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COMPARATIVE STUDY OF SPERM VITALITY IN FERTILE AND INFERTILE MALES

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ABSTRACT: **AIM:** To study sperm vitality in fertile and infertile males, analyze and correlate. **MATERIALS AND METHODS:** Total 100 semen samples were studied in each of the experimental and control groups. Sperm vitality was determined by the dye- exclusion test. **RESULTS:** The percentage of vital spermatozoa in the control group was more than 74% in all the cases which was within the normal limits. However only 65 cases out of the total 100 in experimental group, showed the vitality percentage within normal limits (>74%). In 35 samples of the experimental group, the vitality percentage was in the range of 60 to 73%. The mean vitality percentage of the experimental group was 82 ± 6.78 and that for the control group was 84.45. The difference in the mean and median values of vitality in the two groups was statistically significant. **CONCLUSION:** Sperm vitality was highly significantly more in semen samples of fertile males than those of infertile males.

KEYTERMS: Infertility Male, Semen Analysis, Fertility, Pregnancy.

MESHTERMS: Andrology, Fertility, Infertility, Infertility Male, Pregnancy, Semen, Semen Analysis, Sperm, Spermatozoa.

INTRODUCTION: Infertility is the problem faced by mankind since its evolution on the earth. Infertility of unknown etiology is a condition of 15-20% of the infertile population for which there is no rational therapy.¹ Semen analysis is a part of the standard diagnostic routine investigations for infertile couples. In the literature^{2,3} there is controversy regarding the 'lower limits' of criteria used to classify a sample as fertile. Prediction of male fertility potential on the basis of semen quality remains desirable but an elusive goal. Hence this study was carried out.

METHODS: The present work was carried out by selecting the patients visiting (1) A Private pathology laboratory and infertility centre and (2) the Reproductive Biology Unit of a public hospital in a metropolitan city.

Total 100 semen samples were studied in each of the experimental and control groups, within the age group of 20-35 years. Control group comprised of semen samples in 100 proved fertile^{4,5} males within the age-group of 20 to 35 years. Experimental group comprised of semen samples in the husbands of 100 infertile couple^{6,7} in whom there is no detectable cause of infertility in wives.

Sperm vitality⁸ was determined by the dye- exclusion test⁷. The test is based on the principle that dead cells with damaged plasma membrane take up certain stains. One drop of Eosin solution was mixed with one drop of fresh semen sample on a slide and it was examined at 400x after 30 seconds. All the slides were examined under the binocular research microscope, having photo micrographic attachment, available in the department.

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ETHICS: All the procedures followed in this study are in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration.

STATISTICS:

Sr. no.	Number of sperms seen/400xfield	No. of cases	
		Experimental	Control
1	60	1	0
2	61 to 69	18	0
3	70 to 73	16	0
4	74 to 77	10	17
5	78 to 81	18	22
6	82 to 85	14	28
7	86 to 90	14	21
8	91 to 95	9	12
Total		100	100

Table 1: Showing the percentage of unstained spermatozoa observed in the slides of vitality staining and the number of cases falling in those ranges

Sr. no.	Study groups	Mode	Median	Mean $\pm$ SD ml	'P' value
1	Expt	80	80	82 $\pm$ 6.78	S
2.	Ctrl	85	85	84.45 $\pm$ -6.31	P<0.05

Table 2: Showing the mode, median and mean values of the percentages of unstained spermatozoa in the slides of vitality staining of the experimental and control group

RESULTS: The percentage of vital spermatozoa in the control group was more than 74% in all the cases which was within the normal limits. However only 65 cases out of the total 100 in experimental group, showed the vitality percentage within normal limits (>74%). In 35 samples of the experimental group, the vitality percentage was in the range of 60 to 73%. The mean vitality percentage of the experimental group was 82 $\pm$ 6.78 and that for the control group was 84.45. The difference in the mean and median values of vitality in the two groups was statistically significant.^{9,10,11}

REVIEW OF LITERATURE: Bostofte, E. Serup, J. and Rebbe, H.¹² (1982) studied the relationship between morphologically abnormal sperms and pregnancies obtained during a 20 year follow-up period. They noted various morphological abnormalities and staining abnormalities in the sample. There was significant correlation between increased percentage of abnormal sperms and decreased chances of obtaining living children. 72% men with 25% abnormal sperms obtained living children while 28% could not. There was no statistical relation between abnormal sperms and abortions.

Aitken, R.J., Best, F.S.M., D.W., Djahanbakch, O. and Lees, M.M.⁸ (1982) correlated the fertilizing capacity in normal fertile men with semen analysis. They studied semen of 35 men of presumed fertility within the range of 28 to 45 years of age. In the semen examination sperm motility,

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total count, percentage, normal morphology, percentage motility, and sperm volume were studied. Washed sperms were incubated to effect capacitation.

