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Research Article

Sperm penetration assay and its correlation with semen analysis parameters

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ABSTRACT

Background: Aim of current study was to determine whether the Sperm Penetration Assay (SPA) can be used as a test to discriminate the infertile male from fertile one. We have also correlated the SPA with semen analysis.
Methods: Sperm characteristics namely Semen analysis and the sperm penetration assay were tested in 44 infertile and 10 fertile men. Sperm penetration assay was determined by using zona free hamster eggs.
Results: With decreasing spermatozoa concentration in the semen there was significant decrease in percentage penetration of zona free Hamster eggs ($p<0.001$). There was decrease in Sperm penetration assay with deteriorating progressive sperm motility ($p<0.05$) and no consistent relationship appeared between the sperm morphology and the Sperm penetration assay ($p>0.05$).
Conclusions: The Sperm penetration assay could discriminate the infertile group from fertile group significantly ($p<0.001$). The test appeared to be highly reproducible and probably identifies a truly infertile male.

Keywords: Sperm penetration assay (SPA), Semen analysis, Infertility, Zona free, Hamster oocyte

INTRODUCTION

The ultimate evidence of the fertilizing ability of a spermatozoon is to yield pregnancy. The process of spermatogenesis, quality of the ejaculate, sperm count, sperm concentration, functional properties of the spermatozoa like motility morphology, viability etc. have been evaluated in depth, in male infertility.[1,2]

Variability in each of these parameters may have a different effect on the sperm fertilizing potential. The absolute predictive value of the so-called 'basic' semen analysis is relatively poor in relation to fertility potential through either spontaneous conception or following assisted conception treatment.[3,4] This directs one to contemplate about another test which can actually demonstrate the fertilizing potential of a sperm. Assessing the fertilizing ability of the human sperm would be very worthwhile yielding meaningful information. The use of human eggs will be both unethical and impractical, hence a suitable alternative would be, the substitution of human eggs by animal eggs.[5]

Hamster oocytes are unique in the sense that they present no barrier to fertilization by spermatozoa of any mammalian species.[5] This cross-species fertilization in vitro has evolved to test the fertilizing ability of the capacitated sperms as it evaluates several aspects of sperm physiology including sperm capacitation, acrosome reaction, sperm-oolemma binding and fusion and sperm head decondensation.[6,7]

The sperm penetration assay (SPA) in the hamster oocytes will provide the information about the qualitative aspect of the spermatozoa and therefore it cannot be a replacement of the conventional semen parameters, however this bioassay may be utilized to provide additional information about the sperm in the semen. The possible uses of zona free hamster oocyte test[5] in infertility are adjunct to semen analysis, selection of sperm in a donor programme, prior to human IVF procedure, evaluation of effect of treatment of the male partner, chromosomal analysis of the sperm and many more.

Several authors suggest that SPA could be a useful predictor of male infertility.[8,9] Corson in 1990 has shown that SPA is a more sensitive indicator of fertility than any other seminal parameters.[10] However, there is divergence of opinion regarding the efficacy of SPA in evaluation of infertility and widely differing results have been shown by different investigator.[11,12] Stenchever et al. found that in almost all cases of unexplained infertility, where no female factor was present, the problem could be assigned to the man as evidenced by an abnormal SPA.[13] SPA may be a useful technique for predicting appropriate treatment options. The SPA allows an important differentiation between patients who can have conventional in vitro insemination- IVF and those who require intracytoplasmic sperm injection-ICSI.[3,14]

The test is still being tested and evaluated for its potential clinical application because it is very difficult to standardize due to multiple variables at several steps in the procedure. However, SPA has drastically altered the approach regarding evaluation of infertile couple because it can be used clinically to provide a couple some estimate as to their prognosis for future fertility.

The objective of this study is to determine whether the SPA can be used as a test to discriminate the infertile male from fertile one. We have also tried to correlate the SPA with conventional semen parameters.

METHODS

The present study has been conducted in the department of Obstetrics and Gynecology in concert with the Department of Microbiology, Institute of Medical Sciences, B.H.U., India. The study was approved by the Institutional Review Board.

The couples were registered in infertility clinic. The control Group A consisted of 10 fertile couples (husbands having fathered a child within last 3 years) and Group B included 44 infertile couples consisting of 32 cases of primary infertility (wife has never conceived) and 12 cases of secondary infertility (wife previously pregnant by the same spouse).

