

## In Vitro Fertilization With Intracytoplasmic Sperm Injection Is an Effective Therapy for Male Factor Infertility Related to Subnormal Hypo-Osmotic Swelling Test Scores

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**ABSTRACT:** The aim of the study was to determine if intracytoplasmic sperm injection (ICSI) would be an effective therapy for males with subnormal hypo-osmotic swelling test (HOST) scores, a condition known to prevent implantation of embryos despite allowing normal fertilization and embryo formation. Couples in whom the male partner had a HOST score of <50% and failed to achieve a pregnancy despite at least 3 cycles of intrauterine insemination with chymotrypsin-galactose-treated sperm were treated with in vitro fertilization (IVF) with ICSI. The clinical and viable pregnancy rates were 49.0% and 45.3% (n = 53). The implantation rate was 27.1%. These data thus demonstrate that ICSI is an effective therapy for infertile couples in whom the male partner has a subnormal hypo-osmotic

swelling (HOS) score. Previous studies using conventional IVF without ICSI when HOS was subnormal found normal fertilization rates but a marked reduction in pregnancy and implantation rates. The very acceptable pregnancy and implantation rates demonstrated in this study with ICSI is consistent with the hypothesis that the defect associated with sperm that have subnormal HOST scores is not related to the single spermatozoon that is responsible for fertilizing the oocyte but may be related to a toxic factor associated with the supernumerary sperm attached to the zona pellucida.

Key words: Functional integrity, implantation disorders, sperm membrane.

**J Androl 2001;22:261–265**

One of the properties of a cell membrane is its ability to permit the transport of molecules selectively. Using bovine sperm, it has been demonstrated that when exposed to hypo-osmotic solutions, water will traverse the membrane and enter the spermatozoon in an attempt to reach osmotic equilibrium (Drevius and Eriksson, 1966; Bredderman and Foote, 1969; Foote and Bredderman, 1969; Drevius, 1972). This test was modified for human sperm by Jeyendran, et al (1984), who hypothesized that this test may be useful for detecting male subfertility not only because membrane integrity is important for sperm metabolism, but also because it is required for sperm capacitation, the acrosome reaction, and the binding of the sperm to the egg surface.

Studies have been performed to evaluate the correlation of subnormal hypo-osmotic swelling test (HOST) scores with other semen parameters that are believed to be predictive of fertilization potential, albeit with various conclusions (Chan et al, 1985; Wang et al, 1988; Coetzee et al, 1989; Mordel et al, 1989; Fuse et al, 1991). Probably the best way to determine if a test correlates with oocyte fertilization potential is to compare fertilization rates fol-

lowing in vitro fertilization (IVF) with sperm with subnormal vs normal HOST scores. Although a couple of studies have found lower fertilization rates with subnormal HOST scores (Van der Ven et al, 1986; Hauser et al, 1992; Abu-Musa et al, 1993), the majority have found HOST to be one of the least useful tests to determine low fertilization potential of sperm (Barratt et al, 1989; Sjoblum and Coccia, 1989; Avery et al, 1990; Chan et al, 1990; Enginsu et al, 1992; Kiefer et al, 1996).

A previous in vivo study found a pregnancy rate of 83% in an 8-month treatment period in infertile couples whose male partner had HOST scores of >50% compared with no pregnancies in couples in whom the male partner's HOST score was <50% even if all semen parameters were normal (Check et al, 1989). The use of IVF-embryo transfer (IVF-ET) did not improve pregnancy or implantation rates (Check et al, 1995). The only study that might be interpreted to mean that low HOST scores may not be associated with poor pregnancy rates was our own publication of frozen embryo transfers. However, we believed a more likely hypothesis was that a toxic factor may be attached to the sperm membrane, which impairs the functional integrity of the sperm membrane, and that this factor may be cryolabile (Check et al, 1996a).

A few anecdotal cases were published suggesting that fertilization of oocytes by intracytoplasmic sperm injection

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Received for publication June 5, 2000; accepted for publication September 26, 2000

tion (ICSI) in men with low HOST scores may be associated with successful pregnancies (Katsoff et al, 1997). The study presented here evaluated the use of ICSI for patients with low HOST scores in a much larger series to see if the aforementioned successful pregnancies were merely fortuitous or whether ICSI is an effective therapy for men with HOST scores of <50%.

## Materials and Methods

### Study Design

The study included all cycles of IVF with ICSI performed at the Cooper Center for IVF from January 1, 1997 to June 31, 1998 when the HOST score was <50% in 2 consecutive evaluations. Only the first embryo transfer cycle following retrieval was included so that some patients did not contribute more cycles than others. The results of any frozen embryo transfer cycles were evaluated separately. There was no overlap of patients from the aforementioned pilot study (Katsoff and Check, 1997).