They also studied other parameters related to movement characteristics like percentage of motility, the total mean velocity, the percentage of progressive spermatozoa, the mean progressive swimming speed, the concentration of progressive spermatozoa, the percentage of straight swimming spermatozoa, the concentration of straight swimming spermatozoa, the concentration of amplitude of lateral and displacement, the percentage of progressive straight swimming spermatozoa, and the percentage of progressive unclassified spermatozoa. They further extended the study and calculated a limited number of combined indices of sperm quality like $mv = \text{percentage of motility after capacitation} \times \text{total mean velocity after capacitation}$, $mvn = \text{percentage of motility after capacitation} \times \text{total mean velocity after capacitation} \times \text{percentage of normal morphology of sperm suspension}$, $mvnc = \text{percentage of motility after capacitation} \times \text{total mean velocity after capacitation} \times \text{percentage of normal morphology of sperm suspension} \times \text{sperm density in original sample}$.

They detected sperm density more than $20 \times 10^6/\text{ml}$, sperm morphology more than 40% normal, and percentage of motile sperms more than 40% normal. The sperm movement characteristic exhibited 57% motility and $22.8 \mu/\text{s}$ velocity. The capacitation process raised motility by 34%. On zona-free hamster oocyte penetration test in the mean penetration rate was 44%. Most fertile sample was exhibiting 40% penetration rate while the least fertile sample could achieve 30% penetration. Authors emphasized the requirement of more sensitive semen analysis tests capable of identifying subfertile males. They are of the opinion that the zona-free hamster egg penetration test is a sensitive test to determine the ability of human spermatozoa to fertilize living, human ova in vitro. They concluded that the routine semen analysis test is not a reliable index of fertility.

Rogers, B.J., Bentwood, B.J., Campen, H.V., Helmbrecht, G. Soderdahi, D. and Hale, R.W.¹³ (1983) determined the parameters in the routine semen analysis that can be used as indicators for fertility and infertility. They studied the relationship between morphology, count, motility, and penetrating ability of human semen specimen using zona-free hamster eggs. The mean sperm count was $108 \times 10^6/\text{ml}$, motile sperms 61%, average proportion of normal forms 64%, and the mean penetration value was 69%. There were in the duty who were clinically infertile. This group showed mean sperm count $42 \times 10^6/\text{ml}$. This count was 60% lower than that of the fertile group. In the infertile group the mean motility was 45% and the proportion of normal forms was 32%. This group demonstrated in vitro poor fertilization value (3.2%). The sperm count, the most commonly used diagnostic semen parameter, when correlated with fertilization value (ZFHEP) showed poor correlation, however the correlation of morphology with fertilization value showed the best result. In infertile group 74% men with normal count and motility had less than 50% normal forms. They concluded that a fertility problem in the male could be diagnosed most reliably from the presence of poor morphology, specifically fewer than 50% normal forms.

Gopalkrishnan, K. Padwal, V., Meherji, P.K. Kokral, J.S., Shah, R. and Juneja, H.S.¹⁴ (2000) analyzed semen samples of 32 couples with early pregnancy loss (more than 3 first trimester abortion) and compared the study with 51 couples with proven fertility. The mean values for volume, sperm count, motility, morphology, viability and percentage of total sperms having normal morphology were not significantly different than those of the control. The percentage of head abnormalities was significantly more (41%) when compared with control (22%). The seminal viscosity in the experimental group was significantly different from that of the control. The alteration in viscosity may be associated with anti-sperm antibodies and it alters the motility and there-by the

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function. The high seminal fluid viscosity was due to seminal vesicle insufficiency, leading to relatively high prostatic component with high zinc level, causing increased chromatin stability.

Electron microscopic examination showed that the chromatin material was either not compact or partially compact and had an irregular nuclear border with large vacuoles while control group showed compact homogeneous chromatin material. In these couples they also evaluated genetic, endocrine, anatomical and auto-immune factors and subclinical infections. They claimed that this is the first study detecting loss of chromatin integrity as a possible contributing factor from males to early pregnancy loss inspite of normal seminogram.

DISCUSSION: Immotile sperms may be dead or just dormant. This distinction is essential while using the sperms for in-vitro fertilization. In the experimental group mean immotile sperm number was 21% while the vitality was approximately 82%. This indicates that 18% sperms were dead sperms (100 minus 82) while 3% (21 minus 18) were the immotile but living sperms (dormant). In the control group mean immotile sperm number was approximately 20% while the vitality was 84% approximately. This indicates that the 16% sperms were dead sperms (100 minus 84) while 4% (20 minus 16) were the immotile but living sperms (dormant) Though the difference in vitality in both the groups appears to be marginal it is statistically significant. Infertility is associated with less vitality percentage or more immotile sperm number. This study can be carried out in a cost effective and economical setup. It does not require high end instruments and can be easily and routinely carried out in remote centers.¹⁵ Moreover, viability is important than morphology, in views of recent advances where ICSI and IVF procedures are in vogue. This study reinforces basic semen analysis.¹⁶

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