The Comparative Study of the Human Sperm Hypoosmotic Swelling Test and Routine Semen Analysis

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Abstract: *To ascertain the value of the hypoosmotic swelling (HOS) test in predicting male fertility, semen samples from 213 males of infertile couples were assessed by the HOS test and the results correlated with routine semen analysis. The HOS test for the 138 normal semen samples was significantly higher than for the 75 abnormal semen samples ($p<0.001$). A strong positive correlation was observed between sperm swelling and sperm motility ($r=0.83$, $p<0.0001$), and sperm viability ($r=0.82$, $p<0.0001$). Lower correlation was obtained between sperm swelling and sperm concentration ($r=0.63$, $p<0.0001$), and total count ($r=0.52$, $p<0.0001$). The cut off value of the HOS test that showed the best sensitivity, specificity, and predictive values was 37%. The results indicate that the simple and inexpensive HOS test may be a useful addition to the standard semen analysis. (Thai J Obstet Gynaecol 1993;5:39-44.)*

Key words: human sperm, hypoosmotic swelling test, routine semen analysis

A routine semen analysis has long been the standard laboratory test for the assessment of male infertility. The parameters widely measured include the volume of the ejaculate, pH, sperm concentration, motility, morphology, viability, white blood cells, and sperm antibodies. However, it has become increasingly clear that these parameters may be inadequate for diagnostic purposes[1,2]. Although the in vitro human sperm zona-free hamster ovum penetration assay (SPA), first described by Yanagimachi et al[3], can assess the functional capacity of human spermatozoa, the assay is time - consuming and complex.

The hypoosmotic swelling (HOS) test for investigating the functional integrity of the human sperm membrane has been proposed as a useful assay in the diagnosis of the infertile male[4]. Membrane integrity is not only important for sperm metabolism, it is also imperative for normal fertilization, i.e. for sperm capacitation, the acrosome reaction, and the binding of the spermatozoon to the egg surface. Jeyendran et at[4] found that there was good correlation

between the HOS test and SPA (r= 0.90). Lower correlation was observed between the HOS test and sperm morphology (r=0.30), motility (r= 0.61), and viability (r=0.52).

To ascertain the value of the HOS test in male fertility predictions, the objectives of this study were to correlate the HOS test with standard semen analysis, to compare the HOS test between normal and abnormal semen samples, and to determine the cut off value of the HOS test.

Materials and Methods

A total of 213 semen samples were collected from male partners of couples first attending the Infertility Unit, Department of Obstetrics and Gynaecology at Siriraj Hospital, from March 1989 to June 1990. Semen samples were collected by masturbation after 3 to 5 days abstinence and allowed to liquefy at room temperature. Semen analyses were performed within 2 hours after collection according to standardized methods defined by the World Health Organization[5].

The HOS test on the sperm was performed as described by Jeyendran et al[4] by one of the authors without knowing the results of the semen analyses. The hypoosmotic swelling technique consisted of mixing 0.1 ml of the undiluted ejaculate with 1.0 ml of hypoosmotic solution prepared by mixing 7.35g sodium citrate . $2H_2O$ and 13.51g fructose in 1 litre of distilled water. After incubation at 37°C for 30 minutes, the sample was examined under phase contrast microscope (x 400 magnification). At least 100 spermatozoa were evaluated for swollen tails, and the percentage of swollen tails was calculated for each sample.

Statistical significances were analyzed by Student's unpaired t-test. Correlations between the percentage of swollen sperm and the results of routine semen analysis were analyzed by correlation coefficient.

Results

The results of the HOS test in normal and abnormal semen samples are shown in Fig. 1. The percentage of swollen sperm for the 75 abnormal semen samples varied from 5 to 40 %, and for the 138 normal samples varied from 20 to 90%. Only 10 samples (7.2%) in the normal semen group gave sperm swelling percentages of less than 60.

Table 1 shows the HOS test for normal semen samples and samples with low concentration, poor motility, and both abnormal parameters. The mean values of percentage of swollen sperm were 71.3, 26.1, 23.0, and 19.1 respectively. The HOS test for the normal semen group was significantly higher than for the abnormal semen group ($p<0.001$).

