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Research Article

Improvised Eosin and Leishman as Morphological Stain for Sperm Cell Analysis; Adult Male Wister Rats and Rabbit as a Model of Study

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Abstract

Introduction: Over the years improvement has been made on the staining techniques for the morphological analysis of sperm cells ranging from the normal eosin and nigrosine combination to other more simple or complex single staining technique.

Aim: Using improvised combination of Eosin and Leishman stain as morphological stain for detection abnormalities and viability studies towards future applications in assisted reproductive science.

Method: 22 Wister rats and rabbits sperm cells where stained to analyze their morphology, using both the conventional technique and the hibiscus flower extract to determine the reproducibility of this improved staining technique.

Results: From the staining technique the morphology of the sperm cells where properly noticeable from the head, body and tail which is represented in the pictures as shown.

Conclusion: In a third world country this hibiscus plants are accessible stains for quick and accurate morphological observation of Wister rats and Rabbit spermatozoa as an intervention tool in absence of complex staining techniques and since it is natural may have little or no effect on sperm cells.

Keywords: Wister rats; Rabbits; Spermatozoal; Eosin; Nigrosin; Leishman

Introduction

The morphology of spermatozoa is a device critical for an effective preparation and early embryonic advancement in helped conceptive systems [1]. Last Tygerberg Classification Criteria depicted by Kruger in 1986 and the WHO grouping are the most vital spermatozoa morphological characterizations. As per Kruger's sperm, the head, neck and tail segments ought to be ordinary, the limits of the head ought to be smooth and oval and 70% of head ought to be covered by acrosome. The leader of the sperm head length ought to be 4 - 5m and must have a width of 2.5 - 3.5m. The length of the tail ought to be a normal of 45m. The center part should be thin and long and width ought to be under 1 mm, length ought to be of 1.5 times the length of the head. The tail ought to be more slender than center piece, ought to be level and not wrinkled, and ought not contain broken parts and cytoplasmic flotsam and jetsam. Also, the sperm ought not have the neck, center piece, tail variations from the norm and an expansive cytoplasmic bead the greater part of the spermatozoon head in the neck range [2,3].

Morphological highlights of spermatozoa are communicated in numerical esteems. Techniques utilized as a part of the recoloring may cause a slight change in the estimation estimations of spermatozoa in light of the fact that fixatives can make cells contract a bit. For instance, 3 - 5m length and 2 - 3m wide estimations are viewed as typical for the head of spermatozoa in the strategy for Papanicolaou. These qualities change 5 - 6 and 2.5m - 3.5m in the Diff-Quick recoloring technique. In both the center piece ought to be 1m thick, the length of this esteem ought to be up to 1.5 times of this esteem. The tail segment ought to be 45m length decreasing bit by bit [4]. As a result of these distinctions, we analyzed morphology of spermatozoa with various recoloring techniques and planned to locate the better recoloring strategies for research facilities in our examination.

Materials and Methods

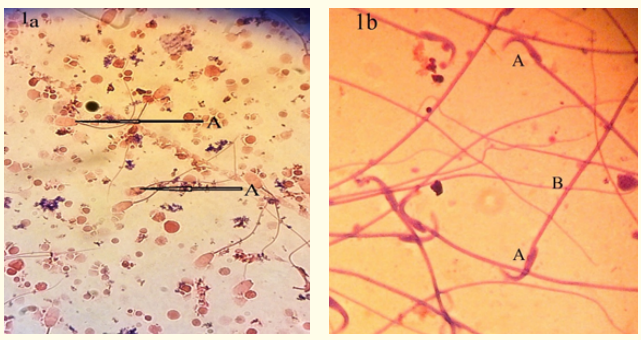
In this examination randomized 10 Wister rats and 10 rabbit's semen tests were acquired from the Wister rats the sperm cells where reaped from the vas deferens from the rabbits, the sperm cells where gathered utilizing a fake vagina. The examination was completed in the University of Benin Health Services Department.

