

A comparative prospective study using matched samples to determine the influence of subnormal hypo-osmotic test scores of spermatozoa on subsequent fertilization and pregnancy rates following in-vitro fertilization

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The achievement of pregnancies *in vivo* is rare in couples where the male partner has defective sperm membranes as shown by hypo-osmotic swelling (HOS) test scores of <50%. However, there have been mixed reports on the value of the HOS test in predicting outcome following in-vitro fertilization; some studies suggest reduced fertilization rates and others find little, if any, predictability of decreased fertilization. The assumption has been made that fertilization rates are proportional to pregnancy rates; however, this may not necessarily be true since defective spermatozoa could lead to a less viable pre-embryo and therefore a decreased viable pregnancy rate. We performed a comparative prospective study using matched controls to evaluate fertilization rates and to determine subsequent pregnancy rates. The mean HOS scores were 70.0 and 36.7% respectively, with mean motile sperm concentrations of 35.7 and 34.0×10⁶/ml in 27 matched pairs. There was no difference in the mean number of oocytes retrieved, fertilization rates or number of embryos transferred between the two groups by HOS score. The clinical and viable pregnancy rates and implantation rates were 25.9, 18.5 and 9.9% for normal versus 3.7, 3.7 and 1.1% for subnormal groups. These data suggest that low HOS scores may be associated with the formation of defective embryos, leading to low pregnancy rates but normal fertilization rates.

Key words: conception/functional integrity/IVF/sperm membrane

Introduction

The hypo-osmotic swelling (HOS) test is a measurement of the functional integrity of the sperm membrane. It was modified for human use in 1984 by Jeyendran *et al.* There are data suggesting that, when a patient has two HOS scores of <50%, in-vivo pregnancies are rare, even if other semen parameters are normal (Check *et al.*, 1989).

One of the first studies evaluating the correlation of the HOS test with in-vitro fertilization (IVF) found that subnormal

scores correlated with reduced fertilization rate following IVF (Van der Ven *et al.*, 1986). IVF was performed with washed but unselected spermatozoa (i.e. no swim-up or Percoll discontinuous gradients processing, etc.). Specifically, ejaculates whose spermatozoa fertilized oocytes possessed at least 60% HOS-reactive spermatozoa; however, no distinct cut-off values were found for the more standard semen parameters (Van der Ven *et al.*, 1986).

Other researchers have also reported similar findings: Liu *et al.* (1988) found the HOS score to average 65% in patients with <50% fertilization rate compared to 77% for those with >50% fertilization rate. Takahashi *et al.* (1990) found that the HOS test showed a stronger correlation with IVF rates than other semen parameters. However, many other studies do not agree that the HOS score is that valuable in predicting fertilization rates (Barratt *et al.*, 1989; Sjoblum *et al.*, 1989; Avery *et al.*, 1990; Chan *et al.*, 1990).

All of these previous studies addressed the correlation of poor HOS scores and fertilization rate, but none of them evaluated their association with pregnancy rates. The study presented herein prospectively evaluated the influence of subnormal HOS scores not only on fertilization rates following IVF/embryo transfer but also pregnancy rates.

Materials and methods

Eligibility for participation in the study group required that, prior to the IVF cycle, the male partner of a couple had had two HOS tests that had <50% tail swelling, and that his own semen was being used for insemination for IVF/embryo transfer. Furthermore, the couple could not be part of the shared oocyte programme (Check *et al.*, 1992b). Also, all female patients were given the same ovarian stimulation protocol, i.e. the 10 mg luteal phase leuprolide acetate-human menopausal gonadotrophin stimulation regimen (Meldrum *et al.*, 1989). Once these criteria were met, the male partner was matched for control purposes with the first male registering thereafter with the programme who had two normal HOS tests (≥50%), was undergoing IVF/embryo transfer within the same month, and had similar motile sperm concentration and normal morphology scores. Similarity of motile concentration was defined as being in the same range: ≤5×10⁶ motile spermatozoa/ml, between 5 and 10×10⁶ motile spermatozoa/ml, between 10 and 15×10⁶ motile spermatozoa/ml, or >15×10⁶ motile spermatozoa/ml. Efforts were made when matching motile concentrations to choose similar sperm concentration and percentage motility rather than matching an individual

with a higher count but lower percentage motility with a patient with lower count but better motility. Similarly, if normal morphology was defined as being in the same range (<4%, 4–14% or >14%) though the group with >15×10⁶/ml concentration and >14% motility would have wide swings in motile concentration, previous data have suggested that it would not be warranted to subdivide this group any further since prognosis is excellent once this level is achieved (Check *et al.*, 1991, 1992a). Patients were excluded from the study if they had positive antisperm antibodies ≥20% as determined by the direct immunobead test.

The HOS test was performed by combining 0.1 ml of ejaculate with 1.0 ml hypo-osmotic solution (fructose/sodium citrate) following precisely the technique described by Jeyendran *et al.* (1984). After incubation of the mixture for at least 30 min at 37°C, 100 spermatozoa were observed with a phase-contrast microscope for tail changes typical of a reaction in the HOS test. The HOS tests were performed on unprepared specimens during standard semen analysis.

