

The best stain for morphological study of human seminal fluid's smears.

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ABSTRACT

Objectives:

There is a high need for proper evaluation of the morphological features of human sperms. The importance of this lies in the field of andrology, male fertility and in vitro fertilization. The wet smears can give rough clue about the shape of the sperms, but it is neither accurate nor reproducible. This study aimed to determine the best stain which can be used for seminal fluid cytology.

Methods:

This study was conducted in Port Sudan, Red Sea State, Sudan in the period from October 2006 to September 2007. The total number of patients was 50.

Samples which were collected from normospermic patients (NSP) were prepared by direct smear technique. Samples which were collected from oligospermic patients (OSP) and azospermic patients (ASP) were prepared by direct smear technique and also by indirect smear techniques (concentration method).

Smear samples were stained by freshly prepared Harris's Haematoxylin, Papanicolaou stain, May-Grunwald Giemsa stains (MGG), supra vital stain, Giemsa stain and leishman's stain.

Results:

In this study, the best stain was Harris's Haematoxylin (80% excellent for the head of sperm, 70% good for the neck, 59% excellent for the tail, 42% very good for cells in background). Harris's stain was followed by papanicolaou stain and the third best stain was supra vital stain. MGG was better than Giemsa in staining of semen smears (75% good versus 25% good) in overall performance. The worst stain was Leishman's stain.

Conclusion:

Stained smears must be used for the morphological study of semen samples. Harris's Haematoxylin is the best stain for semen cytological features. Stains which used for the semen samples should be freshly prepared.

Keywords: Harris's Haematoxylin, Papanicolaou stain, May-Grunwald Giemsa stains, supra vital, Giemsa, leishman's, andrology, azospermic.

The field of andrology has undergone rapid advance, with ever-increasing awareness of the importance of objective assessment of the quality and functional characteristics of spermatozoa and of those variables related to the secretory function of accessory glands. This is essential both in the evaluation of the infertile couples and in the assessment of fertility in men

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whose sperm production is suppressed by potential antifertility compounds or by toxic agents¹. Moreover, objective measurement of semen variables is also of interest to the burgeoning fields of in-vitro fertilization (IVF) and artificial insemination by husband semen (AIH)¹. On the other side, the morphological features of spermatozoa seen with the microscope are not the true morphology of living spermatozoa, but an image we create. This image comprises a number of factors: spermatogenesis, sperm transport, maturation and ageing, smearing technique, fixation, staining, and the optics

and illumination used (the quality of the microscope). Furthermore, it is of great importance that the preparation (smearing and staining) are of high quality when assessing sperm morphology².

A large number of cells are found in the seminal fluid after ejaculation that appears as spermatozoa cells. Normally up to 20% of the spermatozoa in the ejaculate can have abnormal shapes³.

A group of stains can be used to evaluate the morphological and cytological features in seminal fluid. Haematoxylin and eosin stain is probably the most widely used in histological and cytological stain⁴⁻⁷.

The other universal stain for cytological preparations is the Papanicolaou stain, which consists of Haematoxylin, OG6 and EA 50^{8,9}. Romanowsky stains (Giemsa, Leishman, Supravital & MGG) are usually employed for routine staining of the blood and the cytological features in the fluid¹⁰.

The usefulness of sperm morphology assessment as a predictor of a man's fertilizing potential has often been challenged due to different classification systems, various slide preparation techniques and problems with reproducibility because of observer variations¹¹. According to the literature, the importance of sperm morphology as a single and independent predictor of in-vivo and in-vitro fertilization seems to be proven^{12, 13}. A little work was done in seminal fluid staining and examination. In this study, we tried to

find out the best stains for seminal fluid's smears (sperm and other cells).

Materials and Methods:

This was analytical study carried in Port Sudan, Red Sea State, Sudan during the period from October 2006 to September 2007. The total number of the whole population was (739,300) according to the national census of 2002 with adjusted growth rate. There are four localities in this area (Port Sudan, Sinkat, Tokar and Halayib).

The required samples were collected from 50 patients complaining of infertility. 40% of them were normospermic patients (NSP), 40% were oligospermic patients (OSP) and 20% were azospermic patients (ASP). From each sample 60 smears were prepared – 10 smears for each stain – by direct and concentration (centrifugation) methods. In this study, six types of stains were used (Haematoxylin, Papanicolaou, Supravital, MGG, Giemsa and Leishman). The conventional methods of staining were used for each type of the stains. The results of the staining procedure were graded as excellent, very good, good, bad and very bad for each part of the sperm and for the cells in the background.

Results:

Results of Harris's stain were 80% excellent for the head (table 1 & figure 1), while staining by Papanicolaou stain showed 29% excellent staining for the head (table 2 & figure 2).