The correlation between the HOS test and various semen parameters was calculated. A good correlation was observed between the percentage of swollen sperm and sperm

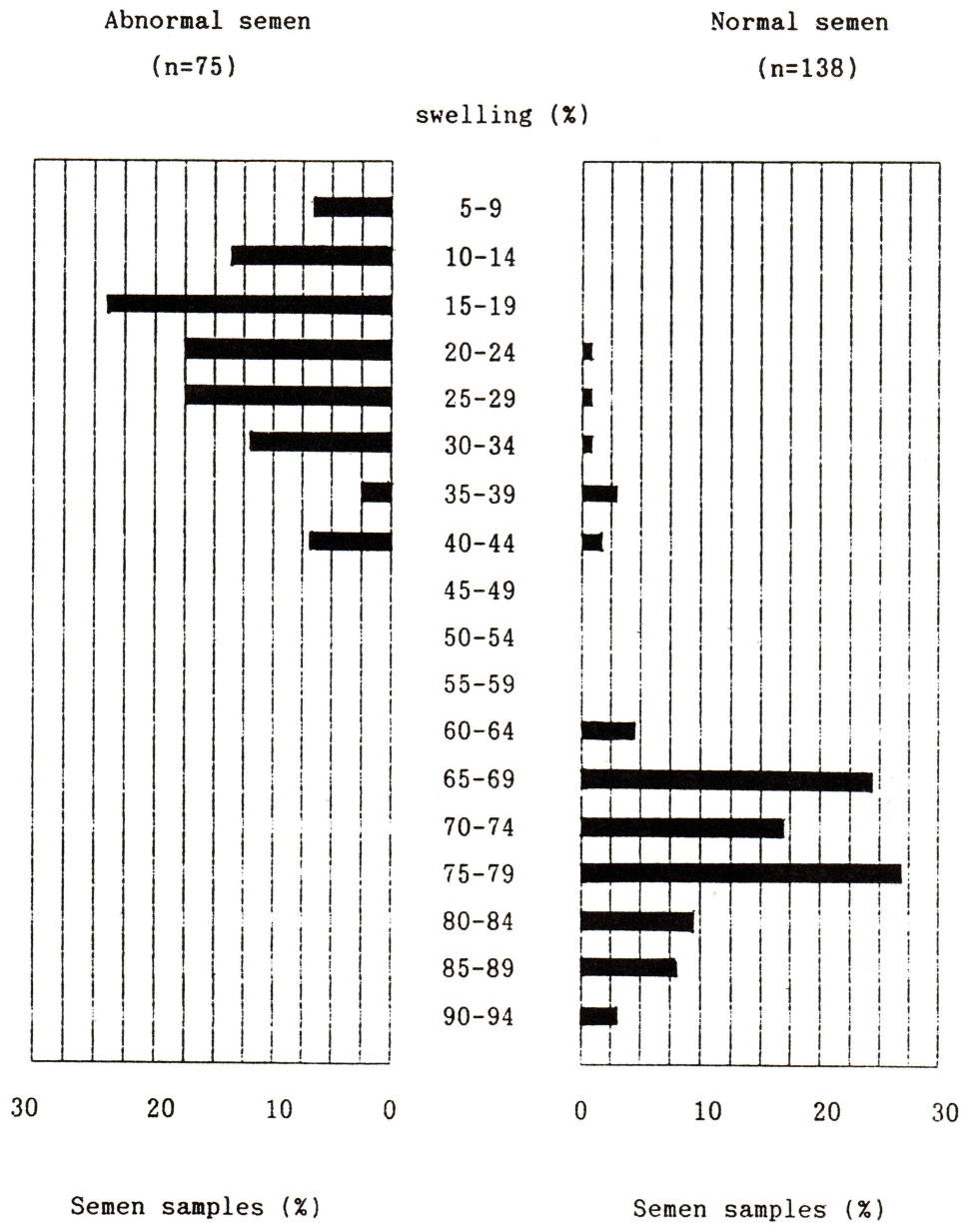

Fig. 1 The hypoosmotic swelling test in normal and abnormal semen samples.

concentration ($r=0.63$, $p<0.0001$), total count ($r=0.52$, $p<0.0001$), viability ($r=0.82$, $p<0.0001$), and sperm motility ($r=0.83$, $p<0.0001$).