The variables measured included number of oocytes retrieved, fertilization rate, number of embryos transferred and clinical and viable pregnancy rates. A clinical pregnancy was defined as one in which a gestational sac was observed by ultrasound. A viable pregnancy was defined as a pregnancy that showed viability by sonography at the end of the first trimester.

Statistical analysis included McNemar's test for matched proportions to compare pregnancy rates and paired *t*-test to compare the mean number of oocytes retrieved, mean fertilization rate and mean number of embryos transferred. All tests were done at the *P* < 0.05 level of significance. A sample of 27 pairs had 80% power to detect a true difference of 30% in the pregnancy rates between the two groups at the *P* < 0.05 level of significance and 80% power to detect a true difference of 25% in pregnancy rate at the *P* < 0.10 level of significance.

Results

Patients were enrolled in the study from January 1989 through June 1994. Although 1270 couples underwent IVF/embryo transfer without donor spermatozoa in our facility during this period, only 27 couples met the eligibility requirements. Thus, the prevalence of subnormal HOS in our IVF population was 2.1%.

There were 27 matched pairs in this study. The mean age ± SD of the female partners of males with low HOS scores was 33.2 ± 4.8 years, compared with 34.1 ± 4.3 years for the female partners of the control group. The mean motile concentration was 35.7 ± 35.3×10⁶ motile spermatozoa/ml in the group with normal HOS scores and 34.0 ± 52.3×10⁶ motile spermatozoa/ml in the group with low HOS scores. The mean normal morphology scores were 6.2 ± 4.0 and 6.8 ± 5.7% respectively. The mean HOS scores were 70.0 ± 9.3 and 36.7 ± 9.0% respectively.

A comparison of IVF outcome variables by HOS scores is presented in Table I. There was no difference in the mean number of oocytes retrieved, mean fertilization rates or mean

Table I. Comparison of in-vitro fertilization outcome variables by hypo-osmotic swelling (HOS) scores in 27 matched pairs

	HOS normal	HOS subnormal
Mean number of oocytes retrieved	11.0 ± 7.5	11.2 ± 7.4
Mean fertilization rate of all oocytes (%)	55.5 ± 32.3	56.0 ± 27.9
Mean number of embryos transferred	3.4 ± 1.6	3.5 ± 1.5

Table II. Clinical and viable pregnancy rates according to hypo-osmotic swelling (HOS) scores in 27 matched pairs

	No. treated	No. clinical pregnancies ^a	No. viable pregnancies ^b
HOS ≥50%	27	7 (25.9%)	5 (18.5%)
HOS <50%	27	1 (3.7%)	1 (3.7%)

^a*P* = 0.034, McNemar test.

^b*P* = 0.10, McNemar test.

Table III. The fertilization and pregnancy rates for the HOS subgroups with abnormal semen parameters in 27 matched pairs

	HOS normal	HOS subnormal
Motile sperm concentration <10 (n = 5)		
Fertilization rate (%)	33.6 ± 30.6	35.4 ± 26.8
Pregnancy rate (%)	0.0	0.0
Normal sperm morphology (strict criteria <4%) (n = 9)		
Fertilization rate (%)	57.7 ± 24.5	49.5 ± 31.2
Pregnancy rate (%)	22.2	11.1
Sperm motility <40% (n = 6)		(n = 7)
Fertilization rate (%)	35.5 ± 27.8	58.0 ± 33.6
Pregnancy rate (%)	16.7	14.3
Sperm count <20 × 10 ⁶ /ml (n = 7)		(n = 4)
Fertilization rate (%)	50.3 ± 31.9	61.5 ± 31.9
Pregnancy rate (%)	0.0	0.0

HOS = hypo-osmotic swelling.

number of embryos transferred by HOS score (paired *t*-test, *P* > 0.05).

There were seven (25.9%) clinical pregnancies in the normal (≥50%) HOS group and one (3.7%) clinical pregnancy in the low (<50%) HOS group (*P* = 0.034, McNemar test) as seen in Table II. There were five (18.5%) viable pregnancies in the normal HOS group and one (3.7%) viable pregnancy in the low HOS group (*P* = 0.10, McNemar test) (see Table II). The implantation rate was higher for the normal HOS group (9.9%, nine of 92) than for the subnormal HOS group (1.1%, one of 91) (*P* = 0.009, χ^2 -test). The fertilization and pregnancy rates for the subgroups with abnormal semen parameters are given in Table III.

Discussion

It is to be hoped that a simple test can be devised which can be performed at the time of routine semen analysis, and that

when a subnormal result is obtained, a poor likelihood of pregnancy can be predicted. There was some optimism that the use of strict morphology might be that long sought after test. One study found that when a high concentration of spermatozoa was used for oocyte insemination normal fertilization rates were achieved; however, the pregnancy rates were still poor (Oehninger *et al.*, 1988). This same group, who also reported very low IVF pregnancy rates with poor strict morphology, recently published data showing a 16.5% pregnancy rate when strict morphology was $<4\%$, compared to 18.4% with morphology 4–14%, and 19.8% with morphology $>14\%$ (differences not statistically significant) (Grow *et al.*, 1994). Even an in-vivo study did not demonstrate ability to predict poor pregnancy rates by reduced strict morphology (Check *et al.*, 1992a). Therefore, strict morphology does not seem to be a highly specific test to determine male subfertility. Thus, the HOS test may be the only semen parameter available at the time of initial semen analysis which, when abnormal, predicts reduced pregnancy rates even when fertilization rates are normal.

