Successful live birth from oocytes after more than 14 years of cryopreservation

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Abstract

Objective To report a birth of a healthy girl after long-term oocyte cryopreservation by slow cooling in sodium depleted medium.

Design Clinical application.

Setting University Affiliated, Private IVF center.

Patient A 38-year-old woman received embryos from IVF by intracytoplasmic sperm injection (ICSI) with her own oocytes that were cryopreserved by slow freezing in a low-sodium medium 14 years and 6 months before, when she was 24 years old.

Main outcome measure(s) Survival, fertilization, cleavage, clinical pregnancy and delivery.

Result(s) From six metaphase-II oocytes thawed, two survived, one was fertilized after ICSI and a cleaving embryo was transferred on day 3. A single term pregnancy was achieved, ending with the delivery of a healthy girl.

Conclusion(s) Cryopreservation after slow freezing in a sodium depleted medium maintained the developmental competence of oocytes after long-term storage and resulted in a successful live birth. As far as is known, this case represents, up to date, the longest storage period of cryopreserved human oocytes resulting in a live birth.

Keywords Choline chloride · Cryostorage · Live birth · Long term · Low sodium · Slow freezing

Introduction

During the late 1990s, our center cryopreserved surplus oocytes of some of the patients from the IVF program in a choline-based, sodium-depleted medium [1–3]. After a certain time, some of those oocytes were thawed, achieving pregnancies with a reasonable success rate [2]. Others were discarded as a request of the couples after achieving pregnancy, and a few still remain in cryostorage.

A patient whose oocytes remained frozen since 1999 returned to the clinic in 2013 for an attempt of fertilization and transfer. The details and results of that cycle are presented in this case report.

Case report

In 1999, a couple was submitted to the study unit asking for treatment after a 5-year-long history of primary infertility. The wife, a 24-year-old woman, gravida 0, para 0, had a normal fertility profile, while her 34-year old husband presented a very low sperm count and motility, showing only about 10,000 motile sperm/ml after several semen evaluations.

An IVF cycle with ICSI was indicated and the couple underwent treatment in our clinic. Since the couple expressed their desire not to inseminate all the oocytes retrieved, oocyte freezing was also programmed together with the IVF therapy.

Both partners signed an informed consent. The Ethical Committee from the clinic advised that Institutional Review Board approval was not required for the freezing of the surplus oocytes.

Ovulation induction was initiated with pituitary desensitization. This was achieved with leuprolide acetate (Reliser®; Serono, Buenos Aires, Argentina) following a long down-regulation protocol. Ovarian stimulation was performed with purified Follicle Stimulating Hormone (Metrodine®; Serono, Buenos Aires, Argentina) and human Menopausal

Capsule 14 years after retrieval and cryopreservation of a mature oocyte using slow freeze technology, a live birth was obtained substantiating the prospect of long term storage for delaying reproduction in humans.

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Gonadotrophin (Pergonal®; Serono, Buenos Aires, Argentina). Preovulatory oocytes were retrieved transvaginally by ultrasound guidance performed 36 h after administration of 5,000 UI of human chorionic gonadotrophin (Profasi®; Buenos Aires, Argentina) Serono, Buenos Aires, Argentina). Fifteen metaphase-II oocytes were obtained. They were all denuded with hyaluronidase and washed several times in HEPES-buffered human tubal fluid with 10 % serum substitute supplement (SSS) (Irvine Scientific®, Santa Ana, CA, USA). Nine were submitted to a standard ICSI procedure, while the remaining six oocytes were slowly frozen as described [2] in a choline-based sodium-depleted medium. Normal fertilization was achieved in five of the injected oocytes. After 3 days in culture, three cleavage stage embryos graded 8A, 8B and 6 B were transferred. The remaining two embryos, arrested in their development at the 4-cell stage, were discarded.

Pregnancy was not achieved, and after this failure the couple lost contact with the clinic for many years. Finally they returned for an attempt with the cryopreserved oocytes in June 2013.

The patient underwent endometrial preparation with incremental oral oestradiol valerate administration (Ronfase®; Pfizer, Buenos Aires, Argentina) as follows: 4 mg/day during days 1–5 of the menstrual cycle, 6 mg during days 6–11 and 8 mg from day 10 onwards. Careful ultrasound monitoring of the endometrial lining's thickness and pattern showed, after 16 days, an endometrial lining of 8.5 mm. Oocyte thawing was decided and vaginal progesterone administration was started at a 600 mg/day dose (Utrogestan®; Ferring, Buenos Aires, Argentina).

