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Role of Sperm Function Tests in Unexplained Infertility

Suvarna S. Khadilkar, Parathe, S.M. Tayde

Department of Obstetrics and Gynaecology, Grant Medical College and Cama & Albless Hospital, Mumbai, Maharashtra, India.

Summary

Unexplained infertility is a challenging problem for treating doctor. The apparent normal semen analysis does not rule out the functional abnormalities of sperms. The role of sperm function by H.O.S. test [Hypoosmotic swelling test] in unexplained infertility has been studied and analyzed here. 35 patients of unexplained infertility were screened for sperm function test by H.O.S. We observed that 20% of patients had abnormal H.O.S. test and the results correlated well with morphological abnormalities. Abnormal HOS indicates poor membrane integrity. Thus it may adversely affect the results of various ARTS.

Introduction

Unexplained infertility is a challenging problem for the treating doctor but is a frustrating experience for the infertile couple. The couple goes from one hospital to another, changing protocols after protocols for months together, just to realize that nothing seems to be working inspite of every thing being apparently normal. Many things need to be looked at in such patients. Nidation failure, luteinized unruptured follicles (L.U.F.) and LPD or luteal phase deficiency are few of the factors being studied all over the world. However "assessment of sperm functions in unexplained infertility" is an important factor that needs to be studied.

The role of sperm function by H.O.S. test {hypoosmotic swelling test} in unexplained infertility has been studied and analyzed here. The routine semen analysis repeatedly comes apparently normal, but one does not know whether the longevity & fertilizing property of sperms is normal or not. There is definitely something beyond "normal routine semen analysis". For all practical purposes, detailed history, clinical examination and routine semen analysis is sufficient for most of the patients. But this spectrum of patients with unexplained infertility needs further evaluation in the form of sperm function tests. H.O.S. or Hypoosmotic swelling test is one important sperm function test which is studied in this paper. The aims & objectives of this paper were to assess sperm function by H.O.S. test & perform detailed analysis of sperm morphology and other routine parameters on patients with unexplained infertility. We studied whether any of the routine parameters of semen analysis correlated well with HOS test results.

Materials and Methods

35 patients diagnosed to have unexplained infertility, attending infertility clinic in Cama Hospital, were selected for the study. All the patients had undergone all routine investigations, specific infertility investigations like D & C, laparoscopy / HSG and routine semen analysis. None of the investigations

showed any obvious abnormality. The couples had regular coitus for period of one year or more which did not result in any conceptions.

The semen samples of these patients were analyzed in different labs and had always shown normal routine analysis as per W.H.O. recommendation for the same. (W.H.O. 1992) [Table I]. All the patients selected had counts above 20 millions/ml, with motility of more than 50% with at least 25% R.L.Ps [rapid linear progressive]. This was not always specified in all the reports. All had more than 30% morphologically normal sperms. All the patients were asked to collect semen samples by masturbation in wide-mouthed sterile glass beakers after the period of 3-4 days of abstinence. After liquefaction of samples a drop was taken on a slide for the initial examination which included initial coiling and initial count. Thereafter the samples were processed in two parts. Routine analysis was done on the first part with great care and Sperm morphology was done on stained smears under higher magnification [1000X] applying 'stricter criteria'. A separate count was kept of head, midpiece and the tail abnormalities.

Table I
WHO Criteria for Normal Semen Analysis [1992]

Seminal Parameter	Normal values
Volume	2ML
Motility	>50% Forward progressive, >25% R.L.P.s [rapid linear progressive]
Morphology	>30% normal
Vitality	> 75% alive
W.B.C. Conc.	<1 Mill/ml
Immunobead	<20% Adherent
Mar Test	<10% Adherent

A separate count was also kept of coiled tails at the initial examination and was labeled as "initial coiling". After noting the "initial coiling" percentage, the semen samples were subjected to the sperm function test by H.O.S. method. [Jayendran et al in 1984].

H.O.S. Test

Principle: Healthy Viable sperm having good fertilizing potential have the property of curling & coiling of tails when exposed to the stress of hypoosmotic pressure. The unhealthy poor quality sperms do not have curling property under the hypoosmotic pressure. The unhealthy poor quality sperms do not have curling property under the hypoosmotic stress. So the test evaluates the physiologic integrity of plasma membrane of sperms. The curling is due to swelling of plasma membrane & retraction of axoneme fibers in the tails.

Technique: HOS solution was prepared in our IUI laboratory at Cama Hospital and was stored at 4°C temp.

