USE OF PHASE CONTRAST MICROSCOPY FOR THE EVALUATION OF HUMAN SPERM MORPHOLOGY

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SUMMARY

Morphology of human spermatozoa was analysed by bright field microscopy and phase contrast microscopy. Sets of 200 spermatozoa each were analysed from 563 semen samples obtained from men to be enrolled for IVF-ET programme. The parameters studied were total abnormal spermatozoa : abnormalities of head, midpiece and tail. The results obtained from the two microscopy systems showed good correlation (r=0.911). The results demonstrate that the easy operation and reduced reportiong time and equal predictability of phase contrast system has definite advantage over bright field microscopy.

INTRODUCTION

The morphological evaluation of spermatozoa is one of the most critical, analytical and quantitative method to judge the quality of semen sample (Gopalkrishnan et al. 1992). Various authors have demonstrated significant correlations between sperm abnormalities and infertility in humans (Amann, 1981, Freund, 1962; Smith et al. 1977, Macleod 1970 and Gopalkrishnan & Anand Kumar 1990). Different microscopic systems like bright field, phase contrast, interference contrast systems have been used (Schmassmann et al. 1979 and Katz et al. 1986) apart from sophisticated computer assisted techniques. Phase contrast is less expensive than differential contrast system and computer assisted automated systems. It is also easier to operate. There are a number of factors which cause variability in morphological evaluation. Among these are the methods of preparing the sample

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Accepted for Publication in July' 96

(unavoidable for bright field microscopy), the microscopy system used, the experience of the operator who analyses the sample, and number of cells analysed. The present study is mainly focussed on the advantages of phase contrast microscopy over the routine bright field optics for evaluation of sperm morphology.

MATERIALS AND METHODS

Semen samples were obtained from men (n=563) enrolling for IVF-ET programme. Samples were collected by masturbation into sterile beakers. After liquefaction, 30 ul was taken, a smear was prepared, allowed to air dry, fixed in 1:1 alcohol ether for 10-20 minutes and stained by Papanicolaou's staining (Papanicolaou, 1942). The morphological analysis was done under oil with 100 X objective. 10 ul drop of semen was taken in a test tube to which diluent (50 g $NaHCO_3$ 10 ml of 35% (V/V) formalin and distilled water were added to give a final volume of 1000 ml) was added. The sperm suspension was taken on a routine haemocytometer and covered with coverslip. The sperm count was done under phase contrast microscopy. Morphological analysis of spermatozoa was done using the same haemocytometer preparation. The morphology was assessed under phase with 40 X objective (Reichert 'Polyvar' microscope). The criteria used for morphologically normal sperm was as described in the WHO Manual (World Health Organization, 1992). 200 spermatozoa were analysed for each microscopy system and for each sample. All smears were assessed by a single observer. Stained smears were seen by bright field optics. Morphological analysis of head, midpiece, tail abnormalities and total abnormalities were done and the results expressed in percentage.

OBSERVATION AND RESULTS

The particulate matter like WBC, germ

Table I

MEAN AND STANDARD DEVIATION OF THE PERCENTAGE OF ABNORMAL SPERMATOZOA AND LINEAR CORRELATION COEFFICIENT VALUE (r) BETWEEN RESULTS OBTAINED USING PHASE CONTRAST AND BRIGHT FIELD MICROSCOPY[1].

Abnormalities	Phase Contrast	Bright Field	Value of r
Head	25.91 ± 29.66	29.69 ± 15.273	0.77
Midpiece	27.51 ± 08.98	25.75[2] ± 08.86	0.66
Tail	22.99 ± 10.03	21.28[2] ± 10.19	0.75
Percentage Abnormal	76.45 ± 14.19	76.70 ± 13.64	0.911

1. Sets of 200 spermatozoa each were analysed from 563 semen samples.

2. $P < 0.05$

cells, epithelial cells and Trichomonas vaginalis were identifiable in the wet smear preparation under phase contrast microscopy. The amorphous particulate matter constituted mainly by the bacterial contamination was graded on a nil- ++++ scale, with phase contrast.

The results of the analysis showed a good correlation between these two microscopy methods (Table 1). Comparison of the percentage of abnormal spermatozoa with determinations for each of the morphological regions of spermatozoa were not significantly different for the two methods of microscopy. The evaluation of sperm quality by the two microscopy systems did not differ. The paired analysis (t-test) showed significant differences ($P<0.05$) only with midpiece and tail abnormalities.

Comparison between the results obtained by phase contrast and bright field showed a high degree of significance. A significant correlation was observed between both the systems ($r=0.9111$) and in abnormalities of different regions of spermatozoa.

DISCUSSION

The study shows that phase contrast microscopy gives comparable result to that obtained with stained smears. This system obviates the need for lengthy staining procedure. Thus it could be recognized as a reliable system for anlaysing sperm morphology a system which is easy inexpensive and above all time saving. The routine study of abnormalities of the shape of spermatozoa is particularly important in analysis of semen (Gopalkrishnan et al. 1992). The identification of structural abnormalities of the spermatozoa specially of head region relates to the acrosomal status which in turn gives us an understanding of the fertilizing capability of spermatozoa in vitro (Gopalkrishnan & Padwal, 1996 and Rogers et al. 1983). Though sophisticated image analysis system have been developed (Gopalkrishnan & Anand Kumar, 1990 and Schmassmann 1979) the cost of purchasing phase contrast system is much lower. This system is slightly more expensive than bright field microscope but this system can be changed to bright field but vice versa is not possible. It would be no exaggeration to say that one would be missing a lot of valuable information by not using this extremely useful tool in appreciating semen characteristics in an unstained state.

Useful observations made by phase contrast includes the grading of amorphous particulate matter. Grades +++ and ++++ are indicative of positive bacterial culture (Gopalkrishnan et al. 1988). Apart from this, the hypoosmotic swelling test (Jeyendran et al. 1984) for sperm plasma membrane integrity and in vitro decondensation test (Gopalkrishnan 1991) for testing the ability of sperm chromatin to decondense in vitro are routinely done by us under phase contrast microscopy. Hence the initial examination of raw sample with phase contrast would indicate what further tests are in order. It is therefore recommended that phase contrast system should be recognised as a reliable system for analysis of seminal fluid specially sperm morphology.

ACKNOWLEDGEMENTS

Authors wish to express their sincere thanks to all clinicians who have referred the patients for semen analysis. We also

acknowledge the help of Mr. D. Balaiah in statistical analysis of the data and Mr. P. Chavan for his secretarial assistance.

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