Pretreatment of sperm with low hypo-osmotic swelling tests with chymotrypsin prior to intrauterine insemination (IUI) and avoidance of unprotectected intercourse results in pregnancy rates comparable to IUI for other male factor problems

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Summary

Purpose: To carry out a retrospectively performed matched controlled study to determine the efficacy of pretreatment of sperm having low hypo-osmotic swelling (HOS) test scores with chymotrypsin galactose prior to intrauterine insemination (IUI) compared to IUI for other types of male factor problems. The women with male partners with low HOS scores were advised not to have unprotected intercourse. *Methods:* All cycles having IUI with chymotrypsin treated sperm for low HOS scores were matched with the very next woman having IUI for sperm with other male factor problems but with normal HOS scores. *Results:* There was a significantly higher clinical pregnancy rate with chymotrypsin treated sperm (32.3% per IUI) vs 21.9% for other male factor cases. The live birth rate per IUI cycle was not significantly different (21.2% vs 15.4%). *Conclusions:* These results now show that pretreatment of sperm with low HOS scores allows very good pregnancy rates following IUI as long as the couple is cautioned about unprotected intercourse. These data support the concept that sperm with low HOS test scores impair fertility by transferring a toxic factor from the sperm to the zona pellucida to the embryo membrane which impairs the embryos from implanting.

Key words: Hypo-osmotic swelling test; Implantation; Intrauterine insemination.

Introduction

In 1989, a study demonstrated that males with normal semen parameters but hypo-osmotic swelling (HOS) scores < 50% did not achieve pregnancies following intercourse [1]. However, several subsequent research publications by other authors have stated that a low HOS score had no adverse effect on fertilization rates following in vitro fertilization-embryo transfer (IVF-ET) [2-5]. Thus, most infertility specialists assumed that this means that a low HOS score has no clinical importance since it was assumed that once the sperm fertilized the egg the role of the sperm ceased.

However, a matched-controlled study performing IVF with conventional oocyte fertilization found no adverse effect of low HOS test scores on fertilization rates similar to these other authors [6]. However, there were hardly any live pregnancies [6]. Another study from the 1990s evaluating single-sperm defects on IVF outcome found a 25% clinical pregnancy rate/transfer with all semen parameters normal and 25% with low motile density, 44% with low strict morphology, but 0% with a HOS test score < 50% [7]. A study where a single pool of oocytes was shared between two male partners with normal semen parameters but one with a normal and the other a subnormal HOS test score was performed. The clinical pregnancy rate/transfer was 50% in the former vs 0% in the latter [8].

The 50% cutoff is critical for the test. Jeyendran *et al.* stated that the grey zone for this test was 50-59% [9]. However no reduced pregnancy rates with male partners with the grey zone HOS scores were found [10]. It has been found that the HOS test abnormality, once it is subnormal, generally tends to stay subnormal [11]. This HOS test abnormality was found in 8% of the male partners aged < 45 in our infertile population, 16% in males aged 45-49, and 33% in males ≥ aged 50 [12].

Fertilization of oocytes by intracytoplasmic sperm injection (ICSI) overcomes the HOS test abnormality [13]. One hypothesis to explain how fertilization is not impaired but pregnancy rates are markedly decreased when a low HOS test score is present is that the impairment of the functional integrity of the sperm membrane, as demonstrated by low HOS test scores, is related to a toxic factor attached to the sperm [14]. This toxic factor can be transferred to the zona pellucida by the supernumerary sperm that attach, which when incorporated into the embryo membrane causes functional impairment of the embryo membrane, which in turn inhibits implantation [14]. The possibility exists that this toxic factor is a protein. If so, then treatment of the sperm with a protein

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digestive enzyme, (e.g., chymotrypsin) might overcome the abnormality.

A pilot study was initiated in 1997 in which eight of 12 men had improved HOS test scores (≥ 50%) after chymotrypsin treatment [15]. Four of these eight couples (50%) conceived in 12 IUI cycles (33% per cycle) [15]. The four whose scores remained < 50% despite chymotrypsin therapy were offered IVF with ICSI, and two of the four conceived.

In this pilot study, the couples were advised not to have unprotected intercourse because otherwise it could not be certain that conception occurred from the treated sperm. On the basis of the pilot study, we recommended intrauterine insemination (IUI) with chymotrypsin as first-line therapy. In-vitro fertilization with ICSI was only suggested if there was no success with IUI. However, a retrospective review of 99 IUI cycles with chymotrypsin therapy found only three live pregnancies (3.0%) [16].

A subsequent study determined whether treating sperm with low HOS test scores with the protein digestive enzyme chymotrypsin before conventional fertilization of oocytes could improve pregnancy rates after ET [17]. Couples in whom the HOS test score was < 50% in two consecutive evaluations were offered free IVF-ET (exclusive of medications) if they would allow half of the retrieved oocytes to be fertilized conventionally with chymotrypsin-treated sperm and the other half to be fertilized by ICSI. The agreement was that the embryos transferred on the retrieval cycle were the ones formed by conventional insemination with chymotrypsin-treated sperm.

