Journal of Andrology, Vol. 23, No. 4, July/August 2002

Usefulness of the Hypo-Osmotic Swelling Test in Predicting Pregnancy Rate and Outcome in Couples Undergoing Intrauterine Insemination

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ABSTRACT: This study was designed to evaluate the usefulness of the hypo-osmotic swelling (HOS) test in predicting successful conception and the pregnancy outcome in couples in whom men are affected by mild infertility and fertile women are treated with ovulation induction and intrauterine insemination (IUI). We retrospectively analyzed the results obtained from 120 couples who underwent not more than 3 consecutive cycles of gonadotropin-induced mono-ovulation followed by IUI. Semen was analyzed by classical parameters, and the HOS test was performed using a hypo-osmotic solution. Using HOS, at least 50% swollen spermatozoa was considered normal. The pregnancy rate obtained in couples with an HOS less than 50% was significantly lower ($P < .05$) than that achieved in couples with HOS values at least 50%. Furthermore, the miscarriage rate was higher when HOS was less than 50%. For prediction of successful pregnancy, the sensitivity and specificity of the HOS test was 64% and 75%, respectively; the predictive value of an abnormal test was 94%, and that of a normal test was 24%. The HOS test may detect subtle damage in spermatozoan structure, which is responsible for the reduced capacity of spermatozoa to induce a viable embryo, with a low rate of pregnancy and a high rate of miscarriage. The results suggest that the HOS test may help in recognizing men who have a poor chance of fertilizing their partners and to have viable pregnancies using IUI.

Key words: Layering technique, male infertility, pregnancy outcome, spermatozoal abnormal membrane, sperm function test, swollen spermatozoa.

J Androl 2002;23:498–502

Standard semen analysis has long been the primary laboratory test of male fecundity. Semen analysis, however, cannot ascertain the functional capacity of sperm and frequently fails to predict the outcome of male infertility. Sometimes fertilization occurs despite an abnormal semen analysis, or it fails to occur when analysis values are normal.

Subfertility is generally defined as a sperm concentration less than 20×10^6/mL, less than 50% showing forward progression motility, and normal morphology in less than 60% (World Health Organization, 1992). It has been reported that more than 25% of men with children have subfertile semen analysis results (Steinberger, 1984).

The most important mechanisms of fertilization, such as capacitation, acrosome reaction, and binding of a spermatozoan to the egg surface are believed to depend on the functional integrity of the sperm membrane. Various tests of sperm function such as the hypo-osmotic swelling (HOS) test (Jeyendran et al, 1984), the zona-free hamster egg penetration assay (Yanagimachi, 1984), the triple stain technique for evaluation of the acrosomal reaction (Aitken et al, 1984), and others, have been proposed for measuring male fertilization potential.

Today, the HOS, modified for human use by Jeyendran et al (1984), stands out as the simplest and least expensive measurement of functional integrity of sperm membrane. Data exist to suggest that in the presence of normal semen parameters, an HOS score less than 50% is rarely associated with pregnancy in vivo (Check et al, 1989). Yet, other researchers contend that the HOS score is not useful in predicting fertilization rate (Chan et al, 1990; Marinko et al, 1996; Milingos et al, 1996).

The aim of our study was to evaluate the effectiveness and clinical usefulness of the HOS test for predicting successful conception and pregnancy outcome in the partners of men who met the criteria of mild male-factor infertility and who were undergoing controlled ovarian stimulation and intrauterine insemination (IUI).

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Received for publication July 2, 2001; accepted for publication January 14, 2002.

Materials and Methods

One-hundred twenty infertile couples in whom the only abnormality identified was the presence of mild male infertility (sperm concentration 10 to 20 $\times$ 10^6/mL, progressive motility 15% to 25%, total motility 30% to 50%, and normal morphology 30% to 50%) and who declined to undergo in vitro fertilization (IVF) were included in this study.

The age of the women ranged from 27 to 36 years (mean age $\pm$ SD, 33.9 $\pm$ 3.4 years). In all women, endocrine profiles were normal for the early follicular phase and progesterone (P_4) assay in the luteal phase (follicle-stimulating hormone [FSH] and luteinizing hormone less than 10 mUI/mL, prolactin less than 15 ng/mL, testosterone less than 0.6 ng/mL, and P_4 more than 6 ng/mL). Commercial kits (Diagnostic System Laboratories Inc, Webster, Tex) were used to conduct analyses; intraassay and interassay coefficients of variation were less than 10%. Hysterosalpingogram, hysteroscopy, and late luteal phase endometrial biopsies were normal in all participants.