In this attempt all the oocytes remaining in cryostorage were thawed as was already described [2]. Only two of the six metaphase-II oocytes appeared healthy after thawing, while the remaining four degenerated. After thorough washing in an embryo culture medium for early development stages (G-1 PLUS Vitrolife®, Englewood, USA) supplemented with 5 % SSS, the surviving oocytes were transferred to 20 µl drops of the same medium under mineral oil that was previously equilibrated in a low O₂ environment (6 % CO₂, 5 % O₂ and 79 % N₂) at 37 °C. These culture conditions were

maintained until embryo transfer. Four hours after thawing, the surviving oocytes were inseminated following the standard intracytoplasmic sperm injection (ICSI) procedure. Fertilization was assessed 14 h later. Only one of the microinjected oocytes fertilized normally, and was transferred 72 h after ICSI, at the 8-cell stage, grade B (Fig. 1).

Intrauterine embryo transfer was performed with the aid of an embryo transfer catheter (Frydman ultrasoft®, CCD Laboratoire, Paris, France) under ultrasound monitoring.

Fifteen days after embryo transfer, serum β-human chorionic gonadotrophin was 1,540 IU/l, after 3 days increased to 4,480 IU/l, and 2 weeks later an intrauterine gestational sac with positive fetal heart tone was detected. A new ultrasound at 8 weeks of gestation confirmed viability. A Caesarean section was performed and a healthy girl weighing 3,150 g was born at 38 weeks of gestation in March 2014. The newborn was healthy with no detected abnormalities.

Discussion

Even though the first pregnancy from cryopreserved oocytes was achieved almost 30 years ago, advances in the field of oocyte cryopreservation have developed very slowly. Only a limited number of successful cases were achieved during the first 20 years. This situation changed in the last decade, firstly through improvements in the methodology of slow cryopreservation, and more recently with the optimization of vitrification. Thus it was that oocyte cryopreservation finally approached the status of a standard technique, and so became available to most in vitro fertilization laboratories.

Probably the steps of cryopreservation more prone to cause damage to the oocyte would be during freezing and thawing. Provided that the cryogenic tank integrity and the appropriate level of liquid nitrogen are properly maintained preserving at a temperature of liquid nitrogen, might theoretically ensure cell viability for long periods of time that probably should easily exceed the reproductive life span of a human being.

Fig. 1 Figure shows oocyte post thawing (a); and day 3 embryo transferred (b). Magnification 20×

There are few reports on long-term storage of slowly frozen human oocytes; however, our experience indicates that when they were left in cryostorage for extensive periods of time they could result in healthy, live births. Nevertheless, more human studies are necessary to improve the safety of long-term storage of oocytes.

Successful human vitrification has a shorter life than slow freezing, and in recent years imposed as an alternative to the slow-freezing methods. Currently even less is known about the long-term effects of vitrification. We will have to wait some years, and more studies would be necessary to determine, whether oocyte vitrification can improve survival and pregnancy rates, as well as to know its impact of long-term storage.

To the extent that the ability to cryopreserve oocytes was improved, many women at risk of losing their fertility, either as consequence of aggressive medical treatments or just by aging, resorted to this technique in order to save some of their oocytes for the future [4].

However, as it was mentioned, given the short time since the generalization of these techniques, there are only few cases that confirm the effective preservation of the viability of oocytes in time.

Previous experience with thawed oocytes [3–7] suggests that cryostorage may allow a good maintenance of oocyte viability over the years. This report is reassuring the ability of long time frozen oocytes to provide healthy babies.

Recently, two cases of live births with oocytes fertilized after 5 years of storage have been published. In one case slow freezing was used [7] and in the other one vitrification [5]. More recently, it was reported [3] the birth of two healthy babies obtained after nearly 12 years of cryopreservation.

As far as is known, this case represents, up to date, the longest storage period of cryopreserved human oocytes resulting in a live birth.

We believe that the present report, which describes a pregnancy and birth after a period of 14 years of storage at -196°C , supports the feasibility of oocyte cryobanking as a clinical procedure, and represents helpful and valuable information for

the assisted reproduction professionals, as well as for future patients who would benefit from this technology.

Ethical standards

Both partners signed an informed consent. The Ethical Committee from the clinic advised that Institutional Review Board approval was not required for the freezing of the surplus oocytes.

All procedures have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Conflict of interest The authors declare that they have no conflict of interest.

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