Spread were set up from the Wister rats and rabbits understudy; the smears were dried at room temperature and settled in methyl liquor.

In the initial segment of the investigation, smears were recolored with the blend of Eosin and Leishman recolor in a proportion 1:5 and it was a homogenized, sifted and left to remain for 24 hours. The stain was poured on the slide and left for (10) ten minutes and it was last flushed in supported typical saline, scratched out and left to air dry.

Result

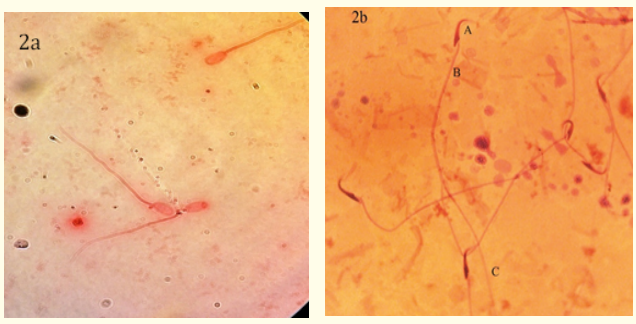
Head, center piece and tails of spermatozoa were seen obviously with GE. Acrosome center rate was to a great degree great as light and dim pinkish (buildup). The stain was homogeneously appropriated on the head and dim pinkish shading was seen. The center piece and tail were seen unmistakably and can be assessed extremely well (Figure 1a and 1b). The perfect picture was acquired by recoloring spermatozoa with Giemsa and Eosin. The buildup of the head can be picked by the whitish blue shading. Buildup and morphology of the head can be chosen obviously (Figure 2a and 2b). (Figure 3)



(a) Rabbit Sperm cell

(a) and (b)
Normal Wister Rat sperm Cells

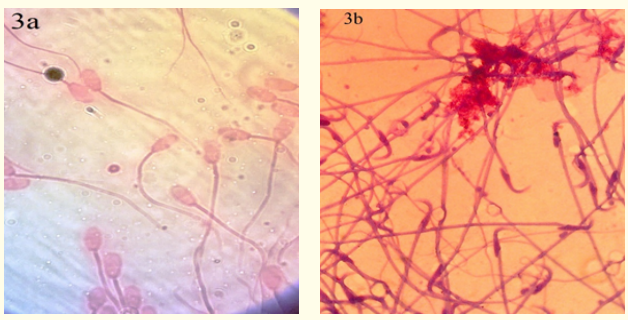
Figure 1 (a and b)



Rabbit Sperm

Wister Rat

Figure 2 (a and b)

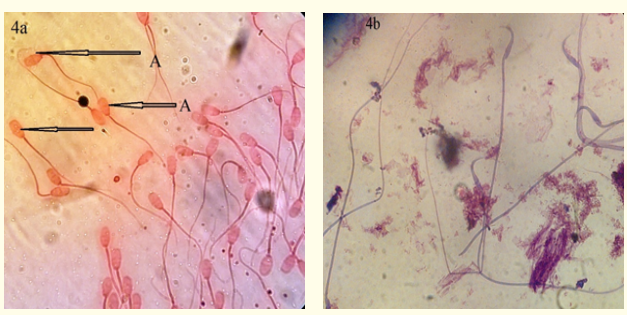


Rabbit Sperm

Wister Rat

Figure 3 (a and b)

The spermatozoa were recolored dull blue purple with Eosin and Leishman recolor with expanded grouping of Eosin recolor without washing in cradled typical saline (Figure 4a and 4b). Head morphology and buildup could be seen extremely well however the center piece and the tail did not appear to be clear.