Considering Fig. 1, the cut off value of the HOS test should be located in the percentage of sperm swelling between 30 and 44. Table 2

Table 1 The hypoosmotic swelling test for semen samples with normal and abnormal parameters*

Semen parameters	N	Sperm count ($x10^6$/ml)	Total count ($x10^6$)	Viability (%)	Motility (%)	Sperm swelling (%)
Normal	138	69.1±26.6	149.4±88.6	82.5±11.9	80.5±12.0	71.3±12.5
Abnormal :						
Low count	14	10.4±6.8	21.5±16.6	66.4±13.7	65.2±15.9	26.1±8.7
Poor motility	25	51.8±24.5	107.9±72.8	32.4±13.9	25.8±12.3	23.0±9.1
Both	36	8.1±6.4	15.5±19.8	28.0±12.0	22.7±11.7	19.1±7.8

* mean ± SD

Table 2 Sensitivity, specificity, positive predictive value, and negative predictive value of the hypoosmotic swelling test

Percentage of sperm swelling	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
30	78.7	98.5	96.7	89.5
31	88.0	98.5	97.0	93.8
32	88.0	98.5	97.0	93.8
33	90.7	98.5	97.1	95.1
34	90.7	97.8	95.8	95.1
35	90.7	97.8	95.8	95.1
36	92.0	97.8	95.8	95.7
37	93.3	97.8	95.9	96.4
38	93.3	95.6	92.1	96.3
39	93.3	95.6	92.1	96.3
40	93.3	94.9	90.8	96.3
41	100.0	93.5	89.3	100.0

shows sensitivity, specificity, and predictive value of the HOS test when the cut off value is set at various percentages of sperm swelling. It was clear that at 37% of sperm swelling, the HOS test showed the best sensitivity and specificity, 93.3 and 97.8% respectively. The positive and negative predictive values at this cut off point were 95.9 and 96.4 % respectively.

Discussion

The development of assays that can assess the functional activity of

human sperm should be of importance in the diagnosis of infertile men. The HOS test, developed for human sperm by Jeyendran et al[4], was found to be highly correlated with the percentage of hamster eggs penetration (SPA) and, thus,fertilizing potential. However, most results obtained on investigating the usefulness of the HOS test only demonstrated low to moderate positive correlations with the different semen parameters; count, motility, viability, and morphology[4,6-9]. In the present study, we found that the HOS test for normal semen samples was significantly higher than for abnormal ones. A strong correlation was obtained between sperm swelling, sperm motility and viability. A lesser degree of correlation was observed between sperm swelling and sperm count. It should be noted that this test is used as an indicator of normal membrane integrity and function. Good motile and live spermatozoa probably require a biochemically intact plasma membrane, thus, showing higher correlation. The sperm morphology was not included in this study because of imperfect data.

A strong relationship between the results of the HOS test and the performance of spermatozoa in an in vitro fertilization (IVF) program has been reported[10,11]. Van der Ven et al[10] found that none of the semen samples that showed less than 60 % swelling in the HOS test fertilized oocytes, and proposed that ejaculates are classified as "normal" when more than 60 % of the spermatozoa respond in the HOS test, less than 50 % is "abnormal", and 50-60 % is a "gray area". Further evidence was shown by Check et al[12] that pregnancies only occurred with ejaculates that possessed more than 50 % of sperm swelling in the HOS test.

Our present study showed that the cut off value for the HOS test was 37 %. None of the abnormal semen samples showed more than 40 % reaction in the HOS test, whereas only 7.2 % of the normal semen samples showed less than 60 % swelling. We agree with the figures proposed by Van de Ven et al[10] that differentiate the HOS test as normal (>60 %), doubtful (50-60 %), and abnormal (<50 %).

Although the standard semen analysis is simple and economical, but it lacks the reliability to predict the fertilizing potential of the ejaculated spermatozoa. The HOS test which evaluates the functional integrity of the sperm membrane, appears to be useful by a number of studies including ours. The test is economical and easy and can be readily performed in any clinical setting. We propose that the HOS test may be a useful addition to the standard semen analysis in the evaluation of male fertility.

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