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Original Research Article

A Rapid, Cost-Effective Approach to Human Sperm Morphology by using Pre-Stained Methylene Blue Slides

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Abstract:

Background: Sperm morphology is a crucial aspect of male fertility, impacting reproductive success. This study delves into the evaluation of a simplified and cost-effective staining method for accurate sperm morphology assessment, addressing the challenges associated with conventional techniques.

Materials and Methods: This study was conducted at St. John's Medical College, Bangalore, with three adult male subjects. The methodology included meticulous sample collection, preparation, and staining of prestained methylene blue slides, emphasizing simplicity and rapidity.

Results: In this study clear visualization of the sperm morphology was achieved. Notably, the stain facilitated the distinct observation of the sperm structure, including the clear identification of the nucleus, middle piece, and tail.

Conclusion: Staining advocates for widespread adoption of this simplified technique, particularly in primary health care settings. The paper contributes to enhancing accessibility and affordability in sperm morphology evaluation.

Keywords: Sperm morphology, Methylene blue stain, Pre stained slides, Male infertility, Primary health care (PHC).

Introduction

Sperm morphology, or the shape and structure of sperm cells, is an important aspect of male fertility and reproductive health. Sperm cells are responsible for fertilizing the egg and are produced in the testes. They are produced in large numbers and are stored in the epididymis until they are needed for fertilization [1].

Sperm cells are characterized by their small size, elongated shape, and distinctive features such as the head, midpiece, and tail. The head of the sperm contains the genetic material and is surrounded by the acrosome, a cap-like structure that aids in fertilization. The midpiece contains mitochondria, which provide energy for the sperm to swim and reach the egg. The tail, or flagellum, propels the sperm through the female reproductive tract [2, 3& 4].

Abnormalities in sperm morphology can affect fertility by reducing the ability of the sperm to reach and fertilize the egg. There are several factors that can contribute to abnormal sperm morphology, including environmental factors, genetics, and underlying health conditions such as infections or hormonal imbalances [5&6].

Ensuring accurate sperm morphology assessment is crucial for fertility evaluation, and it heavily relies on meticulous smear preparation, fixation, and staining processes. These procedures play a pivotal role as they can substantially impact the dimensions of sperm. Various staining methods, including Hematoxylin& Eosin, Giemsa, Papanicolau, Eosin-Nigrosin, and Leishman, have been employed in sperm analysis to enhance visibility and highlight specific features [7]. Among the diverse staining techniques, Hematoxylin& Eosin is commonly used for its ability to differentiate cellular structures, while Giemsa stain aids in highlighting chromatin patterns. Papanicolau staining is renowned for its application in assessing sperm morphology under bright-field microscopy. Additionally, Eosin-Nigrosin staining is valued for its dual capability in assessing both live and dead sperm. Leishman stain is recognized for its utility in differentiating nuclear and cytoplasmic components in sperm cells [8 & 9].

However, it is crucial to acknowledge that the choice of staining method is only one aspect influencing accuracy. The availability and cost-effectiveness of stains must also be considered, especially in resource-constrained settings. Unfor-

tunately, some commercially available stains, such as Shorr, Janus Green, and Sperm Blue, are prohibitively expensive, posing challenges for routine usage in developing countries like India. This financial barrier highlights the need for affordable alternatives that maintain high accuracy, facilitating widespread access to reliable sperm morphology assessment methods in diverse healthcare settings.

Methylene blue staining operates on the principle of the dye's positive charge, as it selectively binds to the negatively charged nucleic acids in cellular structures, particularly DNA and RNA [10]. This staining method is commonly used in histology and cytology to visualize cell nuclei, imparting a blue or violet color under a light microscope, which enhances contrast and facilitates the examination of tissues [11].

The study aims to achieve precise sperm morphology results with minimal time and cost-effectiveness, particularly at the primary health care (PHC) level.

Material and Methods

This descriptive study was carried out on voluntary donor samples in Department of anatomy, at St. John's Medical College, Bangalore, Karnataka. Three male adult subjects' semen samples of aged ≥ 18, years were used in this study.

Study Design: Descriptive observational study.

Study Location: This was performed in Department of anatomy, at St. John's Medical College, Bangalore, Karnataka.

Study Duration: July 2023 to October 2023.

Sample size: 3 samples (from each sample 5 slides were made. Total number of slides analyzed were 15).

Subjects & Selection Method: The study population consisted of individuals from the general urban population, specifically residents of Bangalore South in JP Nagar, who expressed a willingness to voluntarily contribute semen and met the inclusion criteria. Ethical approval for this study was secured from the institutional ethics committee.

