

STABILITY OF THE HYPOOSMOTIC SWELLING TEST OVER TIME

B. S. SHANIS, J. H. CHECK, A. BOLLENDORF,
and D. LURIE

The results of the sperm count, motility, and hamster oocyte penetration (SPA) tests have been found to vary greatly in individuals who have had tests performed on more than one occasion. The study presented herein was designed to evaluate the stability of the hypoosmotic swelling (HOS) test over time. A total of 444 patients were classified into categories according to the time interval between HOS tests: 0-90 days ($n = 267$), 91-180 days ($n = 35$), 181-270 days ($n = 37$), 271-360 days ($n = 30$), 1-2 years ($n = 54$), and >2 years ($n = 21$). A paired t test was used to compare mean HOS scores. The correlation between the HOS test of the first specimen and the HOS of the second specimen was calculated. For those who had 2 tests the only significant difference was found in the >2 year group (69.67 ± 9.13 vs. $64.23 \pm 12.83\%$), $p < .05$. When comparing the first and third HOS test in 74 patients, there was a significant ($p < .05$) decrease in the later test when the interval was greater than 270 days. The difference was not significant when the HOS results were classified as subnormal (<50%) and normal ($\geq 50\%$) for that same time period. It would appear that this test is reproducible, rarely fluctuates, but tends to become less dependable over longer intervals of time.

Key Words: Sperm; Hypoosmotic swelling test; Stability.

INTRODUCTION

Sperm count and motility are known to fluctuate greatly over time in the individual [3, 5]. Similarly, the hamster oocyte penetration test varies greatly, possibly related to the difficulty in performance of the assay, even when repeated after a short interval [6]. The hypoosmotic swelling (HOS) test, which measures the functional integrity of the sperm membrane, has been found to be useful in identifying the subnormal male [2, 4]. In contrast to the hamster test, the HOS test is extremely simple to perform. The study presented herein was designed to evaluate the stability of the HOS test over time.

MATERIALS AND METHODS

In an infertility treatment program, 444 males produced semen specimens on site following 48-72 h of abstinence. The samples were evaluated after 20-30 min of liquefaction using the HOS test described

From the Division of Reproductive Endocrinology/Infertility, Department of Obstetrics/Gynecology, The University of Medicine/Dentistry of New Jersey, Robert Wood Johnson Medical School, Cooper Hospital/University Medical Center, Camden, NJ 08053, USA.

Address correspondence to Bonnie Shanis, MD, 7447 Old York Road, Melrose Park, PA 19126.

by Jeyendran et al. [4]. Repeat testing was done at various intervals depending on patient follow-up. All testing took place between January 1988 and December 1990. Of the 444 males, 74 patients had 3 HOS tests.

All patients were assigned to groups according to the time elapsed between testing: Group 1 had tests performed 0-90 days apart ($n = 267$); group 2, 91-180 days ($n = 35$); group 3, 181-270 days ($n = 37$); group 4, 271-360 days ($n = 30$); group 5, 1-2 years ($n = 54$); and group 6, >2 years ($n = 21$). For each time period a t test (paired, two-tailed) was done to compare the mean difference between the pairs of tests. A p value of $< .05$ was considered statistically significant. Scores for each time period were classified as normal if HOS ≥ 50 and abnormal if HOS > 50 . McNemar's test for related proportions was used to test the hypothesis that the proportion of normal (abnormal) scores obtained was the same for both testings. The Pearson correlation between the HOS of the first and second specimens was calculated.

RESULTS

Table 1 shows the mean and SD of HOS scores at first and second testing. The only repeat testing that was significantly different over time was at a greater than 2-year interval. It is interesting, although not statistically different, that, with the exception of the 0- to 90-day period, all mean HOS scores were decreased on the second test.

Overall, 393 of the 444 specimens (88.5%) had normal HOS scores initially which remained normal for the next specimen (Table 2). The HOS score was $< 50\%$ initially in 34 patients (7.7%) and in 15 (44.0%) it remained abnormal and in 19 (55.9%) it improved to $> 50\%$. There were 17 patients for whom the HOS was normal initially but changed to abnormal on the next test (4.1%). The results were similar when comparing the first to third specimen in the 74 patients (Table 3). The mean HOS test was lower than test 1 in the group greater than 270 days, but there were no significant differences in comparing those with $\geq 50\%$ vs. $< 50\%$ on the HOS test.

