



## DO ANTISPERM ANTIBODIES CAUSE FUNCTIONAL IMPAIRMENT OF THE SPERM MEMBRANE AS MANIFESTED BY A LOW HYPOOSMOTIC SWELLING TEST SCORE?

S. JAIRAJ  
J. H. CHECK  
A. BOLLENDORF

The University of Medicine and Dentistry of New Jersey,  
Robert Wood Johnson Medical School at Camden, Cooper  
Hospital/University Medical Center, Department of Obstetrics  
and Gynecology, Division of Reproductive Endocrinology  
and Infertility, Camden, New Jersey, USA

Low hypoosmotic swelling (HOS) test scores were found to be associated with lower pregnancy rates. The mechanism seems to be related not so much to impaired fertilization but to inhibition of implantation. The defect may be present in males with normal or subnormal semen specimens. However, anecdotal experience suggested that the subset of males with antisperm antibodies (ASAs) have a higher frequency of low HOS scores. The possibility exists that ASAs may impair the functional integrity of the sperm membrane. The study presented herein, artificially added ASAs to sperm to see if this could lower the HOS score. The study would also determine if chymotrypsin, a protein digestive enzyme, could improve HOS scores, if, in fact, they were lowered by the addition of ASAs. The results did not show a reduction in the HOS scores following the addition of ASAs. Thus, it would appear that there is no cause and effect with the simultaneous presence of low HOS scores and ASAs. Possibly, however, longer exposure or a higher concentration of antibodies is needed to lower HOS scores.

**Keywords** chymotrypsin, hypoosmotic swelling, sperm autoantibodies

Males with semen samples demonstrating hypoosmotic swelling (HOS) test scores < 50% rarely achieve in vivo pregnancies, even when other semen parameters are normal [3]. Sperm with this abnormality have no problem with fertilizing oocytes following in vitro fertilization (IVF), but the ensuing pregnancy rates (PRs) are dismal [4, 11].

There appears to be some association between the presence of sperm autoantibodies and low HOS scores. Although most patients with low HOS scores are negative for sperm autoantibodies, patients strongly positive for sperm autoantibodies frequently have subnormal HOS scores. A study of patients undergoing intracytoplasmic sperm injection (ICSI) in 1997 found

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Address correspondence to Jerome H. Check, MD, PhD, 7447 Old York Road, Melrose Park, PA 19027, USA.

that patients who were negative for sperm autoantibodies had an HOS score of 59% despite a mean count of  $2.0 \times 10^6/\text{mL}$  with only 17% motility compared to patients strongly positive for sperm autoantibodies where the HOS score was only 46% despite mean sperm count of  $21 \times 10^6/\text{mL}$  and 46% motility [11]. Interestingly, patients who were moderately positive for sperm autoantibodies (50–80%) had an HOS score of 63% [11]. What is not clear is whether for some reason a higher percentage of patients with sperm autoantibodies also have the same factor that leads to low HOS scores or whether the antibodies themselves have a direct effect on the sperm membrane, causing functional impairment and thus a low HOS score.

The study presented herein attempted to determine if attachment of sperm autoantibodies would directly cause a reduction in the HOS score.

## MATERIALS AND METHODS

### Experimental Design

Semen was provided from 10 healthy volunteers. After liquefaction, each sample was divided into 3 aliquots, and the HOS test and immunobead assay were read twice for each aliquot. The tests were first done on the initial specimen, then on the specimen after exposure to antisperm antibodies, and finally on the ASA-positive sperm after treatment with chymotrypsin–galactose.

### HOS Test

The HOS test was performed by combining 0.1 mL of ejaculate with 1.0 mL hypoosmotic solution (fructose/sodium citrate), following precisely the technique described by Jeyendran et al. [8]. After incubation of the mixture for at least 30 min at 37°C, 100 sperm 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.

### Immunobead Assay

The semen samples were evaluated for antibodies using the immunobead test [2]:  $5\text{--}10 \times 10^6$  sperm were washed 3 times using 0.5% bovine serum albumin (BSA) in Biggers–Whitten–Whittingham (BWW) medium. After centrifugation the final pellet was resuspended in 5% BSA/BWW. The washed sperm were mixed with IgG or IgA beads and read microscopically for the percentage and attachment sites of sperm binding to the beads.

