

# Grey zone score for hypo-osmotic swelling test (HOST) is not associated with embryo implantation defects

M. L. Check, D. Kiefer, J. H. Check, C. Wilson, D. Katsoff

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

## Summary

**Purpose:** To evaluate the clinical significance of the grey zone range (50-59%) of the hypo-osmotic swelling test (HOST) on sperm samples.

**Methods:** Clinical and viable pregnancy rates and implantation rates following in vitro fertilization-embryo transfer (IVF-ET) were determined in couples where the male partner scored between 50-59% on the HOST. The data were further evaluated according to the discrepancy between viability and HOST. Finally, the study assigned whether treating sperm with the protein digestive enzyme chymotrypsin had any effect on pregnancy outcome.

**Results:** Pregnancy and implantation rates were comparable for couples with grey zone HOST scores with the normal IVF rate for the center. Having a large discrepancy between viability and HOST scores had no clinical significance nor did treatment with chymotrypsin.

**Conclusion:** In contrast to HOST scores < 50% where extremely poor implantation rates are noted, grey zone scores have no clinical significance.

**Key words:** Grey zone; HOST; IVF.

## Introduction

Marked reduction in fecundity is related to male partners having semen demonstrating subnormal (< 50%) hypo-osmotic swelling test (HOST) scores [1]. The test measures the functional integrity of the sperm membrane [2]. The abnormality interestingly does not prevent the sperm from fertilizing the oocyte [3-6], but instead prevents the embryo from implanting [7, 8].

The fact that IVF with intracytoplasmic sperm injection (ICSI) has been extremely successful in obviating the HOST defect [9, 10], has led to the hypothesis that the HOST abnormality may be related to a toxic factor attached to the sperm that gets transferred to the zona pellucida by supernumerary sperm. The hypothesis continues that this toxic factor remains a part of the embryo and impairs the functional integrity of the embryo membrane which, in turn, prevents implantation [11]. Sperm with poor structural integrity, as manifested by poor viability, would automatically have low HOST scores but yet theoretically not have the hypothesized toxic protein attached to them.

There has been some suggestion that the hypothesized toxic factor attached to the sperm and transferred to the zona pellucida may be a protein. This is based on some data showing that treating the sperm with the protein digestive enzyme chymotrypsin can in a majority of instances, improve the HOST score above 50%, and anecdotally, four pregnancies were reported as a result of this treatment [10].

Jeyendran *et al.* [2] considered 50-59% as a grey zone, but Check *et al.* [1] in their 1989 publication only found scores < 50% to be clinically significant. The objective of

this study was to re-evaluate the grey zone range of the HOST by not only determining if the IVF pregnancy rates drop when standard insemination of oocytes occurs with sperm with grey zone HOST scores, but also to see if treating the sperm with the protein digestive chymotrypsin prior to oocyte insemination helps subsequent pregnancy rates. Furthermore, the grey zone would be assessed in a different way, i.e., to see if the grey zone score of 50-59% may be associated with lower implantation rates if there is a wide discrepancy between viability and HOST scores. If the sperm has structural damage it follows that damage to the functional integrity of the sperm membrane will also exist. Thus, if viability was in the low 60<sup>th</sup> percentile, the HOST score could be in the grey zone of 50-59% without the existence of the hypothesized toxic protein. However, theoretically, sperm with very good viability, but grey zone HOST scores, may be related to the presence of this toxic protein which can transfer to the zona pellucida. Thus this study was to also determine if sperm with grey zone HOST scores but higher viability may result in a lower percentage of pregnancies.

## Materials and Methods

A retrospective study of IVF-embryo transfer (ET) cycles was performed based on the following criteria: a) the female partners were < 40 years old and used their own gametes, b) at least four oocytes were retrieved, c) the first cycle per patient in the study was used, d) the minimal HOST score was 50%, e) the males also had all other semen parameters normal ( $\geq 20 \times 10^6$  / ml count, motility > 40%, strict morphology > 4%, and negative antisperm antibodies), f) and all semen samples used were fresh ejaculates. All cycles from 1/1/99 to 4/30/00 were reviewed. In

addition cases with HOST scores between 50-59% in period 1/1/97 and 12/30/98 were added to increase the number of patients with grey zone HOST scores. There were 58 couples who met this criteria whose results were used for this study.

The difference between the viability and HOST score was computed: (relative discrepancy = viability score-HOST score/viability score). The data was analyzed according to dividing the differences into four quartiles. Some ejaculates prior to oocyte insemination were prepared by chymotrypsin/galactose as previously described [10], at the discretion of the andrologist. Each oocyte was inseminated by 25,000 sperm. Treatment assignment was not random, but chosen at time of insemination by the physician and embryologist.

## Results

The discrepancy scores ranged from 2.3 to 40. The 25<sup>th</sup> percentile was 9.1, the 50<sup>th</sup> percentile was 11.1, and the 75<sup>th</sup> percentile was 23.9. Patients were stratified into four groups based on the four quartiles of the discrepancy group. The clinical and viable pregnancy rates per transfer, spontaneous abortion rate, and implantation rate were evaluated according to quartile (Table 1). No significant differences or even trends were observed when comparing clinical and viable pregnancy rates or implantation rates.

The clinical and viable PRs and implantation rates per transfer were evaluated in patients with grey zone HOST scores according to whether the sperm was treated with chymotrypsin galactose or not (Table 2).