The data presented herein are in agreement with those studies that did not find the HOS score helpful in predicting poor fertilization rates following IVF/embryo transfer. Interestingly, despite the transfer of a mean number of 3.5 embryos in patients with HOS scores $<50\%$, the clinical pregnancy rate was only 3.7% and the viable pregnancy rate 3.7% per transfer (as compared to 25.9 and 18.5% for patients with an HOS score $\geq 50\%$ with an average of 3.4 embryos transferred). These data are in agreement with another study involving patients enrolled in the shared oocyte programme, thus eliminating possible oocyte factor; clinical and viable pregnancy rates for those patients with subnormal ($<50\%$) HOS scores was one of 23 (4.3%) and none of 23 (0%) respectively, compared to 38 of 149 (25.5%) and 34 of 149 (22.8%) for those with HOS scores $\geq 50\%$ (Check *et al.*, 1994a). These data strongly suggest that pregnancy rates will be extremely low following IVF/embryo transfer if a patient has two subnormal HOS scores.

The prevalence of male partners that have two subnormal HOS scores is low among couples undergoing IVF/embryo transfer, but HOS testing does appear to be able to detect a subfertile male who otherwise has normal semen parameters. It has been shown that there is little variation of HOS scores between different ejaculates from the same individual and the results remain stable over time (Shanis *et al.*, 1992). In contrast to the sperm count and motility, what needs to be determined is how often male subfertility might be responsible for failure to conceive despite adequate fertilization when the HOS score is $\geq 50\%$. More elaborate tests of sperm physiology may prove effective in determining these cryptic subfertile sperm specimens. Of course, subfertile spermatozoa could also be responsible for failed or poor fertilization. Interestingly, one study of failed fertilization using either donor spermatozoa or donor oocytes in subsequent cycles concluded that defective oocytes might be the more likely aetiology for failed fertilization (Coates *et al.*, 1992).

Some large series have been published in which IVF has been considered as a treatment for male factor (Cohen *et al.*,

1985; Enginsu *et al.*, 1992; Ord *et al.*, 1993). The advent of micromanipulation techniques, e.g., subzonal insemination (SUZI) (Cohen *et al.*, 1991; Sakkas *et al.*, 1992; Lippi *et al.*, 1993) and intracytoplasmic sperm injection (ICSI) (Van Steirteghem *et al.*, 1993a,b) have allowed tremendous improvements in the achievement of successful pregnancies for severe male factor. These micromanipulation procedures have allowed patients with severely oligoasthenoteratozoospermic samples ($<500\,000$) as well as males with previous failed fertilization to achieve fertilization and subsequent pregnancies. However, the effect of the HOS score was not assessed in any of these studies. Since the data presented herein found no significant reduction in the fertilization rates with HOS scores of $<50\%$, but a very low pregnancy rate, theoretically, micromanipulation procedures or non-micromanipulation enhancement techniques (Check *et al.*, 1994b) might similarly prove ineffective for improving pregnancy rates for patients with subnormal HOS scores. Hopefully, these data will encourage the programmes with the greatest success with micromanipulation to measure HOS scores so that they can evaluate whether these therapeutic modalities are effective for treating males with this defect.

It may prove that subnormal HOS scores are not indicative of abnormal embryos produced by whichever spermatozoon fertilizes the oocyte, but rather of some damage to the embryo from defective spermatozoa leading to early demise of the embryo. Some support for this concept has been provided by Alvarez *et al.* (1994), who found that processed spermatozoa which did not retain 80% of their motility after incubation at 40°C for 4 h resulted in no pregnancies in 24 transfers, compared to 11 pregnancies in 20 transfers (55%) for those spermatozoa with $>80\%$ motility, and yet there was no difference in fertilization rates. The possibility exists that some males who produce spermatozoa that look normal, fertilize the oocyte but form defective embryos, may produce normal embryos by ICSI when fertilization is with only one spermatozoon and potential toxic sperm effects may be avoided.

We have frequently observed that males with antisperm antibodies have poor HOS scores (one reason for excluding males with antisperm antibodies from the study reported here). However, improved pregnancy rates and improved HOS scores were achieved, despite failure to reduce antisperm antibody concentrations, when the spermatozoa bound with antisperm antibodies were treated with chymotrypsin–galactose (Bollendorf *et al.*, 1994; Katsoff *et al.*, 1994). Recently, we found in a small series a 25% pregnancy rate following intrauterine insemination of spermatozoa not bound with antisperm antibodies with HOS scores $<50\%$ when treated with chymotrypsin–galactose.

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