Composition of H.O.S. Solution:
Fructose:- 1.351 gm
Sodium Citrate:- 0.735gm
Distilled Water:- 100 ml.
1 ml of HOS solution was taken in a tube and warmed at 37°C for 10 minutes. 0.1 ml of semen sample was then added to H.O.S. solution, mixed well and incubated at 37°C for 30 minutes. 10 ul (Micro litre) of incubated mixture was taken on a labeled clean glass slide covered with a cover slip & examined under microscope at 400X magnification.
Percentage of sperms having coiled tails was calculated in two fields and the mean was taken.

Interpretation
HOS Positive % = Total mean percentage post HOS coiling – Initial coiling percentage.
Normal H.O.S. = > 60% sperms with coiled tails. [HOS positive%]
Inference=Good prognosis. Longevity, Viability and fertilization potential is good & normal.
Abnormal HOS=< 60% sperms with coiled tails.
Inference=poor prognosis. Indicates degenerative changes in sperm membrane secondary to either Infection or some inherited disorder.
H.O.S, test results and other seminal parameters were compared and studied in details.

Table II
Sperm Count and HOS Test.

Count Range Mill/ml.	Normal H.O.S. n=28	Abnormal H.O.S. n=7
=20	1	4
21-40	5	1
41-60	11	2
61-80	10	-
>80	1	-

Observations and Results

All the patients (Males) belonged to the range of 25 to 40 years of age. Duration of infertility ranged between 3 to 15 years. Any relevant points in the General Medical, Surgical and Sexual History were noted. Out of 35 patients 28 showed normal HOS test whereas 7 showed abnormal HOS test giving us an incidence of 20%. When the results of HOS test were compared with various seminal parameters we observed that majority of the patients from normal H.O.S. group had counts above 40 million/ml. Whereas 4 out of 7 patients from abnormal H.O.S. group had counts of only 20 million/

ml. That means a majority of patients had counts closer to lower limit of normal.

Sperm motility as assessed by us was > 50% in all samples however careful examination revealed much lower percentage of R.L.P.s (Rapid Linear Progressive Sperms) in 57.2% of patients with abnormal H.O.S. [Table III]. As per W.H.O. (1992) the RLPs should be at least 25%.

Table III
Sperm Motility and HOS Test

Rapid Linear Progressive Sperms [RLPs%]	Normal H.O.S. n=28	Abnormal H.O.S. n=7
>25%	22 [78.6%]	3 [42.8%]
<25%	6 [21.4%]	4 [57.2%]

Table IV shows the correlation of W.B.C. concentration & HOS results. 19 out of 28 had W.B.C. concentration < 1 million/ml in normal H.O.S. group indicating no significant infection. However in abnormal HOS group none of the patients had WBC concentration <1 million/ml. All the patients had significant WBC concentration. Sperm morphology was done in greater details with strict criteria. Head, midpiece & tail abnormalities were noted which included coiled tails also. However no specific part showed higher abnormality. Overall abnormal morphological forms percentage was calculated. If that was above 70%, then it was labeled as abnormal.

Table IV
WBC Concentration and HOS Test

WBC Conc Mill/ml	Normal H.O.S. n=28	Abnormal H.O.S. n=7
0-1	19	-
>1	6	4
>4	3	3

Table V
Sperm Morphology and HOS Test

Abnormal Sperm Morphology %	Normal H.O.S. n=28	Abnormal H.O.S. n=7
0-20	16	2
21-40	7	-
41-60	5	-
61-70	-	-
>70	-	5

Table V shows sperm morphology of normal & abnormal H.O.S. groups. It is quite striking that none of the normal H.O.S. group patients had abnormal morphology of 70% whereas 5 out 7 patients from abnormal H.O.S. group had abnormal morphology of more than 70%. This was found to be highly significant statistically.

Other Parameters like volume, viscosity, agglutination etc. were found to be similar in both the groups. We did IUI on 5 patients of each group. There were no conceptions in abnormal HOS group whereas two conceptions in normal HOS group.

