopreservation
may play a relevant role in treatment outcome, and so far no live births derived from cryopreserved oocytes appear to have occurred in women aged 40 or older. Thus, this study presents the first report of the birth and normal development of a baby conceived from frozen oocytes of a 40-year-old woman.

Materials and methods

In Italy, the insemination of more than three gametes at one time is prohibited, while cryopreservation of surplus oocytes is allowed by the Italian law 40/2004 regulating Assisted Reproductive Technology (Benagiano and Gianaroli, 2004). For this reason, since April 2004 (when the law was introduced), all patients undergoing IVF treatment in the study fertility centre have been included in the centre’s oocyte cryopreservation programme, by being offered the opportunity to have their surplus oocytes cryopreserved. All the women were informed about the procedure, and written consent was obtained from each. The cryopreservation protocol consisted of a slow freezing–rapid thawing method. Oocyte freezing and thawing solutions (OocyteFreeze and OocyteThaw; MediCult, Jyllinge, Denmark) contained Dulbecco’s phosphate buffered saline (PBS) supplemented with human serum albumin (HSA), alpha- and beta-globulins and 1,2-propanediol (PROH) and sucrose as cryoprotectants.

Freezing procedure

After washing in a PBS solution (vial 1, OocyteFreeze), the oocytes were equilibrated for 10 min at room temperature in 1.5 mol/l 1,2-PROH (vial 2, OocyteFreeze) and then transferred into the loading solution of 1.5 mol/l 1,2-PROH and 0.3 mol/l sucrose (vial 3, OocyteFreeze). Between one and three oocytes were loaded in plastic straws (Paillette Cristal 133 mm; Cryo Bio System, Paris, France) and transferred into an automated biological vertical freezer (Kryo 360-1.7; Planer, Sunbury, UK). The cooling process was initiated reducing chamber temperature from 20°C to −7°C at a rate of 2°C/min. Ice nucleation was induced manually at −7°C. After a hold time of 10 min at −7°C, the straws were cooled slowly to −30°C at a rate of 0.3°C/min and then rapidly to −150°C at a rate of 50°C/min. After 10–12 min at stabilization temperature, the straws were transferred into liquid nitrogen and stored for later use.

Thawing procedure

The straws were air-warmed for 30 s and then immersed in a 30°C water bath for 40 s. The cryoprotectant was removed at room temperature by stepwise dilution of PROH in the thawing solutions: the contents of the straws were expelled in 1.0 mol/l 1,2-PROH and 0.3 mol/l sucrose solution (vial 1, OocyteThaw) and the oocytes were equilibrated for 5 min. The oocytes were then transferred into 0.5 mol/l 1,2-PROH and 0.3 mol/l sucrose solution (vial 2, OocyteThaw) for 5 min and then into 0.3 mol/l sucrose solution (vial 3, OocyteThaw) for 10 min before final dilution in PBS solution (vial 4, OocyteThaw) for 20 min (10 min at room temperature and 10 min at 37°C). Finally, the oocytes were cultured at 37°C in an atmosphere of 6% CO₂ in air for 3 h before intracytoplasmic sperm injection (ICSI).

Case report

The patient, a woman with idiopathic infertility, underwent ovarian stimulation for ICSI treatment in the study centre (GynePro Medical Centers, Bologna, Italy) in June 2004, when she was 40 years old. Ovarian stimulation was started on 2 June, on day 2 of a spontaneous menstrual cycle, by daily administration of 400 IU of recombinant FSH (rFSH, Puregon; Organon Italia, Rome, Italy) until the day of ovulation triggering. From day 7 of ovarian stimulation, the gonadotrophin-releasing hormone (GnRH) antagonist ganirelix (Orgalutran; Organon, Rome, Italy, 0.25 mg daily) was administered for 5 days. From day 7 to day 10 of ovarian stimulation, LH activity supplementation in the form of low-dose human chorionic gonadotrophin (HCG), 100 IU/day (Gonasi HP 250; AMSA, Rome, Italy) was administered to optimize follicular maturation (Filicori and Cognigni, 2001; Filicori et al., 2002, 2005). Transvaginal ultrasound guided oocyte retrieval was performed 35 h after ovulation trigger with 10,000 IU of HCG (Gonasi HP 5000; AMSA). A total of 10 oocytes were collected after ovarian stimulation, seven of which were mature (metaphase II; MII) and suitable for insemination. Three fresh MII oocytes were inseminated by ICSI and the remaining four MII oocytes were cryopreserved on 14 June, 2004. Two of the fresh MII oocytes inseminated were fertilized after ICSI and two embryos (one 4-cell and one 5-cell) were transferred on 17 June, but pregnancy did not occur. Following this, a frozen egg replacement cycle was performed. The preparation of endometrium for the embryo transfer was performed by the administration of oestradiol valerate (Progynova; Bayer-Schering Pharma, Milan, Italy, 2 mg orally each day) for 13 days, with pituitary desensitization induced by GnRH agonist triptorelin (Decapeptyl; Ipsen, Milan, Italy, 3.75 mg, depot, starting from day 21 of the previous menstrual cycle). The four previously cryopreserved oocytes were thawed on 26 October, when the endometrial thickness was considered to be optimal for performing embryo transfer (≥8 mm). Progesterone administration (Progestek; Effik Italia, Milan, Italy, 1600 mg daily) was started on the day of oocyte thawing. ICSI was performed in two out of the four metaphase II frozen oocytes that survived after thawing. The two oocytes were fertilized and two embryos were obtained (one 2-cell and one 3-cell). Both embryos were transferred on day 2 (28 October). A singleton pregnancy ensued and a healthy male baby weighing 2950 g was born on 18 July, 2005 by Caesarean section after 36 weeks of gestation.