Technique of SPA

The present study has been performed according to Rogers with certain modifications.[9]

(i) Preparation of semen sample

The semen samples were collected after 3 to 5 days abstinence, by masturbation. Seminal fluid analysis was performed on each sample according to guidelines by WHO (1999).[15] The semen sample was then washed thrice in Biggers, Whitten and Whittingham (BWW) medium by centrifugation at 600 x g for 5 minutes and the final concentration was adjusted between $1x10^6$ to $1x10^7$ sperms per ml. Washed sperms were preincubated in air and 5% CO_2 for 16-18 hours at 37°C.

(ii) Retrieval of hamster oocytes

The female Golden Syrian hamsters of 8-12 weeks age were super ovulated by injecting 30 I.U. i.v. HMG on day 1 and 30 I.U. HCG on day 3 intraperitoneally. The animals were then sacrificed after 15-17 hours of HCG injection and the oviducts removed. The oocytes were teased out of the oviducts using insulin syringe with a 27 G needle. The oocytes were washed twice in the same medium by centrifugation at 600 x g for 5 minutes. To it 0.1% hyaluronidase solution was added and dispersed during a 5-10 minute period to allow removal of cumulus cells. The dissolution of zona pellucida surrounding each oocyte was brought about by adding 0.17% trypsin solution to eggs for 60 seconds. Oocytes were then washed again three times in the same medium.

(iii) Coincubation

Coincubation of 100 microlitres of preincubated sperms with 40 microlitre of the solution with uniformly suspended eggs was done at 37°C for 2 hours. Eggs were washed again with BWW. The eggs so inseminated were transferred to a glass slide pressed with a cover slip resting on a Vaseline paraffin mixture and examined under phase contrast microscope at 400X magnification. At least 20 eggs were examined per sample for the presence of swelling of heads of the sperms with a tail in the egg cytoplasm.

$$\text{Percentage penetration (\%)} = \frac{\text{No. of eggs with swollen heads}}{\text{Total no. of eggs inseminated}} \times 100$$

$$\text{Fertilization Index} = \frac{\text{No. of swelling heads}}{\text{No. of eggs examined}}$$

Statistical analysis: The sensitivity, specificity and predictive value of negative and positive tests were calculated as described by the Chard and Lilford.[16] Fischer exact and t-test were used at appropriate places to analyze the data.

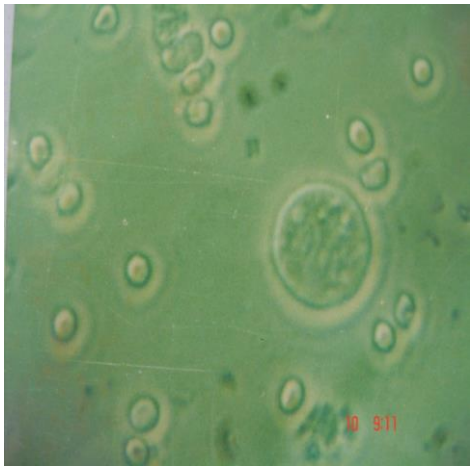

Figure 1: Sperms penetrating the hamster oocyte.

RESULTS

The result of this experiment showed that discrimination between the fertile (Group A) and infertile (Group B) groups can be made by sperm concentration as a parameter of routine semen analysis ($p<0.001$) and to some extent by sperm motility ($p<0.05$). Sperm morphology could not discriminate between the two groups. SPA positivity (>15% penetration) and fertility index however could discriminate these groups very significantly ($p<0.001$) (Table 1).

The sensitivity of the SPA and semen analysis as determined by this experiment were 27.27% and 59.09% respectively. The specificity of both the tests however was 100%. The predictive value of an abnormal SPA was 100% but that of a negative test was 23.81% (Table 2).

Table 1: Correlation of conventional semen parameters, SPA and fertility index in clinically fertile (Group A) and infertile (Group B) subjects.

	Sperm concentration (mill/ml)	Sperm motility (%)	Normal morphology (%)	Percentage penetration	Fertility index
Group A (n=10)					
Range	38-180	50-90	50-90	50-100	0.5-2.2
Average	95.12	72	76	83.5	1.49
Group B (n=44)					
Range	4-108	10-80	10-90	0-90	0-2
Average	36.18	41.59	66.14	37.5	0.605
t-value	4.978	2.268	1.398	6.358	4.565
p value	<0.001	<0.05	>0.1	<0.001	<0.001

Semen analysis according to WHO 1999[15]

Table 2: Sensitivity, specificity and predictive values of a positive and negative SPA and Semen analysis in infertile couples (n=44).