Patients with subnormal HOST scores were not treated with IVF with ICSI unless they had failed to achieve a pregnancy after at least 3 cycles with intrauterine insemination after sperm were treated with the protein digestive enzyme, chymotrypsin. The methodology for treatment with chymotrypsin/galactose has been previously described (Bollendorf et al, 1994) and was used because this treatment has been found to be effective in improving HOST scores, with pregnancies ensuing in some anecdotal reports (Katsoff and Check, 1997).

The data were also evaluated according to whether the male partner also had subnormal semen parameters (eg, sperm concentration or motility according to standards set by the Society of Assisted Reproductive Technology). Morphology was not included in this evaluation because we attempted to use only sperm with normal morphology for the ICSI procedure.

### HOST Procedure

HOST was performed on 2 unprepared semen specimens from each patient presenting for treatment with IVF-ET during standard semen analysis. The test was performed by combining 0.1 mL of ejaculate with 1.0 mL of HOST solution (fructose-sodium citrate, Sigma Chemical Co, St Louis, Mo) based on the technique of Jeyendran et al (1984). After incubating the mixture for >30 minutes at 37°C, 100 spermatozoa were observed with a phase-contrast microscope for tail swelling changes that were typical of a reaction in the HOS test. Based on our previous study (Check et al, 1989), scores <50% were considered abnormal instead of the 60% cutoff reported by Jeyendran et al (1984).

### ICSI Procedure

For each oocyte, a motile sperm was immobilized with an injection pipette in a drop of polyvinylpyrrolidone (Scandinavian IVF Science AB, Goteberg, Sweden) and then injected into the ooplasm. The injected oocytes were placed in human tubal fluid (Irvine Scientific, Irvine, Calif) and 10% synthetic serum substitute (Irvine Scientific) and incubated for >16 hours before

evaluation for signs of fertilization (2 pronuclei; Palermo et al, 1993; Van Steirteghem et al, 1993a, 1993b).

## Results

The male partners of 53 women having IVF were found to have low HOST scores and subnormal semen parameters. Clinical pregnancies (ultrasound evidence of pregnancy) occurred in 26 (49.0%) women and 24 (45.3%) still had viable pregnancies after 12 weeks. The implantation rate was 27.1%.

In only 11 couples did male partners have semen specimens with an HOST score of <50% but otherwise normal semen parameters. There were 3 (27.3%) clinical pregnancies and no spontaneous abortions.

The group with low HOST scores and subnormal semen parameters had 24 subsequent frozen embryo transfers as a result of the first oocyte retrieval cycle. There were 9 (37.5%) clinical pregnancies and the viable pregnancy rate after the first trimester was 33.3%. Three out of 6 (50%) with normal semen parameters and low HOST score achieved clinical pregnancies following frozen embryo transfer and all were viable at the end of the first trimester. Implantation rates for the 2 groups were 20.0% and 14.3%, respectively.

Combining all groups with low HOST scores, irrespective of semen parameters, the clinical and viable pregnancy rates following fresh embryo transfer were 45.3% (29/64) and 42.2% (27/64), respectively. Comparable values for frozen embryo transfers were 40.0% (12/30) and 36.6%, respectively.

The patient profile of the 53 couples participating in the study appears in Table 1.

## Discussion

These data fulfilled the initial objective by convincingly demonstrating that ICSI is an effective therapy for men with low HOST scores. However, because no controls were receiving IVF with conventional insemination one might question whether ICSI was really needed. Indeed, several studies have suggested that conventional insemination of oocytes resulted in normal fertilization rates (Barratt et al, 1989; Sjoblum and Coccia, 1989; Avery et al, 1990; Chan et al, 1990; Enginsu et al, 1992).