Semen collection involves addressing sensitive aspects such as personal, psychological, and social elements. In consideration of this, the Principal Investigator personally approached individuals at their homes to explain the procedure. Sterile bottles (30ml) were provided to donors one day before the scheduled sample collection. Subjects collected the samples at home in the sterile bottles, and the investigator retrieved the sample bottles from the donors' homes. Subsequently, within one hour, the samples were processed in the histology lab within the Department of Anatomy, followed by staining.

Inclusion Criteria: Healthy male, between 20-50yrs

Well-prepared smears that were appropriately fixed and uniformly stained.

Exclusion Criteria: Male with chronic diseases, male who has habit of smoking & alcohol consumption.

Procedure Methodology: After written informed consent was obtained, the sample was processed for histological procedure.

The Preparation of Pre Stained Slides:

The clean slides were kept in agar adhesive fluid for 10 minutes, taken out, and allowed to dry. After complete drying, the slides were transferred to a 1% methylene Blue solution (nuclear stain) for 20 minutes and allowed to dry. These slides were stored in a slide box for the procedure. Once the sample was received, a drop of semen (10 microliters) was placed in the center of the prestained slide and covered with a cover slip. Allowed the slide to take the stain for 5-10 minutes, then observed the slides under light microscope.

Analysis: The slides were observed and analyzed for staining and morphology of sperm using light microscope under high power and oil immersion.

Result

The staining process yielded a discernible presentation of sperm morphology. Specifically, the nucleus, middle piece, and tail were distinctly observable. This outcome underscores the effectiveness of the staining method in providing clear visualization of key components in sperm structure.

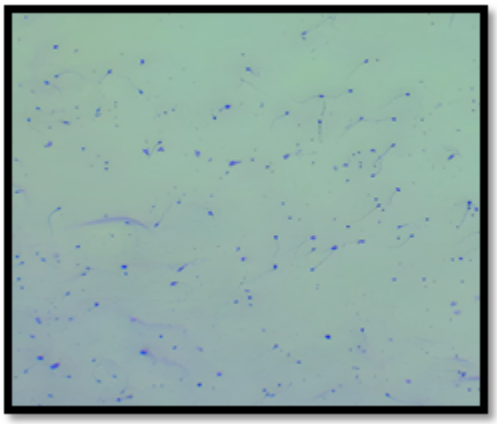

Figure 1: Spermatozoa stained by methylene blue under (400X)

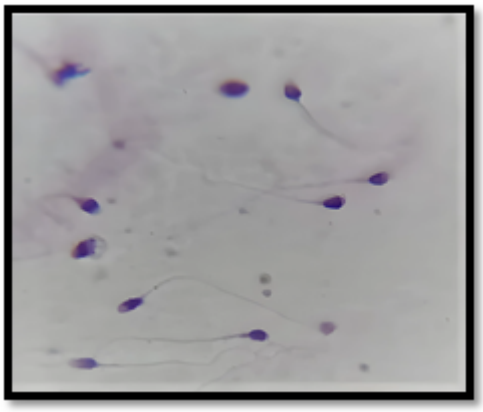

Figure 2: Spermatozoa stained by methylene blue under oil immersion (1000X)

Discussion

Evaluating sperm morphology emerges as a pivotal step in semen analysis, playing a crucial role in the assessment of male infertility [10]. The morphological characteristics of spermatozoa represent a fundamental determinant for the success of fertilization and early embryonic development in assisted reproductive techniques such as In Vitro Fertilization (IVF) [12].

The utilization of nuclear stains is considered an ideal procedures for assessing sperm morphology. Methylene blue stain is a widely employed technique in the analysis of sperm morphology, specifically focusing on the examination of spermatozoa structure. This stain aids in the assessment of the nucleus, encompassing the evaluation of its shape, size, and overall structure. Detection of abnormalities in nuclear morphology provides valuable insights into potential fertility issues.

Contrary to commonly available stains like H & E and Giemsa, which are time-consuming, costly, and require expert assistance, prestained slides offer a practical alternative. They eliminate the need for additional chemicals, technical expertise, and prove to be cost-effective, making them accessible even at the primary health care (PHC) level.

Study limitations include the acknowledgment that while prestained methylene blue slides are valuable for staining and identifying nuclear abnormalities, they may not effectively highlight certain structural or functional sperm defects. Therefore, a comprehensive evaluation may necessitate the use of complementary stains and additional tests.

Conclusion

In conclusion, the utilization of prestained methylene blue stain emerged as a widely adopted technique for scrutinizing the structural characteristics of spermatozoa. This study underscored its efficacy in evaluating nuclear morphology, size, and shape. The findings advocate for the incorporation of prestained slides as a pragmatic alternative to conventional staining methods, offering solutions to challenges associated with time, cost, and expertise. This recommendation holds particular significance for primary health care (PHC) settings, emphasizing the potential of this approach to enhance accessibility and efficiency in spermatozoa assessment within broader healthcare contexts.

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