Infertility duration ranged from 3 to 8 years (mean $\pm$ SD, 4.6 $\pm$ 2.8 years). In couples in whom the woman suffered from tubal damage, anovulatory cycles, polycystic ovary disease, hyperprolactinemia, uterine fibroids, endometriosis; or in whom the men were affected by severe infertility (sperm concentration less than 10 $\times$ 10^6/mL, progressive motility less than 15%, total motility less than 30%, and normal morphology less than 30%) were not considered eligible for the study.

All men underwent andrologic evaluation, with at least 2 semen analyses and HOS tests. Semen samples were collected by masturbation after 48 to 72 hours of sexual abstinence. Semen volume, sperm concentration, motility, and morphology were measured according to standard World Health Organization (1992) criteria. The HOS test was performed after examination standard semen parameters. An aliquot of 0.1 mL liquefied semen was added to 1 mL of hypoosmotic solution prepared by dissolving 3.75 g of sodium citrate and 13.5 g of fructose in 1 L of distilled water in accordance with a previously described technique (Jeyendran et al, 1984). After incubation for 60 minutes at 37°C the samples were examined using a phase-contrast microscope by a single technician throughout the study. One hundred spermatozoa were examined, and the morphological changes in the sperm tail were classified according to the types described by Jeyendran et al (1984). The overall rate of sperm swelling was calculated. Finding at least 50% swollen spermatozoa was considered normal.

Patients were divided into 2 groups according to HOS results: group 1 (n = 49), normal HOS test (ie, swollen spermatozoa at least 50%); and group 2 (n = 71), abnormal HOS test (ie, swollen spermatozoa fewer than 50%).

All women were treated for up to 3 consecutive cycles of controlled gonadotropin-induced mono-ovulation followed by IUI. Only 3 consecutive cycles of the same treatment were evaluated in order to prevent carryover effects of ovarian stimulation treatment from affecting results (Melis et al, 1987; Roh et al, 1987). Ovarian stimulation was conducted with recombinant FSH (rFSH, follitropin beta; Puregon, NV Organon, Oss, The Netherlands), starting at a daily dose of 50–100 IU on the third day of the cycle.

Table 1. *Characteristics of female partners for HOS subgroups with mild male infertility*

	Normal HOS	Abnormal HOS
Age (years)*	34 $\pm$ 3.8	33.8 $\pm$ 3.0
Duration of infertility (months)*	54.5 $\pm$ 14.1	50.4 $\pm$ 12.6
Plasma estrogen (pg/mL)*†‡	310 $\pm$ 4	314 $\pm$ 6
Follicle diameter (mm)*	18.6 $\pm$ 2.5	18.1 $\pm$ 2.4

* Values are mean $\pm$ SD.
† On the day of HCG administration.
‡ Conversion factor to S. I. unit = 3.671.

Before starting controlled ovarian stimulation treatment, transvaginal ultrasound examinations were performed every other day from the 5th day of treatment until the mean diameter of the dominant follicles reached 14 mm; examinations were then performed daily. Human chorionic gonadotropin (hCG; 10 000 IU; Profasi, Serono, Rome, Italy) was administered when the follicle reached a mean diameter of at least 18 mm. Intrauterine inseminations were performed 30 to 36 hours after hCG administration. Sperm for IUI were prepared using a conventional layering technique. For this, approximately 1.0 mL of medium (Sperm Preparation Medium; Medi-Cult, Jyllinge, Denmark) was layered onto 1.0 mL of semen, and the specimen was incubated at 37°C for 45 minutes. The supernatant was removed and was used for treatment. Intrauterine insemination was performed using a Frydman catheter. The cervix was exposed and the catheter was passed into the uterus to about 0.5 cm from the top of the uterine cavity. The sperm were then expelled.

Statistical Analysis

Clinical pregnancy rates and miscarriage rates per patient and per cycle were compared between the two groups. The Fisher exact test and the Yates correct χ^2 were used to compare pregnancy rates (per patient and per cycle) and miscarriage rates; a value of P less than .05 was considered statistically significant. Sensitivity, specificity, and predictive values of abnormal and normal HOS tests for predicting successful pregnancy were also calculated by a 2-by-2 matrix (Stempel, 1982).