Table 1: Degree of semen staining by Harris's Haematoxylin:

	Excellent	V. good	Good	Bad	V. bad
Head	80%	9%	1.2%	8.1%	2%
Neck	16.2%	70%	6.2%	7.5%	-
Tail	59%	21%	8%	8%	3%
Cells in background	42%	37.2%	2.3%	10%	3%

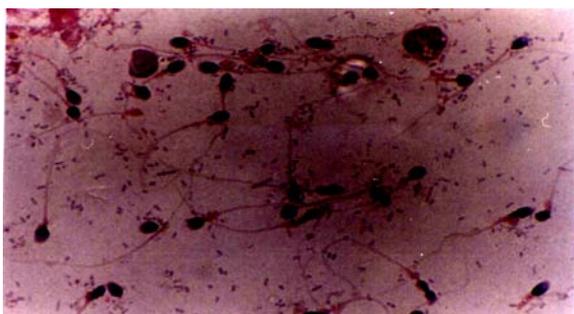


Figure 1: photomicrograph of mear stained by Harris's Haematoxylin. (100 x oil).



Figure 2: photomicrograph of smear stained by Papanicolaou stain. (100 x oil).

Table 2: Degree of semen staining by Papanicolaou's stain:

	Excellent	V. good	Good	Bad	V. bad
Head	29%	57%	1%	12%	1%
Neck	1%	45%	40%	10%	4%
Tail	12%	59%	15%	7%	6%
Cells in background	41%	49%	5%	4%	1%

Using Supravital stain, the percentages were as follow: 69% very good for the head, 86% good for neck, 96% good for the tail, and 87% good for cells in background (figure 3).

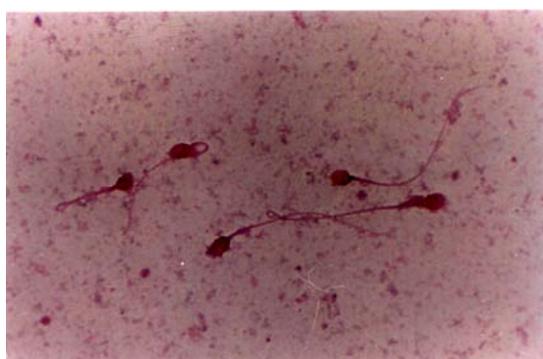


Figure 3: photomicrograph of smear stained by Supravital stain. (100 x oil).

By using MGG stain, it was found that, the head stained good in 74%, the neck stained

good in 57%, the tail good in 49%, The cells in background stained very good in 42% (figure 4) .

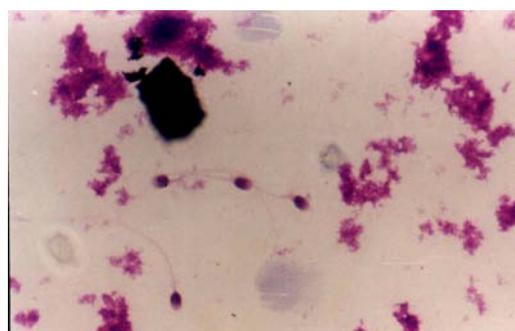
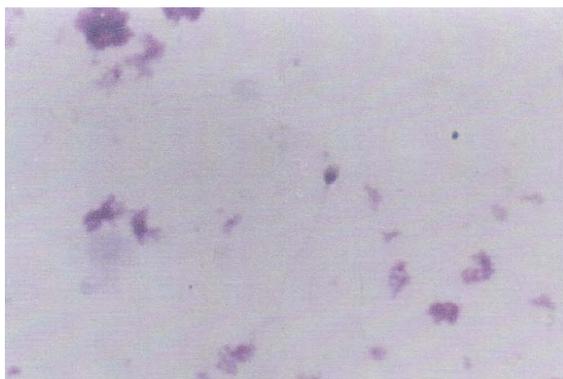
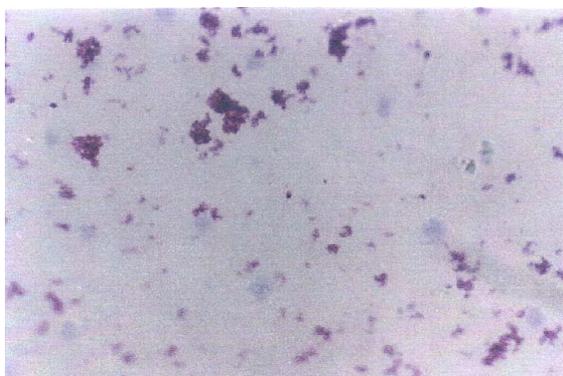


Figure 4: photomicrograph of smear stained by MGG. (100 x oil)

Giemsa stain gave the following results: 77% good for the head, 51% bad for the neck, 47% bad for the tail and for the cells in background 45% was graded as good (figure 5).



