SPERM FUNCTION TESTS - DO THEY PREDICT FERTILITY ?

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SUMMARY

Sperm function tests were studied in 100 cases over a period of 2½ yrs from Sep' 95 to Jan' 97. These tests included viability, hypoosmotic swelling, aniline blue staining and acrosome reaction. Sperm function was compared in oligospermic and normospermic samples. These tests were assessed before and after swim up to determine the effect of swim up of these tests. The presence of varicocoele was noted and sperm function in patients with and without varicocoele was compared. There was no significant difference between the mean values of sperm function tests in oligospermic and normospermic individuals. Swim up procedure significantly enhanced sperm function. Normal viability increased from 47% to 76% and HOS increased from 90% to 95%. There was no significant difference in sperm function in patients with varicocoele and without varicocoele. Viability in men with varicocoele was 50% and in men without varicocoele was 46%. Aniline blue in men with varicocoele was 75% and in men without varicocoele was 87%. The sensitivity and specificity of viability was low but the positive predictive value was high (91%). The sensitivity of hypo-osmotic swelling, aniline blue and acrosome reaction was low but the specificity and positive predictive value was 100%.

INTRODUCTION

Traditionally, semen analysis including sperm concentration, motility and morphol-

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ogy is used as a fundamental indicator of male fertility. However the results do not provide precise diagnostic or prognostic information about male fertility. Some couples conceive quickly, despite abnormal semen analysis results, and converesely men with normal semen analysis results are infertile.

In an attempt to define more accurately what constitutes a normal sperm, other tests have been developed. These test for membrane integrity, nuclear maturity and acrosome status. This study is aimed at assessing the efficacy of these tests in detecting infertility in a male who would be classified as fertile by the standard tests. This is important in light of the development of new techniques of artificial reproduction such as intra-cytoplasmic sperm injection (ICSI). Here sperm assessment must be precise, as it is obvious that repeated attemts at artificial reproduction utilising a sperm which is in fact defective would be futile.

In this study, results of sperm function tests were compared in both oligospermic and normospermic individulas. The effect of swim up technique on sperm function and results of sperm function tests in men with varicocoele were also studied.

AIM

1. To compare sperm function tests in oligospermic and normospermic individuals.
2. To compare sperm function tests before and after swim up procedure.
3. To determine the effect of varicocoele on sperm function.

MATERIALS AND METHODS

Investigations were performed on the semen of 100 husbands whose wives underwent investigations for infertility at the Manipal Assisted Reproduction Centre at Kasturba Medical College Hospital, Manipal, between September 1994 and September 1996.

In both normospermic and oligospermic samples, routine tests and sperm function tests were done. Routine tests consisted of count, motility, and morphology.

Viability is assessed after staining with Eosin Y. A drop of semen is placed on a slide. One drop of 0.5% aqueous yellowish eosin solution is added and covered with a cover slip. After 1-2 minutes, the spermatozoa stained red can be distinguished from the unstained spermatozoa. More than 75% unstained spermatozoa (viable) is normal. Viability tests the stuructural integrity of the sperm membrane. A sperm may be immotile but still viable (Liu et al 1988).

Hypoosmotic swelling is done as follows. Mix equal volume of aqueous solutions of fructose (1.47%) and sodium citrate (2.7%). Keep the mixture at 37°C for 10 min. Add 0.1 ml of semen sample and incubate at 37°C for 30 min. The mixture is taken and smeared on a clean glass slide and covered with a cover glass. The percentage of coiled tails was counted. > 60% is indicative of a normal sample. Hypoosmotic swelling tests the physiological integrity of the sperm membrane (Jeyenderan 1984).

The procedure for aniline blue staining is as follows. Semen sample is washed in phosphate buffered saline and smeared on a slide. It is fixed with 3% gluteraldehyde in phosphate buffered saline. The slide is then stained with 5% aqueous aniline blue in 4% acetic acid. >80% of unstained sperm is a normal sample. Aniline blue tests for the nuclear maturity (Liu et al 1988).

The sample was then subjected to sperm wash and swim up technique and viability and hypoosmotic swelling repeated.

Acrosome reaction was done only after swim up as follows. Spermatozoa were washed twice in phosphate buffered saline to remove culture medium, and air dried smears were treated for 30 sec with methanol to permeabilize the sperm membranes, followed by 30 min of incubation at room temperature in phosphate buffered saline containing 50 ug/mL of P. sativum agglutinum conjugated with fluorescin isothiocyanate (FITC). The incubation were carried out in moisture chambers at room temperature for 30 min. The smears were then washed for 10 min in excess water and air dried and examined. A Leitz Ortholux epifluorescent microscope was used with a UGI excitation filter, giving excitation from the 365 mm emission of the mercury arc lamp, and the 510 K guard filter normally used for fluorescin emmission (Tesarik & Mendoza, 1993).

A sperm that did not undergo the acrosome reaction was identified by a fluorescent head with a clear halo surrounding it. An acrosome reacted sperm did not fluoresce. More than 80% acrosome reacted spermatozoa is normal.

ANALYSIS

Out of 100 semen samples, 73 were normospermic (count > = 20 mill/ml) and 27 were oligospermic (count < 20 mill/ml).