Results

No significant difference was found between treatment groups in age of women, duration of infertility, response to ovarian stimulation (Table 1), and semen analysis parameters before and after preparation but before insemination (except for HOS results, which were significantly lower in group 2; Table 2). Forty-nine couples with a normal HOS test and 71 couples with an abnormal HOS test were evaluated. A total of 345 cycles of IUI were performed; 135 in group 1 and 210 in group 2. The number of cycles completed per patient was not statistically different in the 2 groups; the number of couples who underwent complete cycles (mean $\pm$ SD) was 2.7 $\pm$ 0.7, and 2.9 $\pm$ 0.1 in groups 1 and 2, respectively. No hyperstimulation or ectopic pregnancies occurred.

Table 2. *Standard characteristics of semen for HOS subgroups with mild male fertility before and after sperm preparation for IUI using conventional layering technique*

	Normal HOS		Abnormal HOS	
	Before	After	Before	After
Sperm concentration ($\times 10^6$/mL)*	15.1 ± 2.1	1.8 ± 0.2	16.5 ± 1.2	1.9 ± 0.3
Sperm motility (%)*	44.1 ± 5	85.1 ± 4	41.8 ± 7	81.2 ± 3
Typical forms (%)*	42 ± 4	50 ± 3	39 ± 6	48 ± 4
HOS test (%)*	56 ± 5	70 ± 4	39 ± 3†	49 ± 3‡

* Values are mean ± SD.
† $P < .05$ versus normal HOS group before treatment.
‡ $P < .02$ versus normal HOS group after treatment.

Group 1 achieved 15 clinical pregnancies, which represents a pregnancy rate per patient of 30.6%, a pregnancy rate per cycle of 11%, and a live pregnancy rate per cycle of 8%; 3 patients had miscarriages, which represents a rate of 20% (Table 3). Group 2 achieved 10 pregnancies, which represents a pregnancy rate per patient, pregnancy rate per cycle, and a live pregnancy rate per cycle of 14%, 4.8%, and 1.9%, respectively; 6 patients had miscarriages, which represents a rate of 60% (Table 3).

Pregnancy rates per patient, pregnancy rates per cycle, and live pregnancy rates per cycle were significantly higher in group 1 than in group 2 (Table 3). Similarly, the miscarriage rate in couples with a normal HOS test were significant lower than in couples with an abnormal HOS test (Table 3). The live pregnancy rate per cycle was more than 4 times higher in patients with a normal HOS test compared with those with an abnormal HOS test.

Prediction of a successful pregnancy with the HOS test showed a sensitivity of 64%, a specificity of 75%, and predictive values for abnormal and normal tests of 94% and 24%, respectively.

Discussion

To our knowledge, data on the fertilizing potential of men with mild infertility in cases of mono-ovulation induction are scant. The results of this study suggest that couples in whom men have mild infertility and an abnormal HOS test have a poor prognosis of a successful pregnancy with low-technology assisted reproductive procedures (the predictive value of an abnormal test is 94%); pregnancy rates per patient and per cycle, and live pregnancy rate per cycle were significantly higher in couples with a normal HOS test. However, although a 24% predictive value with a normal HOS test and 94% with an abnormal test clearly suggests that if the test is abnormal, a man would have difficulty fathering offspring with IUI, and if the test is normal, he may or may not have difficulty in procreating.

Standard semen analysis characteristics were similar in the 2 groups, which confirms the poor predictive value of semen analysis. Furthermore, the 2 groups were similar in age distribution, ampules of rFSH employed, days of therapy, and estrogen plasma levels; thus, it is reasonable to postulate that in the 2 groups, spermatozoa interacted with similar quality oocytes.

The results obtained in our study are in contrast with those found by other authors who have reported no correlation between normal or abnormal HOS results and the success of in vitro fertilization (Marinko et al, 1996). However, others (Mahadevan et al, 1984; Ved et al, 1997; Zeyneloglu et al, 2000) have reported the utility of the HOS test for predicting a higher pregnancy rate in women undergoing IVF or intracytoplasmic sperm injection. The type of fertilization procedure, data interpretation, and statistical analysis may account for these discrepancies.