There were 28 oocyte retrievals and 28 ETs. The clinical pregnancy rate per transfer was 42.9% (12/28). The ongoing/delivered pregnancy rate was 32% (9/28). The implantation rate was 21.3% (19/89) [17].

The present study attempted to repeat the aforementioned study of treating sperm with HOS test scores < 50% with chymotrypsin prior to IUI but this time cautioning the couples not to have unprotected intercourse prior to ovulation. Since it seems that based on these studies chymotrypsin-galactose treatment may neutralize the "toxic" factor, it was considered that the aforementioned retrospective review showing poor pregnancy rates despite pretreatment of sperm with chymotrypsin prior to IUI could have been related to sperm with this toxic factor reaching the egg through normal intercourse since the patients were not admonished about having unprotected intercourse [16]. The sperm from the control group was not treated by chymotrypsin.

Materials and Methods

A retrospective matched study was performed. All cycles using IUI for male factor related to HOS test scores < 50% treated with chymotrypsin and protected intercourse were evaluated for clinical pregnancy rate (ultrasound evidence of pregnancy at 8 weeks and miscarriage rate at 12 weeks). During this time period the same number of consecutive cycles treated with IUI for male factor with normal HOS test scores were similarly evaluated.

The HOS test was performed by combining 0.1 ml of ejaculate with 1.0 ml hypo-osmotic solution (fructose/sodium citrate) following precisely the technique described by Jeyendran et al [9]. After incubation of the mixture for at least 30 min at 37°C, 100 spermatozoa were observed with a phase-contrast microscope for tail changes typical of a reaction in the HOS test. The HOS tests were performed on unprepared specimens during standard semen analysis.

For chymotrypsin-galactose treatment, 0.1 M galactose was dissolved in 5 ml of Earle's balanced salt solution and added to 5 mg of chymotrypsin. The patient ejaculated directly into this chymotrypsin-galactose mixture. The semen immediately were mixed to break up the coagulum. Bovine serum albumin (30 mg/ml) was added to stop the enzymatic reaction.

The women receiving IUI for low HOS scores were matched to the very next woman having IUI for other types of male factor with normal HOS test scores. The female partner of the males with subnormal HOS test scores were admonished not to have unprotected intercourse prior to ovulation.

Results

A matched controlled comparison of clinical pregnancies (ultrasound evidence of pregnancy at 8 weeks) per IUI cycle and miscarriage rates by the end of the first trimester in women with male partners with low HOS test scores vs women with male partners with problems with sperm concentration, motility, or morphology is shown in Table 1.

Table 1. — *Comparison of clinical pregnancy rates following intrauterine insemination (IUI) for males with low hypo-osmotic swelling test scores compared to pregnancy rates following IUI for other types of male factor.*

	Number of cycles	Number of pregnancies	Percent of pregnancies per IUI cycle	Number of miscarriages	Miscarriage rate
Abnormal HOS scores (< 50%)	155	50	32.3%	17	34%
Normal HOS scores (> 50%)	155	34	21.9%	10	29%

The treatment of sperm with low HOS test scores with the protein digestive enzyme chymotrypsin, with emphasis on protected intercourse, with subsequent IUI resulted in a very adequate clinical pregnancy rate of 32.3% and an ongoing delivered pregnancy rate of 21.2% per IUI cycle. The clinical pregnancy rate per IUI cycle was actually significantly higher with the HOS abnormal sperm than the clinical pregnancy rates of 21.9% for abnormal sperm with normal HOS scores ($p = .04$), but the ongoing/delivered pregnancy rates were not different (21.2% with low HOS vs 15.4% with normal HOS test scores).

Conclusions

The original pilot study of only eight patients found a clinical pregnancy rate per IUI cycle of 33.3%. The new data show very similar pregnancy rates (32.3%) [15].

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Conclusions

The original pilot study of only eight patients found a clinical pregnancy rate per IUI cycle of 33.3%. The new data show very similar pregnancy rates (32.3%) [15].

This is in marked contrast to the aforementioned study of chymotrypsin treatment prior to IUI without cautioning about unprotected intercourse where only a 3% success was found [16].

Thus these data support the hypothesis that the HOS abnormality is related to a toxic effect that occurs when the sperm attach to the zona pellucida. The assumption is that in the previous large study of chymotrypsin treatment of sperm with low HOS scores prior to IUI, the much lower success rate was related to transferring of the toxic factor from sperm to egg by the untreated sperm present in the cervical mucus that attached to the zona pellucida at ovulation.

Though ICSI is a highly effective treatment for low HOS scores, chymotrypsin treatment and IUI provides an effective alternative that is much less expensive and much less risky [13]. However, the couple must be cautioned about unprotected intercourse prior to ovulation.

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