### Treatment With Chymotrypsin

The reagents used for chymotrypsin/galactose treatment were Earle's balanced salt solution (EBSS) (Irvine Scientific 9208), chymotrypsin type IV-s, 5 mg (Sigma CHY-5s), D(+)-galactose (Sigma G5388), and bovine albumin fraction V powder, low endotoxin BSA (Irvine Scientific 1092). The chymotrypsin/galactose pretreatment protocol was as follows: 0.1 M galactose was added to 5 mL of EBSS, and the solution was incubated at 37°C, sterilized by filtration using a 0.2- $\mu\text{m}$  filter, and added to 5 mg chymotrypsin just prior to semen production. Semen was collected directly into chymotrypsin/galactose in a specimen cup and immediately disrupted with a transfer pipette until the coagulum was liquefied (30–60 s). Rapid addition of 30 mg/mL BSA stopped the enzymatic reaction, and when this was fully dissolved (~5 min) the specimen was layered onto a Percoll column.

### Addition of Autoantibodies to Sperm

Pooled positive ASA serum was added in equal volumes to 1 mL of the sperm specimen, which was incubated at 37°C for 60 min; the mixture was washed with modified BWB medium and resuspended to 1 mL with modified BWB medium. A direct immunobead test (DIBT) was then performed and the percentage of IgA and IgG were recorded; 0.5 mL of the now positive sperm was incubated with equal volumes of TYB for 1 h in a 4°C water bath, 0.5 mL of the sperm was also incubated with an equal volume of human tubal fluid (HTF), as a control. The specimens were allowed to warm at 37°C for 10 min, then the DIBT was again performed and the percentages of the IgA and IgG were recorded.

### Statistical Analysis

The *t* test was used to determine any correlation between the HOS scores and the presence of ASA as related to the chymotrypsin-galactose treatment. A *p* value of <.05 was considered significant.

## RESULTS

The mean HOS score on the initial sample was 87.7%. Following the addition of antisperm antibodies, there was not a significant drop in the HOS score (83.5%) (*p* = .079). The immunobead test, however, demonstrated that 100% of the sperm were bound with antibodies. The addition of chymotrypsin to the sperm now bound with antisperm antibodies improved the HOS score to 89.7% and almost resulted in a significant improvement (*p* = .053).

## DISCUSSION

These data did not show any significant reduction in HOS scores with the attachment of sperm autoantibodies. The mean score of 83% of the sperm now bound with antisperm antibodies was not even close to the critical score of 50% that is associated with male subfertility.

The concept as to how sperm with low HOS scores cause infertility is not that those sperm with low scores are not able to fertilize, because even if that were true, there would still be plenty of sperm left in specimens with other normal semen parameters to achieve a pregnancy. Rather, the concept is that when a sperm sample has a low HOS score without any apparent cause, it may be secondary to a toxic factor attached to the sperm membrane. Some of these sperm may attach to the zona pellucida and transfer the toxic factor to the oocyte and eventually the embryo, and the defective embryo membrane may preclude proper implantation. Thus, it is not the single sperm that fertilizes the egg that is the problem; instead, it is the supernumerary sperm attached to the zona pellucida. The fact that in vitro fertilization (IVF) with ICSI results in high PRs when the sperm is taken from a sample with a low HOS score supports this concept [5, 10].

Functional impairment to the sperm membrane may occur and be associated with a low HOS score without this toxic factor. For example, cryopreservation of sperm has been shown to cause a low HOS score [6, 7]. But these sperm usually have associated decreased viability also. Thus, it seems likely that in the case of cryopreservation, ice crystal formation damages the sperm membrane, resulting in functional and structural impairment of the sperm membrane. However, the hypothesized toxic factor would not be present, and thus the effects on

subsequent achievement of pregnancy are not nearly as devastating as when the low HOS score has an idiopathic etiology.

Since adding antibodies to sperm does not significantly lower the HOS score if a higher association of sperm autoantibodies and low HOS scores are found, it is probably not cause and effect. The use of chymotrypsin to treat sperm coated with autoantibodies has improved PRs following intrauterine insemination (IUI) and IVF [1], but there is no reduction in the level of autoantibodies after treatment [9]. However, some sperm samples with low HOS scores treated with CG do improve over 50% and these samples have resulted in pregnancies even following IUI [5, 10].