(a)



(b)

Figure 5: Photomicrograph of smear stained by Giemsa stain (a by 100 x oil, b by 40x)

Leishman stain showed bad results in 80% for head, and were bad in all of the slides for the neck. In 100% of the slides the tail was stained very bad and in 60% of the smears the cells in background were stained very bad (figure 6).

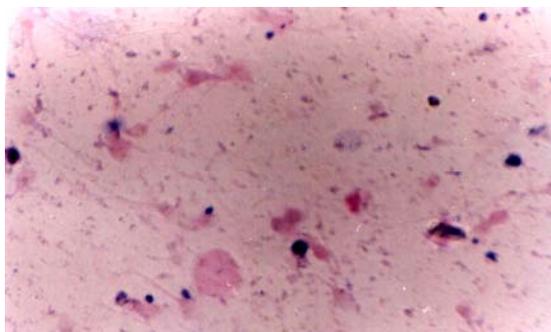


Figure 6: photomicrograph of smear stained by Leishman's stain. (100 x oil).

DISCUSSION:

To the best of our knowledge, very little work was done in the routine and special stains for morphological features in seminal fluid. Our study is consistent with the most famous study in semen staining, which was written in the WHO manual for semen analysis¹⁴. This study concentrated on the result of the semen's morphology without any comparison between the stains. They favored to use Papanicolaou's stain, but they didn't use Harris Haematoxylin in their study. We used six types of stains, which is another advantage to our study.

The common habit of using fresh unstained deposits for seminal analysis, when looking for the motility of the sperm, gives a rough clue about the morphology. This rough estimate is made from the sluggish linear or non-linear moving spermatozoa and dead spermatozoa. However, a phase contrast microscope is more preferable than the light microscope in the evaluation of the unstained deposit of seminal fluid. The use of ordinary light microscope for fresh unstained seminal fluid usually does not give accurate results for several reasons. First, sperm have round or oval uniformly shaped head with a ratio of 2:1 between acrosome and post acrosomal portion and this ratio cannot be assessed in unstained seminal deposit¹⁵. Furthermore, when the acrosome detaches, the post acrosomal portion changes its normal shape (semilunar shaped) and appear ragged or bell-shaped. Also the vacuolated head may not appear in unstained deposit of seminal fluid when light microscope is used. In addition, the identification of the biaxial attachment of mid piece and its cytoplasmic droplet need a phase contrast microscope. Moreover, the tail of spermatozoa sometimes does not appear. On the other Hand, the phase-contrast microscope does not provide sufficient resolution for assessment of sperm morphology due to the presence of the phase ring on the back phase of the lens¹⁶. For all these reasons the use of unstained fresh semen should be restricted only to the study of the motility of the sperms.

Among the six stains used in this study, the best stain was Harris's Haematoxylin. All parts of the spermatozoon stained very clearly; the acrosomal cap stained blue, the nuclear part of the head was stained perfectly. In addition, the mid piece and the outline of the flagellum stained clearly visible and any irregularities in it could be detected. Furthermore, the cells in background were stained perfectly; nuclei appeared sharp and chromatins stained very clear.

Harris's stain was followed by Papanicolaou stain, which had the same advantages, but irregularities in the mid-piece and the flagellum couldn't be detected.

The third best stain was the supravital stain. All parts of the spermatozoon were stained perfectly, the acrosomal cap was stained orange, and the nuclear part of the head stained well. The mid piece and flagellum were visible but the irregularities in it couldn't be detected. The cells in back ground were not stained perfectly.

MGG and Giemsa stains shared the same position, because of the similarity in their features. In both of these stains, the head was stained good and chromatin appeared well. In addition to that, 16.7% of mid piece and flagellum did not appear or appeared faint. The cells in background stained clearly in MGG stain and not clear enough in Giemsa stain.

In this study, the worst results were scored by leishman's stain. All parts of spermatozoon and cells did not appear at all, no acrosomal cap was seen, and visibility of the nuclear part of the head was bad. Also the mid piece and flagellar out line did not appear. It worth mentioning that none of the stains; MGG, Giemsa and Leishman had excellent staining of any part of the sperm or the background.

In conclusion: stained smears must be used for the morphological study of semen samples whereas; wet deposit should be confined to the assessment of motility.

Harris's Haematoxylin emerged as the best stain for evaluation of cytological features in

seminal fluid, followed by Papanicolaou stain and supravital stain.

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