Discussion

Incidence of unexplained infertility in our clinic was 16.7%. This was slightly higher compared to 10% incidence reported by I.R.R. in a similar study conducted in 1989. [ICMR bulletin 1996]. They have reported abnormal H.O.S. test and hamster egg penetration essay result in unexplained infertility to be 9/29 or 31%. But we have slightly lower incidence of 5/25 or 20% of abnormal H.O.S. It was found that samples having abnormal HOS had significantly high (I mill./ml.) particulate debris suggestive of infection which is known to affect sperm function & membrane integrity. Hence long term antibiotic treatment is required. The total sperm count was found to be lower in abnormal HOS groups. But Morphology when studied more strictly & carefully was found to be significantly abnormal in abnormal H.O.S. group as compared with normal H.O.S. group. So careful morphology gives similar prognostic value of semen analysis, however should be supplemented with sperm function tests which are relevant.

HOS is a sperm function test which has a good prognostic value for results of ARTs. Various sperm function tests are described. There is a need for upgrading and standardizing various laboratories. So with the help of trained, skilled pathologists it is possible to do these tests before proceeding for various ART procedures. However H.O.S. is a simple, rapid, less cumbersome test which requires only basic laboratory support and skill. Therefore even a clinician can do this test on his own patient's semen samples. Surface membrane properties are very important in normal gamete fusion.

Brief Review of some other Sperm Functtion Tests:

1. Gelatin Slide test: Acrosomal function test (Gopalkrishnan et al, 1995).
   This tests the ability of acrosomal enzymes to dissolve protein (eg. Gelatin coated on the slide) is seen under microscope as 'haloes' of dissolved

gelatin around sperm heads, when treated with suitable reagents.

2. N.C.D. test: Nuclear Chromatin Decondensation Test: [Gopalkrishnan et al-1991]
   This tests the ability of the sperms to form male pronucleus by decondensation of nuclear chromatin.
3. SMAI test: Sperm Mitochondrial Activity Index test [Gopalkrishnan et al-1990]
   This tests the ability of spermatic mitochondrial enzymes to bring about good motility of sperms.
4. A.O. test: Acridine Orange staining of spermatozoa. I Tejada et al, 1984]
   This test detects the ability of sperm nuclear chromatin to resist denaturation with the help of fluorescent stain.

All the above tests are useful however need special stains & reagents, they are more time consuming & should be done in proper laboratory set up. H.O.S. test gives overall membrane integrity, longevity, so gives a fair idea about the prognosis.

Osmolarity of semen in fertile men ranges between 360-380M osmol (velasquez et al, 1977). Normal Osmolarity of semen is an important factor for successful fertilization. The osmolarity of the HOS solution is about 150 M osmols.

Recently at Loma Linda University in California, Chan et al, 1996 reported a new method combining the supravital stain [Eosine Y] test with the hypoosmotic sperm swelling test (VHOS) which reduced the number of false positive results from HOS tests. We eliminated this possibility by doing the initial coiling and subtracting it from the post HOS coiling.

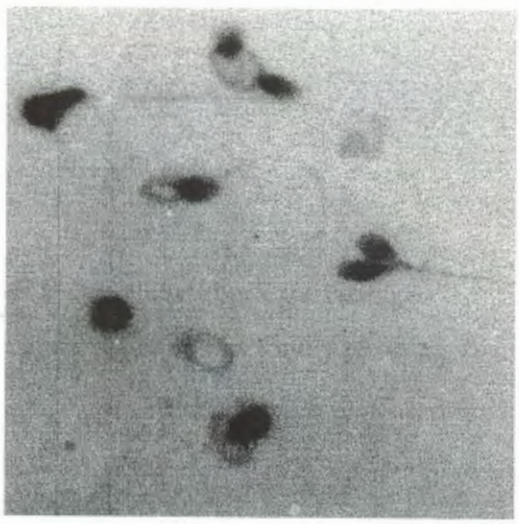

Fig 1: Microphotograph showing post HOS coiled tails

A consideration of the hypoosmotic swelling test in combination with the sperm viability in hypoosmotic solution will enhance the detection of differences in membrane properties on the sperm surface. Sperm specimens failing both the VHOS and HOS tests have been associated with poor fertilizing ability and poor in vitro fertilization outcome. The tests will help identify the specimens requiring special sperm processing. Percoll gradient method should be avoided as it may further damage the weak acrosomal membranes.

Conclusion

Our study emphasizes that in the work up of unexplained infertility, sperm function tests have a definite place. Even though the tests do not have much therapeutic value, they have a good prognostic value. 20% abnormal sperm function assessed by H.O.S. test in this study calls for doing this test and perhaps other relevant tests as a step before selecting the ART protocol for the patients with unexplained infertility. ICSI may be better choice for patients with abnormal sperm function tests.

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