Thus, based on the data presented herein, if a sperm sample is both positive for autoantibodies and has a low HOS score and if chymotrypsin therapy does not improve the HOS score, ICSI rather than IUI should be performed. The reasoning is that the failure to improve the HOS score suggests the continued presence of this toxic factor even if the adverse effects of the sperm autoantibodies have been neutralized. Improved PRs with chymotrypsin for sperm autoantibodies without changing immunobead levels has been hypothesized as being related to damaging the antibody and thus ameliorating its harmful effect but not removing it from the sperm surface. Pregnancies have resulted following CG treatment of sperm with both high levels of autoantibodies and low HOS scores when the HOS score has improved to over 50% [5, 10] and was the impetus to try this protein digestive enzyme for low HOS scores even when autoantibodies were not present [10].

The study was set up to determine if chymotrypsin would also improve the HOS test when a reduction was caused by artificially added sperm autoantibodies. Since a meaningful reduction was never achieved, this additional step turned out to be superfluous. However, it is interesting that a significant improvement following therapy ( $p = .053$ ) was almost accomplished.

Before one completely abandons the concept of a direct effect of sperm autoantibodies impairing the functional integrity of the sperm membrane, there is one other consideration. Although most sperm autoantibodies are believed to attach at the time of ejaculation, the immunobead test is not performed until after liquefaction. In this study the antibodies were added to an already liquified specimen. Thus, the contact of sperm and autoantibodies was much shorter in this study, and a longer exposure might lower the HOS score. A study is ongoing to evaluate this possibility.

## REFERENCES

1. Bollendorf A, Check JH, Katsoff D, Fedele A (1994): The use of chymotrypsin/galactose to treat spermatozoa bound with anti-sperm antibodies prior to intra-uterine insemination. *Hum Reprod* 9:484-488.
2. Bronson R, Cooper G, Rosenfeld D (1984): Sperm antibodies: their role in infertility. *Fertil Steril* 42:171-183.
3. Check JH, Epstein R, Nowroozi K, Shanis BS, Wu CH, Bollendorf A (1989): The hypoosmotic swelling test as a useful adjunct to the semen analysis to predict fertility potential. *Fertil Steril* 52:159-161.
4. Check JH, Stumpo L, Lurie D, Benfer K, Callan C (1995): A comparative prospective study using matched samples to determine the influence of subnormal hypo-osmotic test scores of spermatozoa on subsequent fertilization and pregnancy rates following in-vitro fertilization. *Hum Reprod* 10:1197-200.

5. Check JH, Katsoff D, Summers-Chase D, Swenson K, Choe J (1999): Good pregnancy rates (PRs) and implantation rates following in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) for low hypo-osmotic swelling (HOS) scores. 24th Annual Meeting of the American Society of Andrology, Louisville, Kentucky, 10-13 April 1999. *J Androl* January/February 1999 Supplement, pg 37, abstract #48.
6. Check ML, Check JH (1991): Poor hypo-osmotic swelling test results from cryopreserved sperm despite preservation of sperm motility. *Arch Androl* 26:37-41.
7. Check ML, Check JH, Long R (1991): Detrimental effects of cryopreservation on the structural and functional integrity of the sperm membrane. *Arch Androl* 27:15-160.
8. Jeyendran RS, Van der Ven HH, Perez-Palaez M, Crabo BG, Zaneveld LJD (1984): Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J Reprod Fertil* 70:219-228.
9. Katsoff D, Check JH, Kozak J (1994): Failure of test yolk buffer to decrease antisperm antibodies on sperm. *Arch Androl* 33:137-139.
10. Katsoff D, Check JH (1997): Two methods of achieving pregnancies despite subnormal hypo-osmotic swelling test scores. *Fertil Steril* 68:549-551.
11. Kiefer D, Check JH, Katsoff D (1996): The value of motile density, strict morphology, and the hypoosmotic swelling test in in vitro fertilization-embryo transfer. *Arch Androl* 37:57-60.