

SPERM EXTRACTED AND CRYOPRESERVED FROM TESTES SEVERAL HOURS AFTER DEATH RESULTS IN PREGNANCY FOLLOWING FROZEN EMBRYO TRANSFER: CASE REPORT

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A 38-year-old male died suddenly on his honeymoon. Sperm was extracted from his testes 3 h following his death and cryopreserved. His wife had *in vitro* fertilization (IVF) and the eggs were inseminated by intracytoplasmic sperm injection (ICSI). None of the sperm were motile. Selection was based on softness and pliability. There were 4 embryos formed that cleaved, but only 2 were transferred on the retrieval cycle. The wife failed to conceive, but then had a second transfer of the 2 cryopreserved embryos. She achieved a chemical pregnancy with the β -human chorionic gonadotropin level attaining a maximum level of 107 mIU/mL (rising from 19 mIU/mL). Though this retrieval cycle did not result in a successful pregnancy the achievement of a clinical pregnancy following frozen embryo transfer at least provides cautious optimism for other cases with similar conditions.

Keywords cryopreservation, death, embryo, ICSI, IVF, sperm, testes

Pregnancies have been achieved with cryopreserved-thawed motile testicular sperm using *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) [3, 6, 8]. A case report was recently published where a pregnancy and full-term delivery occurred following ICSI with frozen/thawed immotile testicular sperm, despite the fact that 3 embryos only reached the 2-cell stage, 48 h after retrieval [7].

The case described herein reports fertilization and pregnancy followed by spontaneous abortion also using frozen/thawed completely immotile cryopreserved sperm that was obtained from a testicular biopsy several hours after the sudden death of a 38-year-old male.

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CASE REPORT

History

On his honeymoon a 38-year-old male died of a sudden heart attack. His wife, desperate to at least preserve his genes for future generation and to provide her with his child, made many phone calls to outside sources to attempt to cryopreserve his sperm since the hospital to which he was taken was not equipped for that procedure. Several hours after his death she established contact with a cryopreservation institution and arrangements were made with a urologist to perform a testicular biopsy.

Testicular Biopsy and Sperm Extraction

Seminiferous tubules were excised from both left and right testes 3 h following death and were placed into Ham's F10 medium. The sperm was removed from the tubules by compressing them between glass slides or expressed sperm from the tubules by using subcutaneous needle and syringes with a dissecting microscope. The sperm was prepared with a 2-layer Percoll gradient technique. The supernatant was diluted with an equal portion of TEST-YOLK medium and aspirated into 2 nunc vials.

Sperm Freezing/Thawing

The sperm was first frozen in liquid nitrogen vapor for 30 min and then plunged into liquid nitrogen. Thawing was performed by removing 1 vial and chipping away a portion; the remainder was placed back into liquid nitrogen. The chipped portion was thawed at room temperature. The volume of the specimen was 75 μ L and there was only 0–1 sperm per high-powered field. None were motile.

IVF/ICSI Procedure

Controlled ovarian hyperstimulation used leuprolide acetate for 10 days exclusively starting in midluteal phase and then continued this GnRH agonist at a dosage of 0.25 mg while 300 IU of recombinant FSH (Gonal-F) was given. There were 21 oocytes retrieved from this 36-year-old woman and 15 were deemed mature. ICSI was performed on the 15 mature oocytes [9]. The immature eggs were matured in vitro one more day and ICSI was performed. A total of 6 eggs were fertilized; 1 was discarded and 2 were left to divide to 3 days from retrieval as requested by the patient and 3 were cryopreserved at the 2-pronuclear stage as previously described [4].

Assisted embryo hatching was performed using acidic Tyrode's solution as previously described [2, 5]. A 9-cell and 2-cell embryo were transferred. The 9-cell embryo had uneven and irregular cells and had a <33% fragmentation, and the 2-cell embryo was just slightly uneven and had <25% fragmentation. None of the sperm demonstrated any motility whatsoever after thawing. Using the micro needle, the sperm chosen for injection into the oocyte were those that felt softer and more pliable versus those that were "hard and twiggy."

RESULTS

Cryopreservation of the 2 pronuclear embryos used a simplified protocol with 2 propanediol as the cryoprotectant as previously described [1, 4]. The thawing protocol used a one-step removal of cryoprotectant [1, 4]. Only 2 of the 3 2PN embryos cleaved and at 72 h a 2-cell

embryo and a 4-cell embryo were transferred. The 2-cell embryo had slightly uneven cells with <25% fragmentation and the other embryo was symmetrical with <25% fragmentation.

A positive β -hCG level of 19 mIU/mL was achieved 11 days from transfer, which increased to 64 mIU/mL 3 days later. The level went to 107 mIU/mL 3 days later, then plummeted to 5 mIU/mL 23 days from transfer.

DISCUSSION

A search of the English literature did not reveal any previous publications about fertilization and/or pregnancies following transfer of embryos formed by ICSI from testes of a dead man. There is, however, a case, mentioned in the lay press, from California that apparently had a successful pregnancy. The details of that case are not known so we do not know if there were any similarities to this case, e.g., the significant delay in obtaining sperm postmortem, non-motility of the sperm, the technique for choosing "viable sperm," and the fact that the pregnancy was from frozen not fresh embryo transfer.

This case thus emphasizes that pregnancies can occur from testicular extraction even after death with subsequent cryopreservation. There are still some ethical issues—e.g., does the spouse have the right to use this sperm without the male partner's consent? New techniques are needed to help identify viability even when there is no motility.

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