Live birth from oocytes cryopreserved with slow-freezing protocol and thawed after 6 years of storage

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Introduction

After the birth of more than 900 babies from human cryopreserved oocytes with no apparent increase in congenital anomalies compared to conventional IVF babies [1], oocyte cryopreservation has become a quite widespread technique in Assisted Reproductive Technologies (ART), even in the female fertility preservation programs. Today two procedures are available for oocyte cryopreservation: slow-freezing (SF), the first protocol introduced, and vitrification (VT) [2]. The main goal of VT is to achieve high cryoprotectant concentration in order to increase the viscosity of the cryoprotectant solution and to suppress ice nucleation [3]. Biological and clinical outcomes of SF protocols seem to be less than those obtained with fresh and vitrified oocytes [4–6]. Oocyte cryopreservation is usually used in ART laboratories for supernumerary oocyte storage after Controlled Ovarian Stimulation (COS) [4], prevention of Ovarian HyperStimulation Syndrome (OHSS) risk, oocyte accumulation in poor responder patients [7], and fertility preservation programmes for cancer patients [8–10] or for social reasons [11]. Nevertheless, in 2008 the Practice

Committee of American Society of Reproductive Medicine (ASRM) concluded that “*Oocyte cryopreservation presently should be considered an experimental technique*,” as the data available on the efficacy of oocyte cryopreservation in relation to the length of storage are still limited [1,12]. Instead, the ASRM considers embryo cryopreservation a non-experimental technique [12], with a proven clinical outcome regardless of the length of storage [13,14]. Unfortunately, there are limited data concerning children born from long-term cryopreserved oocytes [1]. The first live birth obtained from oocytes stored for a long period was reported by Yang et al. in 2007: oocytes were cryopreserved by SF for fertility preservation before cancer treatment and thawed 6 years later. In 2008, Parmegiani et al. reported a live birth from oocytes cryopreserved by SF after 5 years of storage in a patient who underwent infertility treatment. Kim and Hong [17] recently described a similar result, reporting a live baby born from vitrified oocytes thawed 5 years later.

Here we describe the case of a woman who underwent ART procedures for both female (tubal) and male (oligoasthenozoospermia) infertility in our non private sterility center. The patient gave birth to two babies in two different thawing cycles from cryopreserved oocytes with SF protocol: the first baby after 11 months and the second baby after 6 years of storage.

Case report

The patient underwent the first ART procedures in 2005, in accordance with Italian law 40/2004, which permitted the insemination of no more than three oocytes and the transfer of all the resulting embryos [18]. Since the oocyte cryopreservation was an experimental procedure, it was performed

Capsule Live birth from cryopreserved oocytes thawed after 6 years of cryobanking.

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after an adequate counselling and only in presence of a informed consent signed by the couple. In this cycle, a total of nine oocytes were collected and 3 MII oocytes were injected as described by Palermo et al. [19]. Two embryos were transferred after 48 h of culture, but did not result in pregnancy.

Four supernumerary MII oocytes were cryopreserved for future thawing cycles. We used a slow freezing–rapid thawing protocol, as described elsewhere [20].

The patient underwent a second ICSI cycle with non frozen oocytes in May 2005 and a total of eight oocytes were collected. Three MII oocytes were injected and 5 MII oocytes were cryopreserved. One embryo was obtained and transferred after 48 h of culture but the patient did not become pregnant.

In September 2005, the patient came back to our Centre asking us to thaw the oocytes. We began the endometrial preparation on the second day of the menstrual cycle, with per os estradiol valerate (Progynova[®], Shering, Milan, Italy) 2 mg twice daily. The first ultrasonographic assessment was performed, on average, on day 12 of the menstrual cycle. When the endometrial thickness ranged between 8 and 12 mm, we added intravaginal micronized progesterone (Prometrium[®], Rottapharm, Milan, Italy) 200 mg twice daily. Oocyte thawing was carried out on the day when ultrasonography showed an adequate thickness of 8–12 mm.

Three cryopreserved oocytes were thawed in May 2005; all survived and were injected. Two embryos were obtained and transferred after 72 h of culture. Treatment with estradiol valerate and micronized progesterone were continued until the day of β -hCG testing (about 12–14 days after the embryo transfer), but the dose of micronized progesterone was increased to 200 mg three times daily, starting from the day of ET. In the case of a positive β -hCG test and the ongoing pregnancy, we continued the hormonal support for 8 more weeks. Once again, however, the patient did not become pregnant.

Three month later, in December 2005, the patient underwent a further thawing cycle: three oocytes (1 cryopreserved in January and 2 in May) were thawed; all survived and were injected, resulting in the transfer of three embryos after 72 h of culture. The pregnancy test performed 2 weeks after ET was positive, and a single gestational sac was observed by ultrasound. Pregnancy progressed without complications, and in August 2006, the patient delivered a healthy girl at 38 weeks of gestation by caesarean section performed for breech presentation. In December 2010, at the age of 42 years, the patient contacted our Centre asking us to thaw the last three oocytes cryopreserved in January 2005. All three oocytes were thawed; one degenerated and the other two were injected. Two embryos were obtained and transferred after 48 h of culture. Two weeks after ET, the pregnancy test was positive, and two gestational sacs were confirmed by ultrasound at seven gestational weeks. After

2 weeks the second ultrasound examination showed only one gestational sac with fetal heart beat.

In June 2011, the patient delivered a healthy boy at 39 weeks of gestation by repeat caesarean section. The newborn was 51 cm long and weighed 3,270 g. Apgar score was 9 and 10 after 1 min and 5 min of neonatal life, respectively. Hospitalization in NICU was not required. To date, the two babies are healthy.

Discussion

Since the clinical outcomes reported in international literature are usually referred to cycles with short-term oocytes cryobanking [1], unlike embryo cryopreservation [13,14], there is not much available information about the children born from long-term cryopreserved oocytes [15–17]. If available, these data could be useful, especially for appropriate counselling to the patients needing fertility preservation before cancer treatment [21]. In this case report we described the first live and healthy baby born from cryopreserved oocytes by SF for infertility treatment thawed after 6 years. Our case supports the hypothesis that SF of human oocyte may be a safe and effective technique for long-term cryostorage, maintaining oocyte competence for successive biologic steps: oocyte survival, fertilization, embryo cleavage and implantation. Nevertheless, it is mandatory to remind that oocyte cryopreservation is still an experimental procedure with not standardized clinical results. The limited efficacy of this technique could be mainly due to the detrimental effects of the exposition of oocytes to the cryoprotectants and to very low temperatures [22].

Studies conducted on the oocyte cryopreservation showed that the oocyte morphology and functionality could be affected, especially involving the meiotic spindle, the cortical granules and the zona pellucida [23].

Other studies are needed to investigate the efficacy of long-term oocyte cryopreservation, including offspring health at birth and later development.

Conflict of interest The Authors declare no potential conflict of interests.

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