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Males With Subnormal Hypo-Osmotic Swelling Test Scores Have Lower Pregnancy Rates Than Those With Normal Scores When Ovulation Induction and Timed Intercourse Is Used as a Treatment for Mild Problems With Sperm Count, Motility, or Morphology

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ABSTRACT: This study was designed to evaluate the effectiveness and clinical usefulness of the hypo-osmotic swelling (HOS) test in predicting successful conception in couples in which men with mild male-factor infertility criteria were undergoing a timed vaginal intercourse protocol. One hundred couples, in which mild male infertility was the only abnormality, were included in the study. Semen was analyzed according to standard World Health Organization (WHO) criteria and subjected to the HOS test. Patients were divided into 2 groups: group 1 (n = 39) with normal HOS test and group 2 (n = 61) with abnormal HOS test. All women underwent three consecutive cycles of follicular growth ultrasound monitoring and timed intercourse. Ten couples were exclude from the study. Ten clinical pregnancies were achieved in group 1 with a pregnancy rate per patient and per cycle of 28.5% and 9.5%, respectively. In group 2, 6 pregnancies were achieved, with a pregnancy rate per patient and per cycle of 10.9% and 3.6%, respectively. Both pregnancy rates per patients and per cycle was significantly higher ($P < .05$) in group 1 than in group 2. The HOS test may be considered an easy and reliable test in identifying among subfertile men those who have a greater possibility of causing pregnancy.

Key words: Hypo-osmotic swelling test, male infertility, timed vaginal intercourse.

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Standard semen analysis consisting of sperm count and evaluation of percent motile spermatozoa and their morphologic abnormalities is still considered the primary laboratory test for assessing male fertility. However, it is not infrequent in clinical practice to see men in which fertilization does not occur despite a normal standard semen analysis. On the other hand, it has been estimated that more than 25% of men with children have subfertile semen (Steinberger, 1984). Subfertility is generally defined as concentrations less than 20×10^6 sperm/mL, less than 50% progressive motility, and less than 60% normal morphology (World Health Organization [WHO], 1992). Therefore, standard semen analysis seems to be a poor predictor of male fertility potential.

The most employed tests for evaluating sperm quality are the zona-free hamster egg penetration assay (Yanagimachi, 1984), the triple stain technique for evaluation of the acrosomal reaction (Aitken et al, 1984), and the hypo-osmotic swelling (HOS) test (Jeyendran et al, 1984). The HOS test, modified for human use by Jeyendran et al (1984), stands out as the simplest and least expensive measure of functional integrity of sperm membrane. In a previous study, we demonstrated that the HOS test is useful for predicting both pregnancy rate and outcome in couples undergoing intrauterine insemination (IUI) (Tartagni et al, 2002).

In this study, we sought to evaluate the effectiveness and clinical usefulness of the HOS test in predicting successful conception and outcome in couples in which men with mild male-factor infertility criteria undergoing timed vaginal intercourse protocols.

Materials and Methods

One hundred couples with at least 2 years of infertility were included in the study. The only abnormality in these couples was a mild male infertility, concentrations of 10 to 20×10^6 sperm/mL, 15% to 25% progressive motility, 30% to 50% total motility, or 30% to 50% normal morphology.

The age of women ranged from 23 to 34 years (mean ± SD, 29.5 ± 2.4 years) and infertility duration from 2 to 7 years (4.2 ± 1.6 years). In all women, the endocrine profile was normal by cycle day 2 measures of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) level (<10 mUI/mL), prolactin level

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Table 1. *Characteristic of female partners in the 2 groups of couples on the basis of the hypo-osmotic swelling (HOS) test result**

	Normal HOS	Abnormal HOS
Age (years)	30 ± 3.6	29.6 ± 3.1
Duration of infertility (months)	52.3 ± 12.1	49.4 ± 11.4
Serum estradiol (pg/mL)†	290 ± 5	295 ± 4
Follicle diameter (mm)	18.4 ± 2.2	18.0 ± 2.3
Body mass index (kg/m^2)	20.9 ± 4.1	21 ± 3.6

* Values are mean ± SD.
† On the day of hCG administration. Conversion factor to SI unit = 3.671.

(<15 ng/mL), and testosterone level (<0.6 ng/mL). In all cases, progesterone (P) levels in the midluteal phase were higher than 6 ng/mL. All women had appropriate late luteal phase endometrial biopsies, normal cervical mucus, and bilateral tubal patency. Body mass index (BMI), plasma estrogen level, and follicle diameter on day of human chorionic gonadotropin (hCG) administration were recorded.

All hormone determinations were performed with commercial kits (Diagnostic System Laboratories Inc, Webster, Tex); intraassay and interassay coefficients of variation were less than 10%.

Semen was collected by masturbation into a sterile container after 3 days of sexual abstinence. Samples were allowed to liquefy for 30 minutes at room temperature before being analyzed according to standard WHO (1992) criteria.

The HOS test was performed by mixing 0.1-mL aliquots of semen with 1.0 mL of a hypo-osmotic solution prepared by mixing 7.3 g of sodium citrate and 13.5 g of fructose in 1000 mL of distilled water in accordance with a previously described technique (Jeyendran et al, 1984). The mixture was incubated for 60 minutes at 37°C, then the samples were examined by phase-contrast microscopy at a magnification of 400×. In this study, standard semen analysis and HOS test were performed by a single technician.

The percentage of HOS-reacted sperm (curled and swollen tails) and nonreacted sperm were calculated by examining 100 spermatozoa. At least 50% swollen spermatozoa was considered normal.

Patients were divided into 2 groups according to HOS results: group 1 (n = 39) with normal HOS test (ie, swollen spermatozoa ≥ 50%) and group 2 (n = 61) with abnormal HOS test (ie, swollen spermatozoa < 50%).

