

## **HUMAN SPERM CRYOPRESERVED AND STORED FOR 30 YEARS, 7 MONTHS AND 6 DAYS RESULTS IN TWO CLINICAL PREGNANCIES**

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**Objective:** Cryopreservation of human spermatozoa is common; however, long term storage for >30 years has not been reported. We describe the cryopreservation, storage and use of human sperm stored >30 years resulting in two pregnancies.

**Design:** Case study at a private fertility clinic

**Materials and Methods:** Cryopreservation of a patient's sperm was performed on 4/30/1982 using the following technique: cryopreservation media (20% Egg yolk, 13.6% Glycerol in a buffer of TRIS, TES, Citrate and Glucose) was mixed 1:1 with semen, placed in 1cc vials and frozen in N<sub>2</sub> vapor for >1 hour followed by plunge into LN<sub>2</sub> for long term storage. The initial ejaculated sample, after 2 days abstinence, had the following characteristics: v=0.3ml, conc=42 M/ml, mot=20% and prog=3. On 12/7/2012 the sample was thawed in a 31°C water bath for 5 minutes, analyzed (v=0.5ml, conc=8M/ml, mot=10%, prog=1.5), separated using a 2-layer gradient, washed and motile sperm were injected into two sets of donor oocytes. Embryos were cultured to blastocysts, trophectoderm biopsy was performed for 24-chromosome analysis and two normal diagnosed embryos were transferred into each of two gestational surrogates. A single clinical pregnancy was confirmed in each of the surrogates.

**Results:** Overall, 2 oocyte donors produced 30-MII oocytes that were injected with 30 year old thawed sperm, 27-2PN zygotes resulted, 15 blastocysts were biopsied, 9 chromosomally normal embryos were diagnosed, 4 embryos were transferred into 2 gestational surrogates (2 embryos/surrogate) and 5 embryos were cryopreserved. Two singleton clinical pregnancies were observed in the surrogates prior to discharge to obstetrical care.

**Conclusions:** Cryopreservation and storage of sperm for >30 years has for the first time demonstrated reproductive potential. Patient's concerns about the useful timeframe of cryopreserved semen and male reproductive tissue can be assuaged, as this abstract demonstrates the longest successful cryopreservation, storage and genetically normal use of human sperm.