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Basic Staining Methods for Sperm Analysis in Oligospermia - A Study in Tertiary Care Hospital

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Abstract: ***Approximately 15% of married couples have infertility problems. Twenty percent of them originate from men, 30% - 40% originate from both men and women. So, one half of the infertility couples have male - factor associated infertility. [1] Assessment of sperm morphology is the most important criterion for determining the quality of a semen sample. The goal of identifying an ideal technique for sperm staining is to ease the visualization of cells and provide a better identification of the abnormalities. This is an important factor for successful fertilization and early embryonic development in assisted reproductive techniques. [2] The accuracy of sperm morphology assessment depends on careful smear preparation, fixation and staining since these procedures can affect the sperm dimensions significantly. Many staining methods such as Haematoxylin & Eosin, Giemsa, Papanicolau, Eosin - Nigrosin and Leishman have been used for sperm staining. Some of the commercially available stains such as Shorr, Janus Green and Sperm Blue are too expensive to be routinely used in developing countries like India. [3] A few stains may cause a slight change in the measurement values of spermatozoa because fixatives may shrink the cells a little. [4] This creates a need for an ideal, simple, and cost effective staining method which also does not alter the sperm morphology for an accurate evaluation of the same. Many of the methods used to stain semen results in very distinct coloring of the sperm but unfortunately they do not distinguish their individual structures which play a key role in the fertilization process. The aim of this study was to identify sperm structures using two staining techniques. This staining technique can be used to stain sperm structures that can not be seen in other methods of slide preparation, which means that it can be considered for routine use in assessing the fertility.***

Keywords: Infertility, sperm morphology, staining techniques, assisted reproductive techniques, cost effectiveness

1. Background

Semen analysis is a laboratory test that is performed to assess male fertility. Infertility is defined as the inability to conceive after 1 year of unprotected sexual intercourse. About 15% of all couples in reproductive - aged couples experience infertility. Infertility in a male is assessed by taking a detailed medical and sexual history, a complete physical examination, and semen analyses. The male factor significantly contributes to 30% of the infertility cases and is a contributing factor in about half.

Recent alarming trends of substantial rise in the number of cases of infertility with as many as 30 - 40% being attributed to male factor associated causes have created a need for further studies and advancements in semen analysis.

It can be used to predict the need and outcome of artificial reproductive techniques such as in vitro fertilisation, gamete intrafallopian tube transfer and intracytoplasmic sperm injection.

A precise diagnosis of ejaculates is necessary to predict male fertility, in humans and is important in optimizing and maximizing their reproductive ability for natural conception as well as in assisted reproduction techniques (ART). While other basic semen parameters i. e. motility and total sperm count, are important in predicting fertility, the morphological structure of spermatozoa seems to be the most significant factor, especially for natural conception and artificial insemination. It has been shown that spermatozoa with abnormal morphology are not able to reach the oocyte. Also spermatozoa with normal motility but with head defects are incapable of fertilization.

Aim

To find the ideal, simple and cost effective basic stain for assessment of sperm morphology in tertiary care setup in oligospermia.

2. Materials and Methods

A study of 60 cases coming to the OPD for semen analysis was conducted. Kruger's strict morphology method was used.

We used the following basic staining methods

1) Eosin and nigrosine
2) Fields stain
3) Leishman stain.

Inclusion criteria:

- Healthy men in the reproductive age group.
- Properly prepared, fixed and evenly stained smears are included.

Exclusion criteria:

- Samples from subjects who have not adhered to the required period of sexual abstinence (2 - 7 days).

3. Result

We found that dual staining method using eosin and nigrosin and H &E was most ideal, simple and cost effective stains for overall assessment of sperm morphology in oligospermia.