All women underwent three consecutive cycles of ultrasound monitoring of follicular growth and timed vaginal intercourse. Examinations started from day 7 of the cycle and were performed every other day until a mean diameter of at least 18 mm in the dominant follicles was reached; then, hCG (10 000 UI; Profasi, Serono, Rome, Italy) was administered.

Patients were requested to avoid intercourse from 4 days before the expected time of ovulation. Vaginal intercourse was suggested 24 hours after hCG administration.

For calculations, we considered only clinical pregnancies, defined as presence of either embryonic heart beat at transvaginal ultrasonography or trophoblast at histologic evaluation after spontaneous abortion.

Rate of clinical pregnancy, delivery per patient and per cycle, and miscarriage rate were compared between the 2 groups by means of the Fisher exact test and the Yates correct χ^2. A value of $P < .05$ was considered statistically significant.

Table 2. *Standard characteristics of semen and hypo-osmotic swelling (HOS) test in the 2 groups of couples on the basis of the HOS test**

	Normal HOS	Abnormal HOS
Sperm concentration (×10^6/mL)	16.8 ± 2.3	15.9 ± 1.8
Sperm motility (%)	42.3 ± 4	40 ± 5
Typical forms (%)	43 ± 3	41 ± 6
HOS test (%)	57 ± 4	38 ± 7†

* Values are mean ± SD.
† Significant at $P < .05$.

Results

Ten couples (4 in group 1 and 6 in group 2) were excluded from the study because of failure to complete the 3 consecutive cycles of treatment. Thus, 90 couples (35 in group 1 and 55 in group 2) completed the trial. Age of women, duration of infertility, BMI, plasma estrogen level, and follicle diameter on hCG day of administration were similar in both groups (Table 1); no significant difference in standard semen analysis parameters between treatment groups was found. By definition, HOS test value was significantly lower in group 2 than group 1 (Table 2).

Thirty-five couples with a normal HOS test and 55 couples with an abnormal HOS test were evaluated; 270 cycles of timed vaginal intercourse were performed: 105 in group 1 and 165 in group 2. Pregnancy rate, delivery rates per patient and per cycle, and miscarriage rate were significantly higher in group 1 than in group 2 (Table 3).

Discussion

This study demonstrates that subfertile men with normal HOS test values compared with men with abnormal test

Table 3. *Number of total cycles and pregnancy rate per patient and per cycle in the 2 groups of couples on the basis of the hypo-osmotic swelling (HOS) test*

	Normal HOS	Abnormal HOS
No. of couples	35	55
No. of cycles	105	165
No. of clinical pregnancies	10	6
No. of term pregnancies	8	2
Pregnancy rate/patient, %	28.5	10.9*
Pregnancy rate/cycle, %	9.5	3.6*
Delivery rate/patient, %	22.8	3.6*
Delivery rate/cycle, %	9.5	1.2*
No. of miscarriage	2	4
Miscarriage rate, %	20	66*

* Significant at $P < .05$.

values have greater probability of causing pregnancy and having a baby with cycles of timed vaginal intercourse. In fact, the ratios for the pregnancy rates per patient and cycle between normal and abnormal HOS test men was 2.1 and 2.6, respectively. Moreover, the ratios for miscarriage rate and delivery rates per patient and per cycle between the two groups was 3.3, 6.3, and 7.9, respectively.

Previous studies showed that the HOS test is able to predict pregnancy rate and outcome in couples undergoing in vitro fertilization and IUI procedures (Check et al, 1989; Check et al, 2001b; Tartagni et al, 2002).

We used 60 minutes instead of 30 minutes of incubation as reported by many authors (Check et al, 1995) because we wanted to evaluate the percentage of swollen g-type sperm, which is easy to calculate and correlates strongly with the percentage of total sperm swollen. The percentage of swollen g-sperm in 30 minutes is significantly smaller than that obtained after more than 1 hour, so if this percentage is to be considered, at least 1 hour of incubation is required (Takahashi et al, 1990).

The HOS test is easy to perform and not expensive; in contrast to sperm count and motility, which frequently fluctuate, the HOS test is stable over time (Shanis et al, 1992). However, at the moment, the HOS test is not included in standard semen analyses and is rarely considered in the workup of unexplained infertility.

Notably, in both groups, conventional semen parameters were similar; this confirms the poor predictive value of conventional semen analysis.

Our results are in agreement with those of other authors reporting a threshold value of 50% overall sperm swelling rate as an indicator of normal fertility potential of human spermatozoa (Shanis et al, 1992).

A recent mechanism proposed to explain how low HOS scores for spermatozoa could alter embryo implantation is that supernumerary defective sperm could damage the oocyte or the pronucleate embryo by altering the physical and chemical properties of the zona pellucida (Check et al, 2001a,b). In fact, it has been hypothesized that some toxic factors contained in the sperm membrane could be transferred to the zona pellucida and subsequently damage the embryo membrane (Check et al, 1995, 2001a; Katsoff and Check, 1997). This could result in anomalies of cell-to-cell communication and finally negatively affect the implantation rate (Denker, 1993).

Sperm for IUI was prepared by a conventional layering technique. Because mono-ovulation induction plus IUI do not give better clinical results compared with mono-ovulation plus timed vaginal intercourse (Melis et al, 1995), it is conceivable that sperm preparation for IUI could remove toxic factors contained in the abnormal HOS test sperm.

The HOS test can be considered an easy, inexpensive, and reliable test for predicting male fertility potential and for identifying among subfertile men those who have a greater possibility of conceiving with timed intercourse following ovulation induction.

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