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The hypo-osmotic swelling test versus routine semen analysis in evaluating the fertility potential of human spermatozoa

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SUMMARY

The hypo-osmotic swelling (HOS) test performed in the male counterpart of infertile couples was found to correlate better with the achievement of pregnancy than with the routine semen analysis. No significant difference in pregnancy rates was found in couples in which the male had a normal or subnormal spermiogram as long as the HOS score was normal. This test thus may be helpful as an ancillary test to help identify "fertile" males who have subnormal routine semen analyses, and "subfertile" males with apparently normal spermiograms.

INTRODUCTION

Males with subnormal spermiograms have frequently achieved pregnancies; in contrast, some men with apparently normal spermiograms have failed to enable his female counterpart to conceive yet switching to donor sperm has resulted in successful pregnancy. Thus, there has been a need to develop a more sensitive sperm assay that would better be able to predict male fertility potential.

The hamster ova penetration test or sperm penetration assay initially offered great promiseas a better predictive test for the assessment of the fertilizing capacity of human spermatozoa than the conventional semen analysis.[1] However, the assay is technically difficult to perform and quite expensive. Furthermore, there have been reports of the failure of the SPA to correlate in clinical infertility situations.[2]

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The hypo-osmotic swelling test, which determines the functional integrity of the sperm membrane, may provide additional assessment of sperm function beyond what is provided by standard semen analysis.[3,4]

A study was designed to determine whether the HOS test could provide any additional information of the fertilizing capacity of the spermatozoa beyond what is provided by the standard semen analysis.

MATERIAL AND METHODS

A total of 917 couples with a minimum of 1-1/2 years of infertility were enlisted in the study. They were divided into 2 groups: 1) 172 couples in which all female infertility factors were corrected and 2) 745 couples included from their initial consultation in our office. Group 1 females were maintained on whatever therapy had been found necessary to correct a defective fertility parameter (if one had been found) for the 6 month duration of study. Group 2 females with regular menses were evaluated using pelvic sonography and serum estradiol and progesterone assays and late luteal phase endometrial biopsies in an attempt to determine if a luteal phase defect was present and if so to ascertain whether these women should be exclusively treated with luteal phase progesterone support or ovulation inducing drugs plus progesterone. Cervical factor was evaluated also by sonography and hormonal assays and treated by either correction of follicular maturation abnormalities or by specific mucus therapy. The females were also given the option of deferring tubal studies while correcting other factors or undergoing either hysterosalpingography or laparoscopy; the latter would be used diagnostically and therapeutically to lyse adhesions and coagulate endometriosis whenever possible.

Two baseline spermiograms and HOS tests were obtained in each male partner after a 48-72 hour abstinence. The HOS test was performed as previously described.[3] HOS results were recorded as percent of sperm that demonstrated swelling and were categorized as normal (swelling in $\geq 60\%$) grey zone (50-59% swelling) or abnormal (<50% swelling). The routine semen analyses were performed with a Makler chamber. The spermiograms were classified as follows: superior (count $\geq 50 \times 10^6$, motility percentage $\geq 50\%$ with progressive forward motion (PFM), $\geq 50\%$ normal morphology); normal (count 21-49) x 10^6/ml, motility $\geq 50\%$ with PFM); abnormal (count $\leq 20 \times 10^6$/ml or motility < 50% or < 50% normal morphology).

Differences in HOS results at the time of initial and follow-up testing and the results of the HOS run in duplicate on each specimen were analyzed using a paired t-test. Achievement of pregnancy among the various groups and sub-groups was determined by chi-square analysis or the Fisher's exact test whenever a cell count was less than 5. Numerical data are presented as mean + 1-S.D. P values less than 0.05 were considered to be significant.

RESULTS

Table 1 provides the pregnancy rate in 172 couples during a 6 month time span beginning from the moment that all female infertility factors were corrected. There was no statistical difference in the percentage pregnancies achieved in men with subnormal, superior or normal spermiograms as long as the HOS tests were $\geq 50\%$ (normal or grey zone). No pregnancies were achieved in the 29 couples where the male had a subnormal HOS test including 7 couples where the spermiograms were normal.

Table 2 indicates the pregnancy rate in 745 couples 6 months from their initial consultation. Infertility factors may have been identified in some of the females and not in others. Therapy may have been initiated in some of the women and not in others. Some but not all females may have had their infertility factors corrected for 1-5 months. As in the couples where all female factors were corrected, there was a statistically significant difference in pregnancy rates in couples in which the male had normal vs those with abnormal HOS scores. No difference in pregnancy rate was found when comparing normal and subnormal spermiograms.