	Age	Primary infertility	secondary infertility	Normal	Oligospermia	Azoospermia	USG
1	38	y				y	bilateral small testes, grade 4 varicocele
2	30	y		y			
3	43		did not liquify				
4	32	y					undescended testes, orchidopexy
5	36	y		y			
6	43	y		inadequate sample < 0.5 cc			
7	39	y	did not liquify				
8	38	y			y		varicocele
9	28	y	did not liquify				
10	35	y		y			
11	35	y		y			
12	32	y	did not liquify				
13	40	y		y			
14	23	y	did not liquify				
15	42		y			y	varicocele grade 3
16	40	y					
17	26	y		y			
18	40	y	did not liquify				
19	60			y			
20	32	y					
21	42		y				
22	32		y				
23	32	y	did not liquify				
24	32	y	did not liquify				
25	28	y	did not liquify				
26	33	y					grade 4 varicocele
27	37	y		y			
28	32	y		y			
29	35	y		y			grade 3 varicocele
30	28	y		did not liquify			
31	28	y		y			grade 1 varicocele
32	40	y		y			
33	36	y		y			
34	32	y		y			
35	30	y		y			
36	31	y		y			
37	35	y		y			
38	34	y		y			
39	34		y	y			
40	35	y		y			
41	29	y		unable to retrieve the sample			
42	32	y		y			
43	48			y			
44	30	y		y			
45	30			y			
46	29	y		y			
47	28	y		y			small right testes
48	30	y		y			ectopic testes, bilateral empty scrotal sac
49	24	y		y			premature ejaculation
50	33	y		did not liquify			
51	30	y		y			
52	40	y		y			varicocele
53	32	y		y			
54	26	y		y			
55	34	y		y			HIV positive
56	27	y		y			
57	26	y			y		bilateral varicocele grade 2
58	28	y					bilateral varicocele grade 2

59	30	Y					Unilateral varicocele grade 1
60	35	y		y			bilateral varicocele grade 1

Age	Primary Infertility	Secondary Infertility	Oligospermia	USG	Abnormal forms
26	y		4million		6%
34		y	13 million		5%
42		y	1million		30%
38	y		3 immotile sperms		80%
35	y		8million	Grade 1 varicocele	20%
28	y		<50, 000	bilateral varicocele	50%
38	y		0.7million	varicocele	60%
34	y	y	<1 million		60%
38	y		0.4 million	bilateral varicocele	40%
52	y		3 lakhs		40%
38	y		1.2 million		20%
38	y		1 million	varicocele	10%
40			2 million		20%

4. Discussion

Assessment of sperm morphology as a component of semen analysis is one of the most important steps in the evaluation of male infertility. Morphology of spermatozoa is a vital criterion for successful fertilization and early embryonic development in assisted reproductive techniques such as Invitro Fertilization (IVF), Gamete Intra Fallopian Tube Transfer (GIFT) and Intra Uterine Insemination (IUI) [5]. Sperm morphology can be evaluated using a number of chemical, biochemical and microscopic techniques. The main problem is that the use of different methods for a given material or type of analysis causes discrepancies in the number of morphologically normal or abnormal sperm identified and in their dimensions As a consequence, a male examined in one laboratory can be classified as having normal sperm morphology, while in another it may be identified as an individual with fertility disorders. [6]This is a major obstacle for doctors of human medicine comparing the results of semen analysis from laboratories using different techniques. Of all the seminal parameters such as concentration, motility and morphology, one of the most powerful indicators of both invitro and invivo fertility remains the morphology. Although the importance of this parameter has been recognised, limitations such as lack of standardization in preparation, evaluation and staining techniques, the true potential of this parameter has not been tapped.

Hence, as mentioned above, the key problem faced when evaluating of sperm morphology and morphometry is the lack of standardization with respect to the choice of staining techniques. The use of dyes with different pH, osmolarity and procedure length may affect the shape and size of spermatozoa, and thus the result of the sperm morphology evaluation. The lack of established standards for the use of different staining techniques remains greater attention in the literature on sperm morphological evaluation. There is a need to establish or develop a staining technique that will enable unambiguous and precise analysis of the morphology and morphometry of spermatozoa. In addition, a standard should be developed for preparing specimens for morphological evaluation. This would allow for comparison of results between laboratories, which would increase the value of sperm morphology analysis in predicting and evaluating fertility

5. Conclusion

While certain staining methods are recommended for assessment of human sperm morphology, the search continues for optimal techniques enabling the reliable assessment of animal spermatozoa. Discrepancies in the reaction of spermatozoa to dyes used may result from differences between species or differences between individuals in the resistance of sperm to external factors. The structure and arrangement of microfibres of the sperm head may also result in different sperm head dimensions. The cytoskeleton of the sperm head consists of nuclear proteins and the nuclear envelope, which are partially responsible for the formation of the nucleus. Depending on the method of fixation and staining, changes may take place in the arrangement of actin fibres in the sperm head. There are no conclusive guidelines recommending specific staining techniques for animal species. Hence sperm morphology assessment proves as the basic necessity for the diagnosis and management of male factor associated infertiliity in oligospermia when advanced techniques are unaffordable, unavailable and inaccessible.

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