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ASSISTED REPRODUCTION TECHNOLOGIES

Live births from frozen human semen stored for 40 years

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Introduction

Low temperature storage in liquid nitrogen is thought to maintain the viability of cells almost indefinitely because reactions that require molecular motion and/or activation do not occur at −196 °C. The adverse effects of background radiation during storage appear to be negligible since exposure of mouse embryos to the equivalent to 2,000 years of background radiation did not have a detectable effect on their viability [1].

Two clinical reports have been published on the successful use of human semen stored for longer than 20 years. Live births have been reported from in vitro fertilization with semen stored for 21 years [2] and from IUI with semen stored for 28 years [3]. In farm animal species, Salamon et al. [4] reported that ram semen maintained its fertility after 35 years of storage. Here we report the birth of twins from in vitro fertilization using semen stored for approximately 40 years.

Capsule Healthy twin girls were born from IVF using frozen semen stored for approximately 40 years.

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Materials and methods

Between January and December 1971, a man aged between 52 and 53 years requested his semen to be frozen for long-term storage. The semen was frozen using TEST yolk buffer with 7 % glycerol in sealed glass ampoules; the exact date of freezing was available on the original paper records, but was not recorded on each ampoule. He made his semen available to women who required donor semen through an agency specializing in gamete donation and surrogacy. His semen was used at the Alta Bates IVF Program between 2009 and 2011.

The directed recipient of the semen was a nulligravid woman in a same-sex relationship. She contacted our clinic after she had established a directed donor/recipient relationship with the sperm provider outside our clinic. The recipient was counseled about the potential adverse consequences of using a semen donor whose age exceeded the 40 years age limit recommended by the American Society for Reproductive Medicine for semen donors [5].

The semen was thawed by keeping the vials in air at room temperature (about 20 °C) until all ice melted. The volume of semen was 0.2 to 0.3 ml per vial. The total motile count of semen per IUI was 7 to 11 × 10 million before processing and 1 to 5 million after processing with density gradient separation. The patient had five IUI cycles when she was between 24 and 25 years old without achieving pregnancy. Subsequently, an IVF cycle with ICSI insemination was performed in order to maximize the chances of a live birth. The patient was 25 years old when she received controlled ovarian stimulation using the short antagonist protocol with leuprolide trigger in October 2011. A total of 14 oocytes were recovered and 13 mature oocytes underwent ICSI resulting in 9 normal zygotes. The semen used for IVF was stored for a period between 39 years

10 months and 40 years 9 months. This was an embryo banking cycle with vitrification of all suitable embryos and no fresh embryo transfer. Four embryos were vitrified on Day 3 (i.e. 3 days after insemination) and another 4 embryos on Day 5 using Cryotips.

Two frozen embryo transfer (FET) cycles were performed using hormone replacement protocol. The first FET was performed on November 17, 2011 when two morphologically good, 8-cell vitrified/warmed embryos were transferred.

The second FET cycle was performed on December 17, 2011. Two Day 3 embryos (6- and 8-cell) were warmed, cultured for 2 days and transferred at the blastocyst stage (their expansion was rated 1 and 2 on the Gardner scale).

Results

There was no implantation in the first FET cycle, but the patient became pregnant after the second FET with dizygotic twins and delivered two healthy girls at the gestational age of 38 weeks. The babies weighed 2,693 and 2,948 g and have exhibited normal development to date.

Discussion

This report shows that human semen stored for approximately 40 years (between 39 years, 10 months and 40 years, 9 month) maintained its ability to result in live birth through ICSI-IVF. This represents the longest reported storage of human semen resulting in live birth. Our finding confirms earlier reports that the viability of frozen semen can be maintained for at least several decades in both humans [2, 3] and farm animals [4], and is consistent with the hypothesis that frozen cells stored suffer little or no damage during extended storage in liquid nitrogen [1].

Men who depend on the use of frozen stored semen to have a child are often concerned whether the viability of their sperm is preserved during long term storage. Our findings should help allay their fears because the required storage is usually much shorter than four decades.

References

1. Glenister PH, Whittingham DG, Lyon MF. Further studies on the effect of radiation during the storage of frozen 8-cell mouse embryos at −196 degrees C. J Reprod Fertil. 1984;70:229–34.
2. Horne G, Atkinson AD, Pease EH, Logue JP, Brison DR, Lieberman BA. Live birth with sperm cryopreserved for 21 years prior to cancer treatment: case report. Hum Reprod. 2004;19:1488–9.
3. Feldschuh J, Brassel J, Durso N, Levine A. Successful sperm storage for 28 years. Fertil Steril. 2005;84:1017.e3–4.
4. Salamon, S, Gillan, L, Evans, G, Maxwell, WMC. Fertility of ram semen after 35 years of frozen storage. Proc 15th Int Congr Anim Reprod. 2004, Porto Seguro, p. 476.
5. The American Society for Reproductive Medicine. Guidelines for therapeutic insemination: sperm. Fertil Steril. 1998;70:1S–4.