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MORPHOLOGICAL STUDY OF SPERMS IN SEMEN DONORS

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Abstract:

Background: The sperm morphology is an indispensable element of human semen analysis as it is one of the most predictive measures of fertility potential.Human sperms are pleomorphic in several aspects,many irregular and abnormal forms are present. Various studies have shown a decline in semen quality over past few decades. The sperm morphology in semen donors was determined in this study in order to augment Indian baseline data.

Aims and objectives: The present study was conducted to define normal spermatozoa and to find out various abnormalities of head, midpiece and tail.

Materials and Methods: Sperm morphology was evaluated in 50 samples of semen donors using papanicolaou staining procedure. The morphological evaluation was according to WHO criteria(1999). The defects were classified as per WHO guidelines into head,neck, midpiece and tail defects.

Results: The % of sperms showing normal oval morphology ranged from 59% to 86% with a mean of 74.72 ± 6.97%.The most common type of head defect was amorphous (6.02±3.68). The small, round heads with small acrosomal area and double head types were comparatively rare. Total neck and midpiece defects were in the range of 0-5 with a mean of 1.80±1.32. Most common type of tail abnormality detected was coiled tails with a mean of 5.58±5.14%. Broken tails, multiple tails and hair pin tails were comparatively rare.

Conclusion: The morphological parameters in normal fertile men were comparable to most of the studies conducted in India and were within the normal range.

Keywords : Sperm morphology, semen donors, Papanicolaou stain.

Introduction

The objective of estimating correctly a man's fertility potential has long been of great interest to researchers. Many rites in history demonstrate how old and strong is human desire for fertility. In cases of infertility, by tradition, it is the female who is held accountable. However male reproductive capacity was found deficient in no less than 50% of infertile couples.[1] When a couple comes for investigations for infertility, it is the male partner who should be investigated first. The male investigations are cheap and painless and results are obtained faster.

Semen analysis is a keystone in the clinical workup of the infertile men .The results of this analysis are the basis for important decisions about treatment by artificial insemination, in-vitro fertilization or other methods.Sperm morphology is considered to be one of the most important parameters in determination of the potential fertility of the semen specimen. Its importance is supported by the fact that spermatozoa with abnormal morphplogy cannot easily penetrate the cervical mucus.[2]

For a spermatozoon to be considered normal,the sperm head,neck and tail must be normal. Abnormal spermatozoa are seen frequently in semen analysis and upto 10% of all spermatozoa have observable defects.[3]

The report of Carlsen et al that semen quality in men worldwide has undergone a decline over past few decades was of concern to the scientific community.[4] Furthermore, a no. of studies in Europe have pointed geographical differences in semen quality, probably related to environmental factors,however, ethnic or genetic differences cannot be excluded.[5,6,7] Only a small no. of such studies have been conducted in Asia or other non-western countries.

In view of the absence of sufficient information on Indian men, the present study was undertaken to contribute towards setting of clinical thresholds for sperm morphology in men of proven fertility. This data would be of use in future to identify any trend towards change in semen quality in Indian men.

Materials And Methods

The study was done on 50 samples of semen donors. The donors selected were men whose wives had conceived within 1 year of marriage and had healthy children. The donors with H/O fever cough,malignancy,undergoing any chemotherapy, having varicocele or any swelling of scrotum,addiction to drugs, on hormonal drugs were excluded from the study. Prior sanction was taken from ethical committee and confidentiality was maintained.

Semen analysis was done on samples collected after abstinence of 3-5 days. Wide mouthed sterile plastic containers were used for specimen collection. Then 5-20µl of well mixed semen after complete liquefaction was pipetted out on a clean slide. The feathering technique was used to make smears of semen, but care was taken not to make the smears too thick. The Papanicolaou staining was done.

Sperm morphology was evaluated in papanicolaou stained smears. The individual parts of spermatozoa such as head, neck and midpiece were studied and analysed.

At least 100 spermatozoa were analysed on each smear using videoplan-11 up microscope under 100x oil immersion objective(N.A=1.25) and 10x ocular lens.The morphological evaluation was performed in a systematic way. Several fields were analysed to cover the whole slide. Overlapping spermatozoa and those lying with head on edge were not assessed.

Sperm morphology was classified according to the standard method recommended by the WHO(1999). The spermatozoa was considered normal when it was having no abnormality of head, neck and midpiece and tail. All the normal spermatozoa were assessed and scored and the defects of abnormal spermatozoa per 100 sperms per slide were noted as head defects, neck and midpiece defects and tail defects.

The raw data obtained was statistically analysed. Range, mean and standard deviation were determined for each parameter.

Results

In the present study, the percentage of sperms showing normal oval morphology ranged from 59% to 86% with a mean of 74.72 ± 6.97%(table1). The most common type of head defect was amorphous(Figure 1) with a mean of 6.02±3.68%. The small, round heads with small acrosomal area and double head types were comparatively rare(table2). Total neck and midpiece defects were in the range of 0-5 with a mean of 1.80±1.32(table3). Most common type of tail abnormality detected was coiled tails(Figure 2) with a mean of 5.58±5.14%. Broken tails,multiple tails and hair pin tails were comparatively rare(table4).