Our results agree with those of Uchida et al (1992) who demonstrated better in vivo ability of a normal HOS test for spermatozoa in interacting with the female reproductive apparatus and in overcoming some steps of fertilization. Increased embryonic loss observed in couples with an abnormal HOS test is consistent with this hypothesis. As stated before, in humans few studies have examined the correlations between sperm function tests and miscarriage rate (Marinko et al, 1996; Katsoff et al, 1999). Check et al (1995) observed an increased incidence of miscarriage in couples with abnormal HOS test results

Table 3. *Number of total cycles, cycle/patients, pregnancy rates, and pregnancy outcome for the HOS subgroup with mild male infertility*

	Normal HOS	Abnormal HOS
Number of couples	49	71
Number of cycles	135	210
Cycles/patient*	2.7 ± 0.7	2.9 ± 0.1
Clinical pregnancies	15	10
Pregnancy rate/patient	30.6%	14%†
Pregnancy rate/cycle	11%	4.8%†
Live pregnancy rate	8%	1.9%†
Live births	12	4
Miscarriages	3	6
Miscarriage rate	20%	60%†

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

undergoing IVF procedures. In this comparative, prospective, controlled IVF study, the clinical and viable pregnancy rates and implantation rates for couples in whom the man's HOS test score was more than 50% were 25.9%, 18.5%, and 9.9%, respectively, compared with only 3.7%, 3.7%, and 1.1%, respectively, when the HOS test score was less than 50% (Check et al, 1995).

It has recently been hypothesized that a defect in the functional integrity of the sperm membrane, which is detectable by the HOS test, may reduce fertility potential by causing implantation disorders rather than fertilization problems (Check et al, 2001a). The defect associated with subnormal sperm that have undergone HOS could be not related to the single spermatozoa that is responsible for fertilizing the oocyte, but to a toxic factor attached to the sperm membrane, which is in turn transferred to the zona pellucida, and then to the embryo (Check et al, 2001b). Supernumerary defective sperm could damage the oocyte or pronucleate embryo by altering the physical-chemical properties of the zona pellucida via the release of toxic metabolites (eg, oxygen radicals; Check et al, 2001b). Subtle abnormalities in sperm detected with the HOS test may lead to subsequent abnormal membrane function in the embryo and anomalies in the cell-to-cell communication and binding that seem to have an important role in the attachment of the blastocyst and subsequent penetration of surface epithelium of the endometrium (Denker, 1993). Human studies indicate that paternally derived proteins are expressed in the embryo at the preimplantation stage (Daniels et al, 1995).

An additional mechanism for explaining the interplay between sperm abnormalities and poor pregnancy outcome is a desynchronization between slower embryonic development and uterine environment (Howarth et al, 1965); accordingly, it is possible to hypothesize that defective spermatozoa could lead to a less viable embryo, and therefore, a low viable pregnancy rate with a high miscarriage rate.

In accordance with a possible detrimental role played by subtle sperm abnormalities and an increased miscarriage rate despite normal semen analysis parameters in terms of pregnancy outcome, has been demonstrated in mammals exposed to a moderate increase in body temperature (Setchell et al, 1988; Mieusset et al, 1992).

In this study we adopted a stimulation scheme with low doses of gonadotropin mainly to obtain a group of mono-ovulatory patients who were as homogeneous as possible; in no cases were multiple pregnancies or hyperstimulation observed. The beneficial effects of ovulation induction already have been demonstrated: improved oocyte quality, improved timing of the ovulatory period, and correction of slight and unknown ovulatory disorders (Melis et al, 1990; Mascarehas et al, 1994).

The pregnancy rates obtained in the 2 groups are lower than those expected for treatment with this technique. Peterson and coworkers (1994) indeed reported a 15% pregnancy rate in women treated with multiple ovulation induction with hMG and IUI. At least 2 reasons could account for the low success rate. The first is that in our couples, due to the very low number of progressive sperm, IVF should have been considered instead of IUI; the second reason is that we report results of induction of mono-ovulation and not of multiple ovulation, which could, by itself, improve the success rate.

In conclusion, the HOS test may be considered an easy, inexpensive, and reliable test for evaluating sperm function, and which is useful in recognizing subfertile men who are likely to make their fertile partners pregnant in IUI programs; it may also be considered capable of predicting the outcome of pregnancy.

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