Table 1. — IVF outcome according to discrepancy between viability and HOST score

	1 <sup>st</sup> quartile discrepancy between 2 & 9.1	2 <sup>nd</sup> quartile discrepancy between 9.2 & 16	3 <sup>rd</sup> quartile discrepancy between 16.1 & 23.9	4 <sup>th</sup> quartile discrepancy ≥ 24
# of embryo transfers	15	14	14	15
Clinical PR/embryo transfer	47% (7/15)	29% (4/14)	50% (7/14)	40% (6/15)
Viable PR/embryo transfer	40% (6/15)	21% (3/14)	36% (5/14)	33% (5/15)
Spontaneous abortion rate	14% (1/7)	25% (1/4)	29% (2/7)	17% (1/6)
Implantation rate	22% (10/46)	15% (7/45)	21% (9/42)	18% (10/56)

p > .05 in all categories

Table 2. — Effect of treatment of sperm with HOST scores in the grey zone with chymotrypsin on IVF outcome

	Group 1 IVF/Chymotrypsin	Group 2 Standard IVF
No. of retrievals	13	20
No. of transfers <sup>a</sup>	9	16
Clinical PR/transfer	44% (4/9)	50% (8/16)
Viable PR/transfer	44% (4/9)	50% (8/16)
Implantation rate	20% (6/30)	21% (11/53)
Spontaneous abortion <sup>b</sup>	0	0

<sup>a</sup> Only transfer cycles were evaluated where it was clearly stated that chymotrypsin was used or not used. We did not make the assumption that if no mention was made of this protein digestive enzyme then it probably was not used.

<sup>b</sup> Rate of miscarriage after clinical pregnancy was established.

The outcome was similar in both groups. The PRs and implantation rates were similar to the normal rates for the IVF center.

## Discussion

These data fail to demonstrate that sperm with HOST scores 50-59% are associated with embryo implantation defects even if there is a wide discrepancy between viability and HOST score. It would appear that when this toxic factor does exist, it is usually sufficient to reduce the HOST score to < 50%. There is probably no advantage in measuring viability when performing IVF as long as the HOST score is measured. There does not seem to be any advantage in treating sperm with grey zone HOST scores with chymotrypsin prior to insemination of the oocytes.

Thus no special therapy is needed when a male has a HOST score in the grey zone. In contrast, ICSI is a necessity when the HOST score is < 50% [9].

## References

- [1] Check J. H., Epstein R., Nowroozi K., Shanis B. S., Wu C. H., Bollendorf A.: "The hypoosmotic swelling test as a useful adjunct to the semen analysis to predict fertility potential". *Fertil. Steril.*, 1989, 52, 159.
- [2] Jeyendran R. S., Van der Ven H. H., Perez-Pelaez B. G., Crabo B. G., Zaneveld L. J. D.: "Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics". *J. Reprod. Fertil.*, 1984, 70, 219.
- [3] Avery S., Bolton U. M., Mason B. A.: "An evaluation of the hypoosmotic sperm swelling test as a predictor of fertilizing capacity in vitro". *Int. J. Androl.*, 1990, 13, 93.
- [4] Barratt C. L. R., Osborn J. C., Harrison P. E., Monless N., Dumphy B. C., Lenton E. A., Codie I. D.: "The hypoosmotic swelling test and the sperm mucus penetration test in determining fertilization of the human oocyte". *Hum. Reprod.*, 1989, 4, 430.
- [5] Chan S. Y. W., Wang C., Chan S. T. H., Ho P. C.: "Differential evaluation of human sperm hypoosmotic swelling test and its relationship with the outcome of in-vitro fertilization of human oocytes". *Hum. Reprod.*, 1990, 5, 84.
- [6] Sjoblum P., Coccia E.: "On the diagnostic value of the hypoosmotic sperm swelling test in an in vitro fertilization program". *J. In Vitro Fertil. Embryo. Transfer.*, 1989, 6, 41.
- [7] Check J. H., Stumpo L., Lurie D., Benfer K., Callan C.: "A comparative prospective study using matched samples to determine the influence of subnormal hypoosmotic test scores of spermatozoa on subsequent fertilization and pregnancy rates following in vitro fertilization". *Hum. Reprod.*, 1995, 10, 1197.
- [8] Katsoff D., Check M. L., Check J. H.: "Evidence that sperm with low hypo-osmotic swelling scores cause embryo implantation defects". *Arch. Androl.*, 2000, 44, 227.
- [9] Check J. H., Katsoff D., Check M. L., Choe J. K., Swenson K.: "In vitro fertilization with intracytoplasmic sperm injection is an effective therapy for male factor infertility related to subnormal hypo-osmotic swelling test scores". *J. Androl.*, 2001, 22, 261.
- [10] Katsoff D., Check J. H.: "Two methods of achieving pregnancies despite subnormal hypo-osmotic swelling test scores". *Fertil. Steril.*, 1997, 68, 549.
- [11] Check J. H., Katsoff D., Check M. L.: "Some semen abnormalities may cause infertility by impairing implantation rather than fertilization". *Med. Hypoth.*, 2001, 56, 653.

Address reprint requests to:

JEROME H. CHECK, M.D., Ph.D.  
7447 Old York Road  
Melrose Park, PA 19027 (U.S.A.)