Live birth in a 46 year old using autologous oocytes cryopreserved for a duration of 3 years: a case report documenting fertility preservation at an advanced reproductive age

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Introduction

The first live birth following oocyte cryopreservation (OC) was documented by Chen et al. in 1986 [1]. OC has since emerged as a potential means to reduce the growing surplus of cryopreserved embryos and provide fertility preservation. In 2008 the Practice Committee of the American Society of Reproductive Medicine (ASRM) labeled OC as experimental due to inferior pregnancy rates compared to fresh in vitro fertilization (IVF) and lack of long term outcome data. Because of its experimental nature, OC was discouraged as a fertility preservation strategy for elective reasons [2].

Since 2008, improvements in oocyte vitrification techniques have led to enhanced oocyte survival and pregnancy rates. Vitrified oocytes have better survival during thaw-cycles than slow freeze protocols [3–5], and vitrified oocytes used in IVF/intracytoplasmic sperm injection (ICSI) have similar fertilization and pregnancy rates compared to fresh oocytes [6–10]. Furthermore, cryopreservation has not been associated with birth defects or developmental abnormalities [11, 12]. The Practice Committee of the ASRM in 2012 removed the experimental label on OC and supports its use for specific situations including fertility preservation among cancer survivors undergoing cytotoxic therapies, women undergoing risk-reducing bilateral salpingoophorectomy, genetic disorders associated with premature ovarian failure, insufficient sperm sample on

the day of oocyte retrieval, or for couples unable to cryopreserve embryos [13].

Despite these improvements, the Practice Committee of the ASRM continues to discourage OC for elective fertility preservation due to insufficient evidence regarding efficacy and safety in this population, especially among patients of advanced reproductive age [13]. Observational studies have suggested that pregnancy rates after OC decline steadily with age [14–16]; however, few studies have compared pregnancy outcomes by age-group between OC cycles and fresh IVF/ICSI cycles. There are also few studies on the longevity of cryopreserved oocytes retrieved at an advanced reproductive age. This is a unique case of a live birth at the age of 46 after IVF using autologous oocytes that were cryopreserved 3 years earlier.

Case report

A 46 year old gravida 0 single woman presented to the New York University Fertility Center (NYUFC) at the age of 42 to discuss fertility preservation. Pelvic ultrasound and physical examination were performed and normal. Day-2 serum estradiol (E2) and follicle stimulating hormone (FSH) values were 79 pg/ml and 5.4 IU/L. Anti-mullerian hormone levels were not routinely performed at this time. Fertility options were discussed including OC, embryo cryopreservation, oocyte donation, and adoption. The patient elected to proceed with OC. Ovarian stimulation proceeded with gonadotropin hyperstimulation (follitropin alfa 300 IU and menotropins injection 150 IU) followed by endogenous luteinizing hormone (LH) suppression using a gonadotropin-releasing hormone (GnRH) antagonist (Ganirelix acetate). Human chorionic gonadotropin (hCG 10,000 IU) was administered when two lead follicles were ≥ 18 mm, and transvaginal oocyte aspiration was performed 35 h later. The patient's estradiol

Capsule This case report documents a live birth in a 46 year old after using autologous oocytes cryopreserved for 3 years. Oocyte cryopreservation is an option for fertility preservation in a select population of patients at an advanced reproductive age.

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level peaked at 2161 pg/mL. During the first OC cycle a total of five oocytes were harvested and three vitrified including two mature (metaphase II: MII) and one metaphase I (MI) oocytes. Two months later the patient underwent a second OC cycle as above, the menopausal injection was increased to 300 IU. The patient's estradiol level peaked at 3657 pg/mL. A total of 12 oocytes were harvested and 10 cryopreserved, including 9 MII and 1 MI oocytes. As was routine laboratory practice at the time, oocytes were divided between slow-cooling and vitrification. 5 MII oocytes were slow-cooled and 5 (4 MII and 1 MI) underwent vitrification. OC methods were performed according to protocols previously described by Grifo and Noyes [17].

The patient re-presented 1 year later with a partner desiring pregnancy. A hysterosalpingogram was performed which showed a normal cavity, patent fallopian tubes bilaterally, and mildly dilated left fallopian tube. She then underwent three consecutive clomid/intrauterine insemination cycles with no pregnancy, followed by five consecutive IVF cycles resulting in one biochemical pregnancy and one missed abortion. A sixth IVF cycle resulted in one embryo, and trophoctoderm biopsy was performed followed by vitrification for pre-implantation genetic diagnosis. Genetic testing revealed 46 XY, monosomy 22, trisomy 16, so the embryo was not transferred. The patient was counseled and advised to consider oocyte donation or use of her cryopreserved autologous oocytes. The patient then took time off from fertility treatments to consider her options.

