

## The hypoosmotic swelling test as a useful adjunct to the semen analysis to predict fertility potential

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The functional integrity of the sperm membrane is believed to be an important factor in the acrosome reaction, sperm capacitation, sperm metabolism, and the binding of the spermatozoon to the egg surface. This factor is not measured, however, by the standard spermogram, but can be evaluated using the hypoosmotic swelling (HOS) test.<sup>1</sup> A normal semen analysis frequently fails to be predictive of male fertility, and fertilization sometimes occurs even in the presence of an abnormal spermogram. In this study, we sought to evaluate the usefulness of the HOS test for the prediction of successful conception in partners of men with normal or abnormal spermograms.

### MATERIALS AND METHODS

One hundred thirty-five couples who had been infertile for at least 1 year were evaluated retrospectively. The female member of each couple had one or more factors believed to be associated causally with the infertility, and all of the problems had been corrected. The female factors included luteal phase defects with mature or immature follicles, anovulation, and abnormal cervical mucus. As part of our routine infertility evaluation, all female patients had undergone laparoscopy, during which endometriotic implants were fulgurated and adhesions lysed, if present. Patients were excluded if tu-

boplasty was required or if all visible endometriotic implants could not be fulgurated. Before inclusion in the study, all women had appropriately dated, late luteal phase endometrial biopsies, normal cervical mucus, bilateral tubal patency, and the absence of endometriosis or adhesions. Once these criteria were met, the pregnancy rate during the next 8 months was determined.

After an abstinence period of 48 to 72 hours, spermograms and HOS tests were performed in duplicate on the same specimen and then repeated in 2 weeks. The initial spermograms were scored as follows: superior (sperm count  $\geq 50 \times 10^6/\text{ml}$ , motility  $\geq 50\%$ , and normal morphology  $\geq 50\%$ ); normal (sperm count 21 to  $49 \times 10^6/\text{ml}$ , motility  $\geq 50\%$ , and normal morphology  $\geq 50\%$ ); abnormal (sperm count  $\leq 20 \times 10^6/\text{ml}$  or motility  $< 50\%$ , or normal morphology  $< 50\%$ ). Hypoosmotic swelling test results were recorded as percent of sperm that demonstrated swelling and were categorized as normal (swelling in  $\geq 60\%$  sperm), grey-zone (50% to 59% sperm swelling), or abnormal ( $< 50\%$  swelling).

Initial and repeat HOS test results were compared using a paired Student's *t*-test. Pregnancy rates among the various groups were compared by chi-square or the Fisher's exact test, where appropriate. The Kruskal-Wallis test was used to evaluate the relationship between motility as a single parameter of sperm function and success of pregnancy. A *P* value  $< 0.05$  was considered statistically significant. Numerical data are presented as mean  $\pm$  standard deviation (SD).

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**Table 1** Pregnancy Rate According to HOS and Spermogram

HOS group	Spermogram group	Conceived	Failed to conceive	P
Normal + grey-zone	Superior + normal	83 (89%)	10 (11%)	0.25
Normal + grey-zone	Subnormal	24 (83%)	5 (17%)	
Normal + grey-zone	All	107 (88%)	15 (12%)	<0.0001
Abnormal	All	0 (0%)	13 (100%)	
Normal + grey-zone	Superior + normal	83 (89%)	10 (11%)	<0.001
Abnormal	Superior + normal	0 (0%)	7 (100%)	
Normal + grey-zone	Subnormal	24 (83%)	5 (17%)	<0.001
Abnormal	Subnormal	0 (0%)	6 (100%)	

## RESULTS

Hypoosmotic swelling replicates were always within 4% of each other, and there were no differences between the initial and repeat HOS test results. There were no significant differences in pregnancy rates between women whose partners had subnormal, normal, or superior spermograms as long as the results of HOS tests were  $\geq 50\%$  (Table 1). There were no differences in pregnancy rates between the women whose partners demonstrated normal (95/108) and grey-zone (12/14) HOS test results, ( $P = 0.88$ ), so these data have been combined. No couple, regardless of results of the spermogram, achieved pregnancy if the HOS test was abnormal. In couples in which the partner had a subnormal spermogram, the pregnancy rate was significantly higher in those with a normal HOS test (24/29) than in those with an abnormal HOS score (0/6).

Men whose partners conceived had a significantly higher sperm motility ( $60.7 \pm 14.4$ ) than those whose partners remained infertile ( $52.9 \pm 15.3$ ) ( $P = 0.007$ ). However, the degree of overlap in the range of sperm motilities observed (17.5 to 86.5 versus 11.0 to 79.5) was such that no threshold value could be identified as useful in predicting the achievement of pregnancy.

The same data analysis was used for the second spermograms performed 2 weeks later, and the same results were found. For the sake of brevity, the actual values are not included in this manuscript.

## DISCUSSION

In our laboratory, the HOS test has proven to be highly reproducible and relatively constant in different individuals over time. The results of the

HOS test were significantly more reliable than the results of the standard spermogram in being predictive of successful conception. It appears that the results of the HOS test are especially useful in males who demonstrate subnormal spermograms, in which case 83% (24 of 29) of those with a normal or grey-zone HOS score were able to fertilize their partners.

The assessment of whether the HOS test provides any better evaluation of the fertility potential of the male partner above the standard semen analysis requires comparison with the best reference standard available. Some data has shown a better correlation of human oocyte penetration with the HOS test than with normal semen parameters.<sup>2</sup> One study demonstrated a good correlation between the HOS test and the human sperm hamster ova penetration assay (SPA).<sup>1</sup> However, other data were unable to demonstrate any significant correlation between the HOS test and the SPA.<sup>3,4</sup>

Whether the results of our studies can be applied clinically for the evaluation of couples in which the woman has no identified infertility factors remains to be determined. Furthermore, since these data were developed from retrospective analyses, additional prospective studies need to be performed.

## SUMMARY

The sperm HOS test was highly predictive of eventual achievement of pregnancy in women in whom other infertility factors had been corrected. No woman conceived whose partner's HOS was  $< 50\%$ . The results of spermograms did not correlate with conception rate.

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