The mean and standard deviation of all the sperm parameters in oligospermic and normospermic samples are given in Table I. It can be seen that the mean for all the variables fall within the normal range except for viability in which the mean is less than 75% in both oligospermic and normospermic semen. This indicates that there is no significant difference between the sperm parameters in oligospermic and normospermic individuals. Also an oligospermic sample may have normal routine and sperm function parameters samples may have normal routine and sperm function parameters and the presence of low count alone does not indicate infertility.

Each of the sperm function tests were further correlated with count, motility, and morphology. The results are shown in Table II. It can be seen that no routine semen parameter correlates with all the sperm function tests. Although motility correlates with viability. HOS and aniline blue it does not correlate with the acrosome reaction. Thus normal routine sperm parameters do not imply normal sperm function.

In order to assess if there was any improvement in sperm function

TABLE I
SPERM PARAMETERS IN OLIGOSPERMIA AND NORMOSPERMIA

	Oligospermia		Normospermia	
	Mean	Standard Deviation	Mean	Standard Deviation
Morphology	41:30	17.67	54.23	14.79
Motility	59.19	18.37	68.95	12.58
Viability	64.41	20.20	71.37	10.99
HOS	68.04	16.05	76.30	9.35
Aniline Blue	83.78	15.07	88.37	10.70
Acrosome Reaction	80.89	22.02	93.48	10.41

TABLE II
CORRELATION BETWEEN SFT AND OTHER SPERM PARAMETERS

	Count	Motility	Morphology
Viability	-	+	-
HOS	+	+	-
Aniline Blue	-	+	+
Acrosome Reaction	-	-	-

TABLE III
BSU AND ASU RESULTS OF SPERM FUNCTION BEFORE & AFTER SWIM UP

	Before swim up		After swim up	
	Normal	Abnormal	Normal	Abnormal
Viability	47%	53%	76%	24%
Hypo-osmotic swelling	90%	10%	95%	4%

TABLE IV
EFFECT OF VARICOCOELE ON SPERM FUNCTION

	Varicocoele Absent N = 82	Varicocoele Present N = 18	P Value	Significance
Viability	38 (46.3%)	9 (50%)	0.07931	0.77823
HOS	76 (93%)	14 (78%)	3.69348	0.05629
Aniline Blue	26 (87%)	6 (75%)	1.11497	0.57265
Acrosome Reaction	25 (86%)	6 (86%)	0.64887	0.96044

TABLE V
PREDICTIVE VALUE OF SPERM FUNCTION TESTS

	Sensitivity	Specificity	PPV	NPV
Viability	60%	61%	91%	18%
HOS	13%	100%	100%	14%
Aniline Blue	11%	100%	100%	14%
Acrosome Reaction	10%	100%	100%	10%

following swim up procedure the results of sperm function before and after swim up were compared. The before swim up and after swim up results of the viability and hypoosmotic swelling tests are analysed in Table III. It can be seen that swim up procedure significantly enhanced sperm function.

Sperm function tests were also compared in patients with varicocoele and without varicocoele and the results are shown in Table IV. It was found that there was no significant difference between sperm function tests in patients with or without varicocoele.

Out of the 100 patients, 13 conceived within the 2 1/2 year period of the study. Of these 13 patients, 1 had a low count of 11 mill/ml and poor viability, and another had only poor viability

with a normal count. The remaining 11 patients all had normal count and viability. All 13 patients had normal aniline blue, hypo-osmotic swelling, and acrosome reaction tests.

The sensitivity and specificity of each of the sperm function tests was determined. The results are shown in Table V. It can be seen that for viability, the sensitivity and specificity are similar, but the positive predictive value is high. For aniline blue, hypoosmotic swelling, and acrosome reaction, the specificity is 100% and positive predictive value for infertility is also 100% as no semen in which these tests were abnormal have resulted in conception.

DISCUSSION

Liu and Baker (1992) evaluated all the sperm function tests to determine which is the most important for fertilization in vitro by a process of logistic regression analysis. Liu et al (1988) found a low significance of viability, moderate significance of acrosome reaction and no significance of hypoosmotic swelling and aniline blue to IVF rates. Similarly in the present study, the pregnancy rate in samples were 18% with normal viability, 14% with normal hypoosmotic swelling, 14% with normal aniline blue, and 10% with normal acrosome reaction.

Thus both, in the study done by Liu and Baker (1992) and the present study, the correlation of normal sperm function and pregnancy rates is low.

This suggests that the negative predictive value of these tests is low, which means that a normal sperm function does not mean that the sample is fertile and definitely will result in pregnancy.

In a study done by Check & Epstein (1988) to determine the predictive value of hypoosmotic swelling test in fertility, 88% with normal HOS conceived but none of the patients with abnormal HOS conceived. In the present study also none of the patients with abnormal HOS conceived. This demonstrates the high positive predictive value of abnormal hypo-osmotic swelling for infertility. The present study also demonstrates the high positive predictive value of aniline blue and acrosome reaction tests.

CONCLUSION

It is concluded that a normal sperm function is not predictive of fertility. However a sample with an abnormal sperm function has a very poor prognosis in terms of fertility. Viability is a simple test that can be done as a screening procedure and confirmation can be done by hypo-osmotic swelling, aniline blue and acrosome reaction tests. All the tests have high positive predictive value for infertility. The swim up procedure enhances sperm function. The presence of varicocoele is not associated with poor sperm function.

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