	SPA	Semen analysis
Sensitivity	27.27%	59.09%
Specificity	100%	100%
Predictive value of a positive test (Abnormal SPA)	100%	100%
Predictive value of a negative test (normal SPA)	23.81%	35.71%

*Criteria for SPA - Normal SPA ≥15%; Abnormal SPA <15%[3,16,17]

**Criteria for normal semen analysis (WHO 1999)[15]

The probability of a positive SPA increases with increasing sperm concentration (Table 3) and increasing sperm motility (Table 4). Such correlation was however, not observed with sperm morphology.

The type of infertility correlates with abnormal SPA. There was 31.25% abnormal SPA in the group of primary infertility and only 16.67% of male partners of secondary infertility showed an abnormal test. The increasing duration of infertility showed positive correlation with abnormal SPA. These observations however were statistically insignificant.

Table 3: Sperm concentration as a variable of Semen analysis and its correlation with SPA in infertile couples (n=44).

Sperm concentration (millions/ml)	Number of cases	Negative SPA	
		Number	Percentage
a. <5	3	2	66.67
b. 5-20	15	6	40.00
c. 20-40	13	4	30.77
d. 40-80	7	Nil**	0.00
e. >80	6	Nil**	0.00

**When compared with a, b & c; $p<0.001$

Table 4: Sperm motility as a variable of Semen analysis and its correlation with SPA in infertile couples (n=44).

Motility (%)	Number of cases	Negative SPA	
		Number	Percentage
<20	6	3	50.0
20-49	17	6	35.29
≥50	21	3	14.28

DISCUSSION

We have found significant differences in the sperm penetration ability in populations of fertile and infertile men which is in conformity with the findings of other workers.[9,17] The mean percentage penetration was 37.5% in the infertile population while it was 83.5% in the control group.

Similarly the mean fertility index in infertile group was 0.65 as compared to 1.49 for the control group. Our results tally with Rogers et al in 1979 and Karp et al 1981.[7,18] However, in our series the mean percentage penetration was much higher in the infertile group (37.5%) such high rates in our results are probably due to the inclusion of couples with unexplained infertility and secondary infertility with near normal semen parameters and fertilization data.

In our study we have found significant correlation between the SPA and sperm concentration ($p<0.001$) and progressive sperm motility ($p<0.05$). With diminishing concentration or motility of sperms in semen, the SPA score decreases. We have however not found any significant correlation between the sperm morphology and the SPAs (Table 1).

It has been suggested that although individual sperm parameters have individually as well as together been correlated with clinical infertility, heterogeneity in each of these parameters is characteristic of human semen and this is the reason why accurate determination of the fertilization capacity of any semen sample is difficult.[19]

In our study the sensitivity of SPA was 27.27% while specificity was 100%. The predictive values of an abnormal and normal test were 1.00 and 0.238 respectively (Table 2). In most of the studies the specificity and predictive value of an abnormal SPA was 100% while the sensitivity varied from 28% to 100% and the predictive value of a normal test varied from 0.33-1.00.

The reason for such diversity of results in various studies is probably due to variation in the definition of a known fertile/infertile male as well as lack of any universal criteria regarding fertilization data in each of these groups. We have taken <15% penetration rate as a negative SPA as in previous studies.[3,17,18]

There should be a consensus about definitions assigned to normal and abnormal before the results of different studies could be compared. The technique also needs to be standardized since there are multiple factors at interpretation of the results which may affect the SPA. Until large studies on semen samples from general fertile population are under taken discrete definitions and range may not be laid down. It is also possible that with a large control group the present concept of normal range may be altered.[13]

The precise efficacy of the SPA can be determined only when long term follow up is undertaken and a final conclusion determined regarding the association of infertility with a low or negative penetration rates and whether the potentially fertile male eventually impregnates his life or not.

CONCLUSION

To get better insight into the problem of infertility, the SPA has evolved as the test to supplement the conventional semen analysis to define the precise functional capability of the spermatozoa. It is clear that an abnormal SPA can influence the management of infertile couples and assisted fertilization techniques with donor spermatozoa with positive assay may be utilized.

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