She returned 11 months later and decided to proceed with IVF/ ICSI using her previously cryopreserved oocytes. She underwent saline infusion sonography revealing a normal uterine cavity. Endometrial preparation was achieved using sequentially increasing doses of oral estradiol beginning on cycle day-2 until endometrial diameter reached 8 mm. Intramuscular progesterone in oil (50 mg/day) was added on the day of the oocyte thaw. All 13 cryopreserved oocytes were thawed, and 12 oocytes survived the thaw process. Twelve oocytes underwent ICSI and 11 were successfully fertilized (9 MII and 1 MI oocytes).

On day-3 post-thaw, the embryos in culture included three 8-cell embryos and one 7-cell embryo, one 6-cell embryo, two 5-cell embryos, one 4-cell embryo, one 3-cell embryo, one 2-cell embryo, and one one-cell embryo. The one-cell embryo was discarded, the remaining 10 embryos had <20 % fragmentation. Embryos were cultured until 5 days post-thaw resulting in one stage 3–4 grade BC embryo, one stage 3–4 grade CB embryo, one stage 2–3 grade CB embryo, one stage one embryo, five cleavage-stage embryos, and one morula. The cleavage-stage embryos and morula were discarded. Embryo status was discussed with the patient along with the number of embryos to transfer as suggested by ASRM guidelines [18]. Four blastocysts were transferred (one from the first OC cycle and three from the second OC cycle). Cycle day-28 and day-35 hCG levels were 270 and 322 mIU/mL,

respectively. The patient returned on cycle day-45 for ultrasound revealing a fetal pole with fetal heart beat. The patient underwent sequential screen with a Down's syndrome risk of 1:9200. She declined chorionic villous sampling and amniocentesis, but underwent a MaterniT21 chromosome analysis which was negative for trisomy 21. The pregnancy was uncomplicated and she delivered a viable female infant at 39 6/7 weeks weighing 8 lb 1 oz.

Discussion

There is sparse data on the success rates of OC at or beyond the age of 42 years old and the viability of oocytes cryopreserved at this age; therefore, autologous OC at this age is generally discouraged. There have been documented cases of live births after OC of surplus oocytes from IVF cycles at ages up to 51 years old as well as cases of live births after OC for extended periods of time [19–24], but the idea of elective OC for fertility preservation at advanced reproductive ages is a novel concept. Patients in this age group are encouraged to become pregnant on their own using sperm donors or electively freeze embryos. For many single women, these are not acceptable options. OC allows women the flexibility to use their own oocytes if they find a partner in the future. Some women may not want to start a family on their own, or deal with the ethical dilemma of storing embryos. When discussing OC for fertility preservation, patients should be counseled about the declining success rates with age and realistic expectations should be discussed.

Overall success rates of OC based on age have been investigated in a few observational studies. The following studies were performed in an infertile population of patients undergoing OC for supernumerary oocytes in IVF/ICSI cycles. Borini et al. compared fresh to frozen oocyte cycles in 2046 women undergoing IVF/ICSI. In accordance with Italian law, a maximum of three oocytes were fertilized per cycle. When they stratified their thaw cycle data by age (≤ 38 years or > 38 years) they found no difference in post-thaw survival rate (58.5 % vs 58.8 %, $p=0.88$) or fertilization rates (73.4 % vs 75.5 %, $p=0.66$) between age groups. However, there was a significantly decreased pregnancy rate per embryo transfer (18.7 % vs 10.1 %, $p=0.012$) and implantation rate (10.9 % vs 6.5 %, $p=0.021$) with increased age [14]. This is consistent with data from Bianchi et al. who studied 342 patients undergoing 443 oocyte cryopreservation cycles. When divided into age groups (≤ 34 , 35–38, and ≥ 39 years old), pregnancy rates (27.7 %, 21.4 %, and 17.6 %) and implantation rates (11.8 %, 8 %, and 7.5 %) declined with age [15].

A recent individual patient data meta-analysis by Cil et al. investigated the probability of a live birth after OC based on patient age [19]. They collected data from 2265 OC cycles using supernumerary oocytes. They similarly found no

association between patient age and oocyte survival or fertilization rates, but implantation rates did decline with age. Furthermore, they predicted that the probability of a live birth after oocyte vitrification and transfer of 1–3 embryos at age 42 to be 4.7–13.2 %. This study included live births after 29 OC cycles from individuals aged 42–51; however, the duration of OC is not documented and the numbers were too small to calculate live birth probabilities beyond age 42 [19].

Ubaldi et al. compared pregnancy outcomes in 182 patients undergoing fresh ICSI cycles and oocyte vitrification cycles, including 104 first and 11 second oocyte thaw cycles in order to estimate pregnancy outcomes by age [25]. Women ages 41–43 had a clinical pregnancy rate per embryo transferred of 25 % for a fresh cycle and 22.2 % for an OC cycle. In this same age-group the ongoing pregnancy rate per fresh cycle was 19 % compared to 16.6 % for an OC cycle. Women ages ≤ 34 years old had a significantly higher cumulative pregnancy rate after undergoing a fresh cycle followed by two thaw cycles of 62.5 % compared to 33.3 % for women ages 41–43 years old, $P=0.006$ [25].