However, it is interesting that the aforementioned studies (Barratt et al, 1989; Sjoblum and Coccia, 1989; Avery et al, 1990; Chan et al, 1990; Enginsu et al, 1992) only evaluated HOST scores and fertilization rates but did not assess correlation with pregnancy rates. Thus, one alternative explanation for very poor in vivo pregnancy rates with low HOST scores despite relatively normal fertiliza-

## Patient profile

Characteristic	(Range) Median	Mean $\pm$ SD
Female's age, y	(23–48) 36	35.7 $\pm$ 5.1
No. oocytes retrieved	(2–34) 11	12.3 $\pm$ 8.5
No. mature oocytes retrieved	(1–27) 8	9.3 $\pm$ 6.3
No. oocytes inseminated	(1–32) 10	10.6 $\pm$ 6.9
No. fertilized	(1–26) 5	6.8 $\pm$ 4.9
% fertilization (all oocytes)	(25–100) 66.7	66.7 $\pm$ 22.2
% fertilization (mature oocytes)	(27.3–100) 70.0	69.3 $\pm$ 22.7
No. embryos available per patient	(1–27) 6	7.6 $\pm$ 5.3
No. embryos transferred	(1–6) 3	3.4 $\pm$ 1.1
Etiology		
Male factor only*	17 (32.1%)	...
Female factor only†	25 (47.2%)	...
Male and female factors	11 (20.7%)	...
Ovarian Stimulation Used		
Gonadotropins only	2 (3.8%)	...
Luteal phase LA/gonadotropins	26 (49.1%)	...
Follicular phase LA/gonadotropins	25 (47.2%)	...

\* Low count or motility.

† Except for low HOST score.

tion rates following IVF could be that somehow the defect in the functional integrity of the sperm membrane leads to implantation failure rather than fertilization failure. Unfortunately, this hypothesis could not be substantiated or refuted by the aforementioned studies showing no or little correlation with low HOST scores and fertilization rates because pregnancy rates were not provided (Barratt et al, 1989; Sjoblum and Coccia, 1989; Avery et al, 1990; Chan et al, 1990; Enginsu et al, 1992).

Credence to this concept of a dissimilitude of fertilization and pregnancy rates with sperm with subnormal HOST scores was demonstrated in a comparative, prospective, matched-control IVF study in which the clinical and viable pregnancy rates and implantation rates for couples in whom the man's HOST score was  $>50\%$  were 25.9%, 18.5%, and 9.9%, respectively, compared with only 3.7%, 3.7%, and 1.1% when the HOST score was  $<50\%$  (Check et al, 1995). Further support for this concept that men whose semen specimens demonstrate low HOST scores have poor fecundity potential despite normal fertilization potential and apparently normal embryo formation was demonstrated in a study of shared oocytes in which 1 of the 2 male partners involved had a subnormal HOST score and the other male partner a score of  $>50\%$  (Katsoff et al, 2000). The male partners of the recipients or the donors with normal HOST scores achieved a clinical and viable pregnancy rate of 50.0% and 40.9% versus 0% and 0% for couples in whom the HOST scores was  $<50\%$  despite equal fertilization rates (67.2% vs 60.9%) and similar embryo morphology (Katsoff et al, 2000).

Based on these studies we hypothesized that the sperm samples with low HOST scores may have associated toxic

factors attached to the sperm membrane, which is transferred to the zona pellucida and then to the embryo when the supernumerary sperm attach. If this hypothesized mechanism is correct, then theoretically, the problem could be corrected by the introduction of only 1 sperm to the oocyte through the process of ICSI (Palermo et al, 1993; Van Steirteghem et al, 1993a, 1993b).

Initially, one of the coauthors proposed a study in which couples having a male partner with HOST scores  $<50\%$  would be randomized to conventional IVF vs IVF with ICSI. Such a study would require approval by the investigational review board (IRB). However, the study was first submitted to the Cooper Center for IVF ethics committee and the majority opinion was that this would not be an ethical proposal because IVF is an expensive and potentially risky procedure and there was overwhelming evidence from previous studies that conventional IVF without ICSI is an ineffective therapy (Check et al, 1995; Kiefer et al, 1996; Katsoff et al, 2000).

Furthermore, the committee argued that because this potentially controversial concept of sperm leading to embryo implantation defects had been published in a major peer review journal in 1995 (Check et al, 1995), and because there have been no studies published subsequently attempting to refute these previous conclusions, the proposed study should be sufficient to evaluate IVF with ICSI as a method of treating couples when the HOST score is subnormal to be sure that the previously reported anecdotal success was not merely fortuitous (Katsoff and Check, 1997).

An alternative proposal for a control group was to randomly compare one group receiving IVF with ICSI with a group treated by intrauterine insemination after expo-

sure to the protein digestive enzyme, chymotrypsin galactose, because there had also been some anecdotal reports of this treatment resulting in improved HOST scores and pregnancies (Katsoff and Check, 1997). This type of treatment had been found to also improve pregnancy outcome when sperm autoantibodies were present (Bollendorf et al, 1994; Katsoff et al, 1995). The ethics committee suggested that it would not be fair to subject the female partner to the intensity, potential risks, and expense of IVF if mere intrauterine insemination with chymotrypsin galactose-treated sperm could be effective. Thus, it was decided that IVF with ICSI would only be attempted if a couple failed to conceive after 3 attempts with intrauterine insemination following chymotrypsin galactose treatment.

