

Purposeful cryopreservation of sperm prior to intrauterine insemination to overcome the embryo implantation defect associated with sperm with a subnormal hypoosmotic swelling test

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Summary

Purpose: To determine if the toxic protein causing sperm to have low hypoosmotic swelling (HOS) test score and subsequent embryo implantation defects caused by this abnormality is cryolabile, and thus possibly purposeful cryopreservation followed by intrauterine insemination (IUI) could result in a pregnancy. **Materials and Methods:** The etiologic factor for a two-year history of infertility failing to respond to IUI and two cycles of in vitro fertilization-embryo transfer (IVF-ET) using conventional insemination was found secondary to a very low HOS test (32% with abnormal <50%). With IVF-ET with intracytoplasmic sperm injection (ICSI), the patient conceived in her first cycle but she had a miscarriage. She failed to conceive in cycle 2 (actually her 4th IVF-ET cycle). Treatment with the protein digestive enzyme chymotrypsin corrected the HOS defect (HOS test 75-80%) on 3 different treatment specimens but she failed to conceive despite IUI with treated sperm. She had switched to IUI for financial reasons. Unfortunately the manufacturer temporarily stopped making chymotrypsin. A unique treatment was attempted for the first time – cryopreservation of the sperm prior to IUI hoping the toxic protein was cryolabile. **Results:** A viable pregnancy was achieved following the first IUI cycle with frozen thawed sperm. Unfortunately there was fetal demise found to be secondary to trisomy 14. **Conclusion:** Sperm with low HOS tests do not inhibit fertilization but instead causes embryo implantation defects hypothesized to be related to the supernumerary sperm attached to the zona pellucida. This is the first reported case of a pregnancy achieved by IUI using cryopreserved sperm with low HOS scores.

Key words: Cryolabile; Cryopreservation; Sperm; Hypo-osmotic swelling test.

Introduction

In 1989 data were published demonstrating a very high correlation of infertility in couples whose male partners had sperm with subnormal hypo-osmotic swelling (HOS) tests even when other semen parameters were perfectly normal [1]. This test is performed by placing sperm in a hypo-osmolar solution and then evaluating the functional integrity of the sperm membrane by assessing the ability of the sperm membrane to pump the higher concentration of water from the high side to the low side by osmosis. If the membrane is functionally intact, at least 50% of the sperm should demonstrate tail swelling [1].

It is generally considered that the function of the sperm is to fertilize the oocyte and then the “job” is done. When evaluating in vitro fertilization-embryo transfer (IVF-ET) and comparing motile density, morphology, and the HOS test when performing conventional oocyte insemination, the HOS test was found to be the least predictive test for poor fertilization [2]. However, it was the best by far in predicting pregnancy rate per embryo transfer [2, 3].

One study evaluated 22 paired IVF-ET cycles where one woman supplied oocytes for herself and for a recipient who was sharing the IVF costs, but where, even though both male partners had normal standard semen parameters, one had an HOS test score $\geq 50\%$ (normal) and one $< 50\%$ (ab-normal). Despite the same number and quality of embryos transferred, the pregnancy rate was 50% for the couple with normal HOS test scores and 0% for those subnormal [4]. Thus, it was concluded that sperm with subnormal HOS test scores cause infertility by causing embryo implantation defects [5].

If sperm with low HOS test scores allow normal fertilization but cause embryo implantation defects, it seems likely that the factor associated with the sperm membrane must transfer to the zona pellucida by the supernumerary sperm that remained attached (the zona becomes incorporated into the embryo membrane). Thus, it seems possible that performing intracytoplasmic sperm injection (ICSI), and thus bypassing any sperm attached to the zona pellucida, could overcome the problem. Indeed ICSI was shown to be an efficacious method to overcome the problem [6, 7].

It was concluded that the toxic factor on the sperm was likely a protein. Treating the sperm with a protein digestive enzyme chymotrypsin was able to provide live pregnancies following intrauterine insemination (IUI) [8, 9] and also with conventional oocyte insemination with IVF [8, 10].

Though pregnancy rates are higher with IVF-ET and ICSI vs. chymotrypsin treated sperm used for IUI with avoidance of unprotected sex [11], many couples prefer IUI for financial reasons [11]. Unfortunately, for a period of eight months, chymotrypsin was not being manufactured. Back in 1996 we published an article showing some pregnancies occurring following frozen/thaw embryo transfer of embryos created by conventional oocyte insemination with sperm with low HOS swelling test scores [12]. This suggested that the toxic protein may be cryolabile. We thus offered freezing the sperm prior to IUI in an infertile couple who did not want IVF with ICSI but IUI with chymotrypsin treated sperm (which was not available at that time). They decided they would be willing to try cryopreservation of sperm before IUI for low HOS, although this had never been done before.

Case Report

A couple with a two-year history of infertility who failed to conceive after five cycles of IUI and two cycles of IVF and embryo transfer where conventional oocyte insemination was used at another reproductive practice, sought a second opinion from our reproductive center. Despite normal conventional semen parameters his HOS test was very low at 32%. The woman (age 40) did conceive following her first IVF-ET-ICSI cycle in our IVF center but she had a miscarriage (trisomy 18). She failed to conceive on her 2nd IVF cycle with ICSI. For financial reasons she switched to IUI with chymotrypsin treated sperm. Though this therapy corrected the HOS score all three times with levels at 75-80%, unfortunately she failed to conceive with the three IUI cycles performed on natural cycles with progesterone support in the luteal phase.

The manufacturer stopped making chymotrypsin for eight months. We tried for the first time to see if purposeful cryopreservation of sperm prior to IUI could achieve a pregnancy. A viable pregnancy was achieved but unfortunately she had a miscarriage at the end of the first trimester. Chromosome analysis revealed aneuploidy – trisomy 14. Chymotrypsin became available again. She conceived on her first IUI with chymotrypsin in a natural cycle with just luteal phase progesterone support. She delivered a live healthy baby.

Discussion

Despite many articles published about this test since 1989, including recent ones, most reproductive centers never measure this test despite the simplicity and inexpensiveness of the technique when they perform semen analysis. The odds are that the large majority of those reading this manuscript are not familiar with the test.

Treating the sperm with chymotrypsin is inexpensive but a little tricky. It may be easier for some infertility specialists to freeze the sperm and then perform an IUI for low HOS test. Freezing sperm may be easier for

those clinicians practicing infertility who do not have an extensive laboratory. At present, all one can say is that it can work, but its efficacy compared to chymotrypsin treatment is unknown.

Conflict of Interest

The authors declare no competing interests.

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