A recent retrospective analysis comparing OC freeze/thaw cycles and fresh IVF cycles performed by Goldman et al. found that there was no significant difference in live birth rates per embryo transferred and live birth rates per mature oocyte retrieved (LBR-MOR) in women less than 40 years old [26]. When autologous cycles were stratified by age (<35 and ≥ 35 years old), the LBR-MOR did, however, decline with age. The LBR-MOR was 5.8 % in donor OC cycles, 3 % for autologous OC cycles in women <35 years old, and 2.1 % for autologous OC cycles in women ≥ 35 years old. This was compared to 5.1 %, 4.2 %, and 4.1 % for fresh IVF cycles. Women over age 40 were excluded from this study, so the LBR-MOR in this age group is unknown [26].

Many of the above pregnancy outcome findings can be explained by aneuploidy rates that are known to increase with age. Aneuploidy has been linked to lower implantation rates and increased miscarriage rates [27–32]. Ata et al. estimated the risk of not having any euploid embryos at age 42 to be approximately 75 % [31], and Harton et al. found that 84.8 % of embryos biopsied on day 5 of culture were aneuploid in women >42 years old [32]. These results have discouraged providers from promoting OC in women >42 years old due to the increased risk of not producing any euploid embryos once oocytes are thawed and fertilized.

Most of the studies on OC and aneuploidy at advanced ages are derived from a population of infertile patients undergoing IVF/ICSI, and in many cases only supernumerary oocytes are cryopreserved. Furthermore, many of the large observational studies are out of Italy where providers are only able to fertilize up to three oocytes by law. Our patient may have had a better prognosis because she did not have a fertility diagnosis at the time of OC, she had normal day-2 E2 and FSH levels, and she responded robustly to gonadotropin stimulation producing 13 oocytes, 11 of which were successfully fertilized, over two OC

cycles. There are also no studies comparing rates of aneuploidy between potentially fertile/good prognosis patients and infertile patients because most of the studies are performed on infertile patients undergoing pre-implantation genetic diagnosis. Potentially fertile patients, despite an advanced age, may have decreased rates of aneuploidy and thus higher implantation rates.

The lifespan of cryopreserved oocytes is not well documented in the literature, but there are several case reports of successful live births after 5–12 years of cryopreservation [20–24]. A study by Parmegiani et al. in 2009 sought to determine the effect of OC duration on oocyte survival, fertilization rate, cleavage rate, and embryo quality. Oocytes were cryopreserved for 1–3 months, 4–6 months, 7–9 months, 10–12 months, or 13–48 months. They found no difference in outcomes based on duration of OC. The average age at OC was 33–35 years old, so this data may not reflect survival of oocytes cryopreserved at advanced ages [33].

Another alternative to OC for fertility preservation is to utilize donor oocytes if infertility ensues at an advanced reproductive age. Donor oocytes are on average from women 28 years old and thus have significantly decreased rates of aneuploidy and pregnancy rates similar to the age of the oocyte donor [34]. The rate of oocyte donation cycles has increased in the past decade from 10,801 cycles in 2000 to 18,306 cycles in 2010 [34]. Despite its favorable pregnancy outcomes, oocyte donation poses many social implications for the recipient. Patients must decide whether to disclose their use of donor oocytes to family, friends, or offspring. This decision may be difficult. On one hand parents do not want their children to be stigmatized by others for their origins, but some parents feel that non-disclosure creates an environment of dishonesty and distrust [35]. Another difficult decision arises regarding the choice of an anonymous donor or an open-identity donor. Some patients who choose anonymous donation do so to prevent donor interference in their family or because they do not consider the genetic connection between the donor and offspring to be significant [36]. Other patients feel it is important for offspring to know their genetic origins and have the choice of contacting their donor or donor siblings [36]. OC avoids these ethical and social dilemmas by allowing the patient to, in essence, become her own oocyte donor.

As the above studies illustrate, the actual pregnancy rate and live birth rate among women over 40 years old undergoing OC is unknown. This makes counseling difficult. OC should be included when counseling women over 40 years old about fertility options, but realistic expectations must be clearly expressed. Patients without a pre-existing infertility diagnosis and a normal initial work-up, such as normal day-2 E2, day-2 FSH, and AMH levels, may have better pregnancy outcomes with OC. Patients in this age group with indicators of poor ovarian reserve such as low AMH levels, low antral follicle counts, or poor response to gonadotropin stimulation are more likely to have poor outcomes with OC and should be counseled towards alternatives such as

egg donation. Future studies are needed to determine the outcomes of OC in this patient population. Studies should incorporate patients with potential fertility in addition to those with infertility in order to identify patient characteristics that are associated with favorable outcomes. This information would help providers to select appropriate candidates for OC and improve counseling.

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