Rabbit Sperm

Wister Rat

Figure 4 (a and b)

Discussion

Appraisal of spermatozoa morphology is an imperative parameter in the analysis of barren men. Because of the utilization of expanding measures of IVF (in vitro preparation) procedures, contemplates are likewise centered around the morphology of spermatozoa and its critical part in treatment. Assessment should be possible, recoloring spermatozoa with an assortment of techniques from the new semen by electron, quick differentiation and light magnifying lens. There are many recoloring strategies to assess the morphology of spermatozoa [5]. García-Herreros., *et al.* [6] utilized Hemaklor, Harris haematoxylin and PanOptic quick recoloring strategies to influence institutionalization of the examining techniques, to test planning and recoloring for the leader of the spermatozoa with PC helped morphometric investigation (CASMA). They presumed that Haematoxylin and Hemaklorun are the best recoloring techniques for assessment of sperm heads.

Soler., *et al.* [7] looked at 3 changed color techniques for appraisal of spermatozoa morphometry with mechanized Integrated Semen Analysis System (ISAS). They assessed 200 spermatozoa cells recolored with Hemacolor, Diff-Quik and harris haematoxylin. While they discovered all strategies were likewise valuable in that utilized as a part of recoloring of spermatozoa yet Diff-Quick stain demonstrated a critical distinction.

TB is utilized as a part of the assessment of the atomic chromatin of spermatozoa by recognizing the nonattendance or crack of disulfide bonds. Beletti and Mello [8] inspected connection between spermatozoa morphology and spermatozoa chromatin buildup with TB and Feulgen response. TB is a successful stain for the assessment of changes in spermatozoa chromatin. Our examination is good with the learning of the writing. TB color recoloring technique is a straightforward strategy and permit synchronous assessment for morphology and buildup of spermatozoa. TB is a perfect color for routine use around there.

Morel., *et al.* [9] arbitrarily chose 47 patients connected for semen examination, they analyzed the decent variety of basic morphological issue in the human spermatozoa among people. Keeping in mind the end goal to demonstrate their association with the atomic development, sperms recolored with Aniline Blue, Spermatozoa morphology deserts contrasts seen amongst people and they found a critical connection between regular of deformities and level of atomic maturate. Our examination is perfect with the information of the writing. Aniline Blue color gives the perfect picture to gauge the rate of acrosome head. Buildup and morphology can be evaluated together.

Tartaglione and Ritta [10] inspected the prognostic spermatological estimations of solidified broke down spermatozoa to evaluate in vitro richness. Assessing plasma film and acrosome trustworthiness are critical esteems for typical elements of spermatozoa, for this point, they inspected smears recolored with Trypan Blue and Giemsa. They reasoned that these stains can be utilized for the guess of the ripeness in the semen utilized for IVF.

Our assessment was predictable with the writing of Giemsa and Wright stains. Buildup and head morphology of spermatozoa were well-selectable. The center piece and tail could be seen. An unforeseen finding that tail and head totally recolored pink in the a portion of the spermatozoa which had harmed head morphology. Additionally think about was recommended to clarify the reason. Spermatozoa morphology and buildup were clear and smooth with Orange G recolor. Be that as it may, recoloring process was too long that is the reason Orange G recolor had no prevalence.

Shorr procedure is favored in numerous research centers because of the inadequate relationship in the consequences of IVF. Henkel., *et al.* [11] assessed diminished motility of spermatozoa in elderly men with Shorr strategy. The level of recolored spermatozoa with ordinary and irregular flagella's was assessed and discovered a negative connection between the recolored strange flagella's and speed proportion and motility of the spermatozoa in elderly man.

Convincingly In our investigation, spermatozoa heads showed up as Pinkish globules with the adjust of fixation with time light pale pinkish shaded, center segments and tails were recolored dim pinkish. Our examination was had all the earmarks of being perfect with the writing and as the aftereffect of our investigation. Spermatozoa center area which is a rich segment for mitochondria's were uncovered plainly and was shown by and by the Eosin and Leishman recolor which is proposed by this examination as a straightforward and available stain for spermatozoal with Wister rats and Rabbits as a model [12-23].

Acknowledgement

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Conflict of Interest

The were no conflicting interest during this study.

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