

# The majority of males with subnormal hypoosmotic test scores have normal vitality

**A. Bollendorf, J.H. Check, D. Kramer**

*The University of Medicine and Dentistry of New Jersey, Cooper Medical School of Rowan University  
Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology  
Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)*

## Summary

**Purpose:** To determine if males with low hypoosmotic swelling (HOS) test scores may have normal vitality tests. **Methods:** Retrospective nine-year review of initial semen analyses evaluating vitality and HOS tests. **Results:** Only 12.5% (45/361) of males with low HOS test scores have low vitality. **Conclusions:** WHO seems to treat vitality and HOS as equal tests. We disagree and find that structural defects of the sperm membrane are much less common than functional impairment of the sperm membrane. Since a male with an HOS test score < 50% will rarely achieve a pregnancy with intercourse, intrauterine insemination, or IVF with conventional insemination it is important to check for the HOS test not viability because the HOS test can be easily corrected with ICSI.

**Key words:** Vitality; Sperm membrane; Hypoosmotic swelling test; Implantation defects; Functional vs structural.

## Introduction

Men with low hypoosmotic swelling (HOS) scores (< 50%) rarely achieve pregnancy after natural intercourse or intrauterine sperm injection [1]. Sperm with low HOS scores fertilize oocytes at a normal rate; however, the embryos formed have extremely low odds of implanting [2-4].

The HOS test measures the functional integrity of the sperm membrane whereas dye exclusion vitality measures the structural integrity. Nevertheless, some andrologists believe that if you measure viability and it is normal, then there is no reason to perform the HOS test. The sperm vitality test is able to distinguish between live and dead sperm. Sperm that swell under hypoosmotic conditions have intact membranes and will exclude stain. However, dead sperm will uptake the dye and not swell due to some type of damage to the membrane.

The World Health Organization (WHO) laboratory manual (1999) suggests performing a sperm vitality (dye exclusion test) or HOS test every time the sperm motility is abnormal or less than 50% in a semen analysis. The HOS test detects a functional impairment of the sperm membrane which may be related to a toxic factor that transfers to the zona pellucida by the supernumerary sperm that attach and may thus lead to functional impairment of the embryo membrane thus preventing implantation [2-4].

The present study evaluated whether these two tests are indeed interchangeable. Our working hypothesis was that we would find a large percentage of males with subnormal HOS scores who have normal viability.

## Materials and Methods

A retrospective review of initial semen analyses from all patients between May 1999 and May 2008 were included in this study. A subset of those males with HOS scores < 50% was identified with their viability results. To perform the dye exclusion test [5] the reagent was 5% eosin in a sodium chloride solution. The procedure involved mixing one drop of liquefied semen with one drop of 5% eosin solution on a microscope slide and read at 400x. One then counts 200 sperm and calculates the percentage of live sperm (unstained).

The WHO 3<sup>rd</sup> edition suggested a normal vitality of 75% or more whereas the WHO 4<sup>th</sup> edition has changed the normal percentage for vitality to 50% or more sperm which exclude dye.

For the HOS test the reagents were 0.735 g sodium citrate, and 1.351 g fructose in 100 ml distilled water [6]. The procedure involved mixing 0.1 ml liquefied semen with 1 ml HOS solution. One then incubates for 30 min at 37°C. After mixing well one drop is placed on a slide. A phase contrast microscope is then used to determine the percentage of sperm with swollen tails in 200 sperm. Then one subtracts the percentage of swollen tails initially seen in the raw sample to obtain the percentage of tails that have swelled from the HOS solution. A result of less than 50% is considered abnormal [6].

## Results

There were 361 males with low HOS scores. Only 12.5% (45/361) of these males had a subnormal vitality.

## Discussion

Another study did show a correlation with poor motility and low HOS scores [7]. However, there are males with normal motility and low HOS scores. Because of the severity of this implantation defect and the simplicity and lack of expense of this test, we recommend it should be performed routinely.

There were no males with subnormal vitality who had normal HOS tests. Thus we therefore could eliminate the

vitality test and just do the HOS test. By only doing the vitality, 28.8% (104/361) of abnormal males whose infertility could be corrected by either IVF with ICSI or treatment of sperm with chymotrypsin-galactose and IUI would have been missed [8-10].

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Address reprint requests to:  
J.H. CHECK, M.D., Ph.D.  
7447 Old York Road  
Melrose Park, PA 19027 (USA)  
e-mail: laurie@ccivf.com