TABLE 1 - Pregnancy rate within 6 months of correction of all female infertility factors. Males evaluated by HOS test and conventional spermiograms but not treated.

HOS Group	Spermiogram Group	Conceived	Failed To Conceive	Percent Conceiving	P
nl + grey	sup + nl	100	23	81	.55
nl+ grey	subnl	29	7	80.5	
nl + grey	all	129	30	81	<.0001
abnl	all	0	13	0	
nl + grey	sup + nl	100	23	81	<.0001
abnl	sup + nl	0	7	0	
nl + grey	subnl	29	7	80.5	<.0001
abnl	subnl	0	6	0	

Group Key:
nl = normal sup = superior (see text for group details)
grey = grey zone subnl - subnormal
abnl = abnormal all = sup + nl + subnl

TABLE 2 - Pregnancy rate within 6 months of couples registering in our infertility clinic. Diagnostic tests and therapy initiated on the female counterparts. Males evaluated by HOS test and conventional spermiograms but not treated.

HOS Group	Spermiogram Group	Conceived	Failed To Conceive	Total	P	Percent Conceiving
nl + grey	sup + nl	165	288	453	.39	36.4%
nl+ grey	subnl	86	175	261		32.9%
nl + grey	all	251	463	714	<.0001	35.2%
abnl	all	0	31	31		0%
nl + grey	sup + nl	165	288	453	<.01	36.4%
abnl	sup + nl	0	8	8		0%
nl + grey	subnl	86	175	261	<.0001	32.9%
abnl	subnl	0	23	23		0%

The average motile sperm percentage in those patients with normal HOS tests was 40.3% compared to 32.2% for those with subnormal spermiograms and abnormal HOS scores. These means are not significantly different (paired t-test).

CONCLUSIONS

The assessment of whether the HOS test provides any better evaluation of the fertility potential of the male partner above the standard semen analysis requires comparison to the best reference standard available. Some data has shown a better correlation of human oocyte penetration with the HOS test than with normal semen parameters.[5] One study demonstrated a good correlation between the HOS test and the human sperm hamster ova penetration assay (SPA).[3] However, other data was unable to demonstrate any significant correlation between the HOS test and the SPA.[6,7]

Furthermore, the SPA test itself may be questioned as to whether it is the best test to determine male fertility potential. Some of these differences may be related to the quality of the laboratories performing the SPA test since it is technically difficult to perform and therefore costly (whereas the HOS test is technically quite easy and inexpensive).

However, recently using the same laboratory, three different conclusions were reached as to the clinical significance of the SPA; one study showed a correlation between subnormal SPA scores and infertility[8] whereas another study failed to demonstrate any correlation with the SPA scores and subsequent fertility.[2] Finally, the same group suggesting a correlation of SPA scores and subsequent fertility failed to demonstrate any correlation with pregnancies achieved by in vitro fertilization.[9]

Though a correlation of the HOS test with in vitro fertilization has been suggested,[5] the latter may require lesser numbers of "fertile" sperm than "in vivo" fertilization. Thus, the best method to evaluate the clinical significance of the HOS test would be to compare this test to the standard semen analysis as far as the ability to predict pregnancy outcome. Though there was a higher percentage of pregnancies achieved by men with normal and superior semen analyses than men with subnormal spermiograms the difference was not statistically significant.

Thus the HOS test seems to help identify those men, who despite subnormal semen parameters have potential "fertile" sperm. Some of the previous data concerning efficacy of various therapeutic modalities, e.g. varicocelectomy or clomiphene citrate therapy did not take into account concomitant therapy of the female factor such that 61% of the 42 men with subnormal spermiograms (but where all female factors were corrected) achieved a pregnancy without any therapy directed to the male. However, all of those achieving a pregnancy had either a normal or grey zone HOS test score. Nevertheless, the data does support meticulously evaluating the female factor right from the beginning rather than delaying further investigation pending correction of the male factor.

The HOS test seems to have some value also in predicting those men with apparent normal spermiograms who are subfertile. Considering the large percentage of pregnancies achieved in the female corrected group by men with normal HOS test scores (124/159 = 81%), versus no pregnancies by men with subnormal HOS test scores, perhaps the males from this latter group should be treated (e.g. varicocele ligation) even if the semen analysis seems normal.

The achievement of pregnancies was just as good in males with grey zone HOS scores as men with normal scores. Thus, our data would support eliminating the grey zone and having just the two categories of normal and abnormal HOS test scores.

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