DISCUSSION

The results of these comparisons show stability over time of both HOS scores and HOS normality. The downward trend of HOS scores on the later testing may be a result of a slow decrease over time or a change in laboratory policy. When the HOS tests were evaluated in our

TABLE 1 HOS Scores, First and Second Testing ($N = 444$)

Time Period Between Tests	<i>N</i>	Mean HOS First Specimen	Mean HOS Second Specimen	Mean Difference HOS Score	Pearson Correlation Coefficient
0-90 days	267	65.4 \pm 13.8	66.4 \pm 13.2	1.0 \pm 12.8	.56
91-180 days	35	71.6 \pm 9.5	69.1 \pm 11.6	-2.5 \pm 13.6	.18
181-270 days	37	72.7 \pm 10.6	70.8 \pm 12.0	-1.9 \pm 11.7	.47
271-360 days	30	71.5 \pm 11.5	68.9 \pm 9.1	-2.6 \pm 10.3	.52
1-2 years	54	71.6 \pm 8.9	70.2 \pm 11.9	-1.3 \pm 11.0	.48
>2 years	21	69.7 \pm 9.1	64.2 \pm 10.8	-5.4 \pm 12.0 ^a	.29

Note. Data are means \pm standard deviations.

^a $p < .05$ (paired t test).

TABLE 2 Distribution of HOS Scores (Normal vs. Abnormal) over Time

Time Period Between Tests	No. of Times Both Tests Normal	No. of Times Both Tests Abnormal	No. of Times HOS1 Normal HOS2 Abnormal	No. of Times HOS1 Abnormal HOS2 Normal
0-90 days	227	14	10	16
91-180 days	32	0	2	1
181-270 days	34	1	2	0
271-360 days	28	0	1	1
1-2 years	51	0	2	1
>2 years	21	0	0	0
Overall	393	15	17	19

Note. McNemar test for related proportions showed no difference between the proportion of normal (abnormal) scores detected by the two tests in each of the time periods studied.

andrology lab by year, the mean HOS for 1988 was $68.5 (\pm 9.5)$, and for 1989 it was 68.5 ± 12.6 . However, for 1990 when we switched from glass to plastic specimen containers, it was 61.3 ± 15.1 . A previous study established a negative effect of plastic on HOS [1]. The variation from specimen 1 to specimen 2 ranged from 10 to 13.5 within the different time periods. If the normal range of HOS is defined as the mean ± 2 SD, the normal range would then fall between 42.11 and 93.29.

Although statistically HOS values remained constant for up to 2 years, a large percentage of initially abnormal tests did change. Twelve of the 19 patients whose tests went from subnormal to normal had latter values in the gray zone (50-60%). Of those who changed from normal to subnormal, 4 of 17 were initially in the gray zone. The HOS, in contrast to other semen parameters, appears to remain stable over time. Cases in which initial results fall within the gray zone or are subnormal should be retested depending on the clinical situation.

TABLE 3 Comparison of HOS1 to HOS3 ($N = 74$)

Time Period Between Tests 1 and 3	<i>N</i>	Mean HOS First Specimen	Mean HOS Third Specimen	Mean Difference Score
0-90 days	14	63.9 ± 13.4	64.8 ± 14.1	0.9 ± 8.6
91-180 days	8	64.1 ± 13.7	69.0 ± 6.8	4.9 ± 12.9
181-270 days	8	71.9 ± 10.4	72.2 ± 7.7	0.4 ± 10.0
271-360 days	10	70.0 ± 12.9	59.5 ± 18.2	-10.5 ± 19.1^a
>360 days	34	72.0 ± 6.8	64.7 ± 11.0	-7.3 ± 11.5^a

Note. Data are means \pm standard deviations.

^a $p < .05$ (paired *t* test).

REFERENCES

1. Check JH, Shanis BS, Wu CH, Bollendorf A (1988): Evaluating glass, polystyrene, and polypropylene containers for semen collection and sperm washing. *Arch Androl* 20:251-255
2. 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
3. Giblin PT, Poland ML, Moghissi KS, Ager JW, Olson JM (1988): Effects of stress and characteristic adaptability on semen quality in healthy men. *Fertil Steril* 49:127-132
4. 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
5. Poland ML, Moghissi KS, Giblin PT, Ager JW, Olson JM (1986): Variation of semen measures within normal men. *Fertil Steril* 44:396-400
6. Rogers BJ (1985). The sperm penetration assay: its usefulness reevaluated. *Fertil Steril* 43:821-840