The possibility exists that certain changes in current IVF methodology could obviate the problem caused by low HOST scores that would now allow normal fertilization and implantation rates with conventional insemination of oocytes with IVF. For example, one of the potential mechanisms by which supernumerary defective sperm could damage the oocyte or pronucleate embryo could be the altering the physico-chemical properties of the zona pellucida via the release of toxic metabolites (eg, oxygen radicals). Possibly, the use of assisted embryo hatching could overcome this problem.

Presently, our IVF center uses assisted embryo hatching with acidic Tyrodes solution on most embryos, similar to the technique described by Cohen et al (1992) and Hoover et al (1995). The aforementioned comparative prospective study using matched samples concluding that low HOST scores result in poor pregnancy rates despite normal implantation rates (Check et al, 1995) included patients enrolled from January 1989 through June 1994. According to another publication on assisted embryo hatching of frozen-thawed embryos, assisted embryo hatching was first started at the Cooper Center for IVF in November 1993 (Check et al, 1996b). Thus, at least 15% of the study group from the aforementioned study (Check et al, 1995) had assisted hatching performed with transfers on 72-hour-old vs 48-hour-old embryos. The pregnancy rates were so poor with conventional insemination and low HOST scores that we do not think that assisted embryo hatching and 3-day transfers would overcome the problem. However, given the fact that only such a small percentage of the previous study group did have assisted hatching performed, we hope this will allow an IRB from another IVF center to approve a prospective randomized study comparing pregnancy and implantation rates following Day 3 embryo transfer and assisted embryo hatching of conventionally fertilized vs ICSI fertilization of oocytes with sperm with HOST scores of <50%.

Along the same lines, one interesting question is whether the HOST defect prevents the Day 3 embryo from

reaching the blastocyst stage. We cannot answer that question because we routinely use ICSI when HOST scores are low. However, many IVF centers that attempt blastocyst transfer do not routinely evaluate HOST scores in the male partners. Perhaps this study will encourage some of these centers to re-evaluate the male partners' semen analyses retrospectively and examine the HOST score to see if those embryos created by fertilization with sperm from men with low HOST scores have any greater difficulty reaching the blastocyst stage. This study might also encourage IVF centers that have performed reactive oxygen species studies in IVF cycles using blastocyst culture to re-evaluate HOST scores to see if subnormal HOST scores correlate with the presence of these potential toxic factors.

If low HOST scores are found not to inhibit blastocyst formation, the possibility could still exist that these embryos, similar to 48- and 72-hour-old embryos, are inhibited from implantation when the partner has low HOST scores. Thus, a similar prospective, comparative study should also be conducted with blastocyst transfer. Possibly, the use of Day 5 embryo transfer could somehow overcome the implantation defects seen when Day 2 or Day 3 embryos, derived from couples whose male partners have low HOST scores, are transferred.

Defective sperm, as assessed by low HOST scores, might also have damaged DNA. Therefore, the use of ICSI in these patients could potentially lead to poor embryo quality, implantation failure, or early spontaneous abortion (Aitken et al, 1998). This would be particularly relevant in older women whose DNA repair mechanisms in the oocyte are more likely to be defective (Aitken et al, 1998). Thus membrane damage in sperm could also be associated with DNA damage and therefore these couples should be counseled about the potential risks of achieving pregnancies that may have been difficult to establish *in vivo* but are now achieved by IVF with ICSI (Aitken et al, 1998).

The highly successful pregnancy and implantation rates and low spontaneous abortion rates following IVF with ICSI are consistent with the hypothesis that the cause of poor outcome with low HOST scores is not via the single spermatozoan that fertilizes the oocyte but the supernumerary sperm attaching to the zona pellucida. It is hoped that these data will encourage more IVF centers to perform the very simple and inexpensive HOST routinely with each initial semen analysis. Furthermore, it is hoped that these encouraging results will generate interest in performing some of the aforementioned prospective comparison trials and retrospective studies to better understand how this sperm abnormality can lead to embryo implantation disorders and whether newer, modern IVF techniques can overcome the problem for the embryo caused by sperm with low HOST scores without necessarily even needing ICSI. We hope these data will stimulate interest in looking for other circumstances in which some male factor can cause poor em-

bryo implantation rates while still producing what appears to be normal embryos with appropriate morphology (Alvarez et al, 1996).

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