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*Original Research Article*

# A Pilot Study for A Simple, Rapid Direct Staining Technique for Human Sperm Morphology

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## ABSTRACT

An assessment of the morphological characteristics of the human spermatozoa is as important for complete evaluation of the semen sample as count and motility. In the present study we have used equal quantity of semen and haematoxylin stains mixed in a micro-tube and allowed to act for 5 minutes, after which a small aliquot of the mixture was placed on a slide and a smear was made using a second slide (like a blood film). The slide was allowed to air dry. After drying the smear was examined with a bright field microscope with oil immersion. Normal and abnormal sperms were clearly viewed.

***Keywords:*** Sperm morphology, semen analysis, Haematoxylin, smear slides

## INTRODUCTION

Semen analysis forms an integral part of infertility investigation. Today about 60% of the infertility problems are due to male factor. [1] Sperm count, motility and morphology play an important role in fertilization. [2]

Wet films only give a rough idea about the shape of the sperm. A number of stains have been tried to improve visualization of the sperm to study its morphology in greater detail. [3]The morphology seen with the microscope is not the true morphology of a living spermatozoon, but an image we create. This image comprises of a number of factors. Spermatogenesis, sperm transport, maturation, and ageing, time in seminal plasma, smearing technique, fixation, staining, mounting and the optics and the illumination used i.e. the quality of the microscope. With standardized and controlled methods we can minimize the technique dependent sources of errors. It is of great importance that the preparations are of high quality when assessing sperm morphology. Even the small artifacts influence the appearance of the sperm. [4]

In the present study the authors have tried to minimize the errors due to staining and fixation and have tried a simple technique to visualize the morphology of the sperm.

## MATERIALS AND METHODS

A fresh sample of semen was used. A standard solution of heamatoxylin stain was prepared.[5] After complete liquefaction, the sample was mixed thoroughly and a drop of semen was placed in a micro-tube. A drop of haematoxylinwas added to the semen and mixed thoroughly and allowed to stand for 5 minutes, after whicha small aliquot of the mixture was placed on a clean slide. The aliquot was then pulled out into a smear with a second slide (like a blood film). The smear was air dried and examined with a bright field microscope with oil- immersion objective.

Certain precautions need to be taken to get a good smear. Minimal force should be used while pulling out the aliquot with a second slide. This is to prevent broken tails. The slides used should be very clean and preferably cleaned with 95% or absolute alcohol before use. [6]

## RESULTS

Normal and abnormal sperms were clearly visible in the smears as can be noticed in the photographs. (Figure1 and Figure 2)

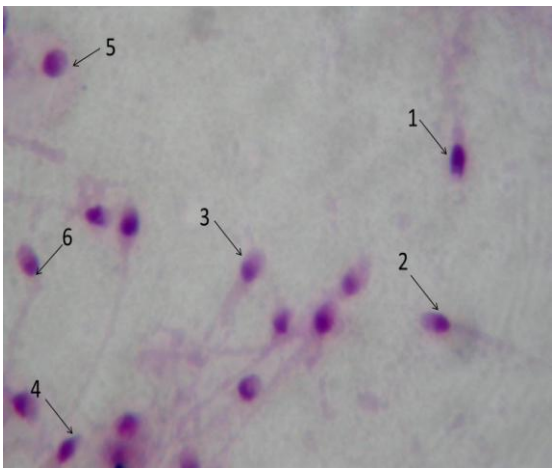


Figure 1: Haematoxylin stain of semen (x 1000).
1 – Abnormal, < 40% acrosomal region,
2 – Normal ,
3 – Abnormal , excess residual cytoplasm > 1/3 head,
4 – Abnormal, tapering head with < 40% acrosomal region,
5 – Normal,
6 – Normal head but bent neck

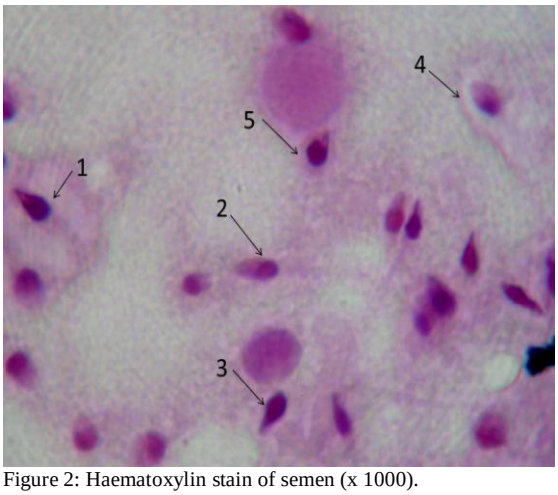


Figure 2: Haematoxylin stain of semen (x 1000).
1 – Abnormal, tapered head with < 40% acrosomal region,
2 – Abnormal tapered head,
3 – Abnormal, tapered head with no acrosome,
4 – Abnormal coiled tail,
5 – Abnormal amorphous