Tables

Table 1 :
Distribution of percentage of normal sperms

% Normal sperm	Number of donors	Percentage
55-65	5	10
65-75	14	28
75-85	31	62
85-95	2	4

Mean ± SD normal sperm = 74.72 ± 6.97

Table 2:
Distribution of head abnormalities

Type of defect (in %)	Mean	S.D.	Range
Large	2.06	2.23	0-9
Small	0.72	1.03	0-4
Tapered	2.10	2.58	0-12
Pyriform	1.38	1.83	0-8
Round	0.20	0.60	0-3
Amorphous	6.02	3.68	0-14
Vacuolated	0.96	1.53	0-6
Small acrosomal area	0.16	0.42	0-2
Double head	0.28	0.60	0-2
Total head defects	14.08	6.88	0-29

Table 3 :
Distribution of midpiece and neck abnormalities

Type of defect (in %)	Mean	S.D.	Range
Bent neck	0.80	1.16	0-5
Thick midpiece	0.80	1.01	0-3
Thin midpiece	0.00	0.00	0
Abaxial insertion	0.20	0.45	0-2
Total	1.80	1.32	0-5
Cytoplasmic droplet	2.32	2.81	0-14

Table 4 :
Distribution of tail abnormalities

Type of defect (in %)	Mean	S.D.	Range
Short tails	0.40	0.96	0-5
Multiple tails	0.06	0.31	0-2
Hairpin tails	0.04	0.19	0-1
Broken tails	0.02	0.14	0-1
Bent tails	2.14	2.21	0-12
Coiled tails	5.58	5.14	0-24
Total	8.20	6.13	1-25

Figure 1:
Spermatozoa with bent neck-arrow(PAPx100)
Amorphous head-double arrow
Neutrophil—arrow head

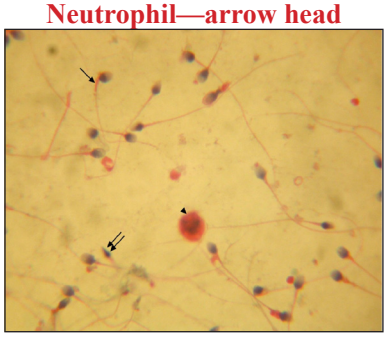
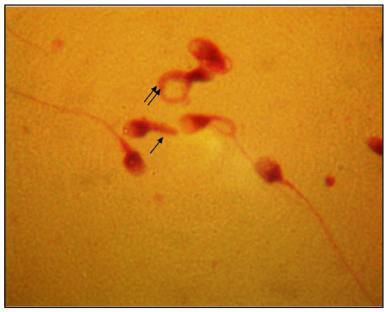


Figure 2:
Spermatozoa with short tail (arrow PAPx100)
Coiled tail –Double arrow



Discussion:

Ideally, semen quality should predict the male fertility potential. However, Tomlinson et al debated the diagnostic value of semen analysis due to difficulties in giving thresholds able to differentiate between fertile and infertile men.[8] Furthermore, semen parameters are considered in different ways on the basis of clinical requirements: as part of infertility investigations or follow-up of infertility treatment, for selection for appropriate method of assisted reproduction, in reproductive toxicology or in contraceptive studies. Several reports describe differences in semen quality between fertile and subfertile groups in order to define clinical cut off values.[6,9] In these and other studies, the reference ranges for semen variables given in WHO manual 1992,1999 were discussed.[10,11] Due to geographical differences, a common set of reference values may not be appropriate to use world wide.[5]

In line with this, it is stated in the WHO manual 1999 that each laboratory should determine its own reference range for each semen variable. The reference ranges given in this manual are "based on clinical experience of many investigators who have studied populations of healthy fertile men". Regarding sperm conc., total sperm count in

the ejaculate and sperm motility, the reference range is the same as in WHO manual 1992 but an interval for sperm morphology is not given. In clinical settings that are ill-equipped to perform detailed semen analysis including a fertilization assay, clearer criteria for diagnosis of male infertility based on sperm morphology would be invaluable as this test is very cost effective also.

The objective of present study was to determine the sperm morphology baseline parameters in proven fertile donors in Indian populations as there are very few published reports in this regard. The result is comparable with findings of the studies conducted in India. Pal et al and Dua and Vaidya reported the % normal oval morphology in Indian population as 72 ± 5.3 and 70.1 respectively.[12,13] The values quoted by Gunalp et al and Haugen et al are much lower than the present study.[9,7] This may be explained by the fact that they used different staining procedures and different sperm morphological classification criteria. The racial variation may be another important factor for the lower values.

The presence of abnormal sperm forms found in our study shows that fertile semen usually contains an important number of abnormal spermatozoa. Historically, male fertility was associated with the proportion of abnormal sperms in semen samples.[14] It was believed that a high proportion of abnormal forms may be a reflection of pathological spermatogenesis. However, the emphasis has gradually shifted and the proportion of normal forms more recently has been used instead to determine the fertility potential of a semen sample.

The % of head, neck and midpiece abnormalities is relatively similar to that found by Panidis et al but considerably lower than that reported by Haugen et al.[15,7] Tail abnormalities were higher as compared to Panidis et al.[15] This discrepancy could be explained by the fact that each author has his own specific way of defining abnormal spermatozoa and another reason could be racial differences. For the sperm morphology and classification of abnormal sperm forms, we have followed the guidelines of WHO 1999.

In view of heterogonous nature of Indian population, climatic differences, it would be worthwhile to conduct a longitudinal study to assess baseline sperm morphology parameters in normal fertile Indian men in different geographical locations.

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