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Case Report

Successful pregnancy outcome after 5 years cold storage of blastocysts

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Introduction

In vitro fertilization (IVF) centers practicing assisted reproductive technology (ART) often face the paradoxical situation of having to produce large number of embryos and then to transfer a minimum number of embryos to avoid the complications of multiple pregnancy. HFEA in UK and Indian Council of Medical Research (ICMR)[1] restrict the number of embryos transferred, to three during any treatment cycle regardless of the procedures used. Embryo freezing is an established way of preserving surplus embryos and it also helps in augmenting the cumulative pregnancy rate of IVF/ICSI patients. When there are overwhelming reasons to cancel the embryo transfer (ET) due to severe ovarian hyperstimulation syndrome (OHSS), embryo freezing comes in handy. In various countries, practice and law stipulate the period of storage of embryos in the range of 1 year up to a maximum of 3 to 10 years. It is strongly argued that embryos should not be stored beyond this period. We have been offering embryo cryopreservation facility since its inception in 1999.

Here we present the case report of a patient who had a successful outcome after her first IVF cycle more than 5 years ago and achieved a further pregnancy from thawing of her frozen embryos from the initial harvest.

Case report

A 28 year old woman with a 3 year history of secondary infertility and her 30 year old spouse were referred to our IVF program in 1999. She had left salpingectomy in 1998 following an ectopic pregnancy. Subsequent diagnostic laparoscopy showed hydrosalpinx of the right fallopian tube.

After the preliminary investigations, ovarian stimulation was achieved under long protocol with rFSH 750 IU for 5 days, followed by human menopausal gonadotropin (hMG) 600 IU for 6 more days and egg collection was fixed on 16th day of the cycle after triggering with hCG 10000 IU on the 14th day. Under sedative analgesia, seven eggs were collected transvaginally through a 17G double lumen Cook's egg collection needle. Eggs were subjected to IVF with husband's washed sperms after 5 hours of culture. Next day, all the seven eggs showed two pronuclei and were

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further cultured with sequential IVF/blastocyst culture medium (IVF Science, Sweden) till day 5.

On the day 21 of her cycle, two expanded blastocysts were transferred under ultrasound guidance with Jansen Anderson embryo transfer catheter (Cook, Australia). On this day, four embryos ranging from morula to blastocysts were cryopreserved in a prelabeled 0.25 mL straw with conventional freezing protocol with glycerol and sucrose in required concentration with the help of CryoLogic Freeze Control (Australia) Freezer and plunged into our Embryo storage container.

After 14 days of ET, serum βhCG levels were 160 mIU/mL and 48 hours later there were 380 mIU/mL confirming a viable pregnancy. At 5 weeks, 8mm sac was seen in the uterine cavity on vaginal sonography, while cardiac activity was seen at 6 weeks confirming clinical pregnancy. From then on the pregnancy progressed uneventfully. After 32 weeks of pregnancy, presentation remained breech. At term a baby girl weighing 2.8 kg was delivered by cesarean section.

After 5 years and 5 months, the patient returned with a request for frozen embryo transfer cycle. This frozen thawed ET cycle was done with long protocol with suitable estrogen/progesterone supplementation as per our protocol. On the day of ET, frozen blastocysts at 5 years and 5 months were thawed. Out of the four embryos frozen, three were recovered with 100% survival. These three were cultured in blastocyst medium for about 2 hours and transferred into the patient's uterus under ultrasound guidance through Sydney IVF ET Catheter (Cook, Australia). After 14 days of ET, serum βhCG level was 322 mIU/mL and 48 hours later it was 760 mIU/mL.

Discussion

In this case after 1975 days of freezing, embryos were thawed with 75% survival. Our overall embryo freezing-thaw survival rate has been 67% (135/201) and pregnancy rate after frozen thawed ET, 14.8% (4/27).

A patient who does not achieve a pregnancy from a fresh transfer, but does achieve it later from the same harvest has raised the pregnancy rate from 0 to 100%[2]. A patient who has one pregnancy from her first transfer and achieves a further pregnancy from the thawed embryos like our patient, augments her pregnancy rate from the same harvest.

A good cryopreservation program is an indispensable component of modern ART. The cryo-stored embryos demand a topping up with liquid nitrogen every 10 days Supernumerary embryos are worth freezing since they can lead to successful implantations and live births after many years of freezing. It is reported that a Spanish woman had a baby from 13 years of frozen storage of embryos [3].

The recommended practice, in some western countries of destroying embryos which are in storage far beyond the stipulated period (Austria, Denmark – 1 year; Netherlands, Russia – 2 years; Norway Sweden – 3 years; other countries – 5 years) is questionable from ethical / moral point of view. Ironically, in India, rules have not been framed as to the maximum period of embryo cryo-storage.

In the past, there have been concerns regarding the safety of cryopreservation and the outcome of subsequent pregnancies after thaw and also development of the offspring. Several key studies have now negated these fears. No detrimental effects of long-term storage of cryopreserved human embryos have been shown. Frozen embryo thawed pregnancies show similar characteristics and terms of gestational age, birth weight, incidence of congenital anomalies and perinatal mortality when compared to fresh embryo transfer or even natural conception when adjusted to age and parity [4].

As of now, there is no scientific evidence to disallow the maintenance of embryos in cold storage beyond 5/10 years. From the point of view of fertility preservation partners, it makes eminent sense for them to pay for the complete cryogenic maintenance of a cohort of embryos.

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