## DISCUSSION

An assessment of the morphological characteristics of the sperm is as important for complete evaluation of the semen sample as are the count and motility. [6] Sperm morphology is considered to be one of the best discriminators for fertilization potential. [7,8] Until the 20th century very little attention was given to sperm morphology assessment. There was a lack of uniformity in human sperm morphology evaluation. Multiple rating modules were published, many of which have made an important contribution towards standardization. [9, 10]

An important breakthrough was noted in 1999 with the publication of the WHO guidelines for semen analysis. [11] According to the WHO publication the spermatozoon is considered normal when the head, neck, mid- piece and the tail was normal. Sperm morphology has been described and identified as an excellent bio-marker of human sperm dysfunction and helps clinicians identifies the source of infertility among male factors in men.

The process of preparing a slide from the semen is time consuming and a number

of studies have been conducted to try to reduce the time taken.[12] For sperm morphology, routine procedure is to make a smear with a small aliquot of semen placed on a clean slide. The aliquot is pulled out into a smear with a second slide. After air drying the slide is fixed with a fixative. Then the slide is stained with a stain of choice. In the present study which is a direct method, fixative is not used. The stain (haematoxylin) is directly applied to the aliquot of semen. The smear is then made and dried and then viewed under an oil immersion microscope. Studies have used Harris's Haematoxylin, Papanicolaou stain, May-Grunwald Giemsa stains (MGG), supra vital stain, Giemsa stain and leishman's stain to stain a semen smear. Harris's Heamatoxylin has proved to be the best and hence this stain was used in the present study. [3]

The advantage of this direct staining method is that fixative is not necessary, easy to perform and time saving. The slide can be immediately examined, no further steps are needed.

The authors also noticed that on a few occasions the smears were not of good quality. The cause could have been quantity of the stain used, time allowed for the mixture to stand or faulty preparation of the smear.

## CONCLUSION

This is a pilot study to evaluate a novel method to stain smears for sperm morphology. Further studies for standardizing the technique will need to be done before this method can be applied as one of the methods useful for assessing sperm morphology.


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## REFERENCES


1. Esteves SC, Miyaoka R, AgarwalA. An update on the clinical assessment of the infertile male ( Invited review) Clinics 2011;66(4): 691-700
2. Bongso A, Soon-Chye Ng. A Laboratory Manual on Assisted Reproductive Techniques, Department of Obstetrics & Gynaecology, National University Hospital of Singapore. 1996 2nd edition: p.13
3. *AK Ageep, SA Ali, AoAlmobarak.* The best stain for morphological study of human seminal fluid's smears. Sudan Journal of Medical Sciences. Open access journal. 2009;4(1).
4. NAFA- Manual for basic semen analysis, NAFA Andrology Laboratory Quality group of semen analysis 2000: p.19-21.
5. Bancroft JD, Marilyn Gamble. Theory and practice of histological techniques. 6th edn- Churchill Livingstone Elsevier 2008:p.121-22.
6. Mortimer D. Practical Laboratory Andrology. Oxford university press. New York 1994: p.71.
7. Nikolettos N, Kiipker W, Demirel C, Schopper B, Blasig C, Sturm R, Felberbaum R, Bauer O, Diedrich K, Al-Hasani S.Fertilization potential of spermatozoa with abnormal morphology. Human Reproduction. 1999; 14 (Suppl. 1): 47-70.
8. Kruger TF, Menkveld R, Stander FS, Lombard CJ, Van der Merwe JP, Van Zyl JA, Smith K.Sperm morphologic features as a prognostic factor in in vitro fertilization. FertilSteril. 1986 Dec;46(6):1118-23.

9. Freud M. Standards for the rating of human sperm morphology. Int. J. Fertil.1966; 11:97-118.
10. Eliasson R. Standard for investigations of human semen. Andrologia. 1971;3(2):49–64.
11. World Health Organization. WHO laboratory manual for examination of human semen and semen cervical mucosa interaction.4th edition. Cambridge University Press. Cambridge UK. 1999;17-19.
12. Kruger TF, Ackerman SB, Simmons KF, Swanson RJ, Brugo SS, Acosta AA. A quick, reliable staining technique for human sperm morphology. Arch Androl. 1987;18 (3): 275-7.

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