So far, the growth of the child has been monitored and has achieved normal scores according to World Health Organization standards (World Health Organization, 2006). The psychomotor development of the child was assessed by Denver Developmental Screening Test (Frankenburg and Dodds, 1967). The test was performed on 24 April, 2008 when the child was almost 3 years old; scores were normal.

Discussion

The application of human oocyte cryopreservation as a clinical procedure represents an extremely valuable tool in the world of IVF. In recent reports, oocyte cryopreservation pregnancy rates by both slow freezing–rapid thawing protocol (Parmegiani et al., 2008a) and ultra-rapid cooling (vitrification) (Kuwayama et al., 2005; Antinori et al., 2007) were found to be comparable.
with those obtained with cryopreserved embryos. Oocyte vitrification, in particular, seems to be a very promising technique (Koutlaki et al., 2006), with an increasing number of live births reported (Antinori et al., 2007; Chian et al., 2008). Current and recent studies on the limits and safety of human oocyte cryopreservation are helping to define the potential of this cryobiology technique. For example, recent studies have shown that a short time lag between oocyte retrieval and freezing (≤2 h) can yield improved clinical results, when using a slow freezing/rapid thawing protocol with high sucrose concentration (Parmegiani et al., 2008a). Reports regarding the safety of long term cryostorage (Yang et al., 2007; Parmegiani et al., 2008b) are a critical step for the development of oocyte ‘cryo-banking’ programmes, whether for homologous fertility preservation or for oocyte donation purposes (Nagy et al., 2007; Cobo et al., 2008). Another critical factor which needs to be established is the age limit for oocyte freezing. Considering that oocytes from aged women appear to be more fragile and genetically flawed than in young women, the female age at cryopreservation may play a relevant role in treatment outcome. The progressive decline of female fertility with advancing age means that fertility reaches almost zero between age 45 and 50 (Menken et al., 1986), due to the poor oocyte quality (Navot et al., 1991) and the progressive depletion of ovarian reserve (Richardson et al., 1987). Successful pregnancy after IVF with homologous fresh oocytes can be achieved in women over 45: a delivery after IVF has been reported in a 46-year-old woman (Dal Prato et al., 2005). Regarding cryopreserved oocytes, few studies on human oocyte cryopreservation have systematically assessed the influence of female age limit on thawing cycle clinical outcome. In a meta-analysis of 2006, Oktay et al. observed that implantation rates using frozen–thawed oocytes for a 33-year-old woman matched those for a 40-year-old woman undergoing IVF with fresh oocytes. This observation may indicate that pregnancies from oocytes frozen at age >38 could be very rare. In a study regarding 90 thawing cycles of slow freezing/rapid thawing, no pregnancies were observed in women aged ≥39 by Bianchi et al. (2007). In 93 thawing cycles performed with slow cooled oocytes from women of ≥38 years old, La Sala et al. (2006) reported one live birth, but no information was given regarding the exact age of the mother. Fasolino et al. (2008) observed a continuous decline in pregnancy and live birth rates with age increasing in a study performed on 553 thawing cycles, concluding that the age factor started to significantly affect results at very early stages.

So far as is known, there are no specific reports in the literature regarding live births from cryopreserved oocytes in women aged 40 years or older. In this case report, normal growth (World Health Organization, 2006) and psychomotor development (Frankenburg and Dodds, 1967) of the 3-year-old child have also been observed. Developmental screening tests are in widespread use, but few reliable and valid tests are available. The most frequently used screening instrument for detecting developmental delay in young children is the Denver Developmental Screening Test (DDST). The DDST has excellent test specificity and the score obtained by this child was normal.

In conclusion, this first report of birth and normal development of a baby conceived from frozen oocytes of a 40-year-old woman suggests that oocyte cryopreservation could be successfully applied to other women who might otherwise be denied access to this technique on grounds of age. If this report is confirmed by others achieving pregnancies and births from oocytes frozen at age ≥38 years, this may open new perspectives for women who want to preserve their reproductive potential for family planning reasons or in the case of gonadotoxic therapies that may affect future fertility.

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