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Review article

The effect of selected staining techniques on stallion sperm morphometry

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ABSTRACT

A problem in evaluating the morphology and morphometry of sperm is the lack of standardization of staining techniques. The method of staining and evaluating specimens can significantly affect the results of morphometric measurements. The aim of the study was to determine the effect of two techniques for staining stallion sperm on the morphology and morphometric dimensions of sperm cells and the formation of artefacts. The subject of the study was the sperm of Polish Half Bred stallions, isolated post mortem from the tail of the epididymis. Morphometric measurements were made of the following: length, width, perimeter and surface area of the sperm head, surface area of the acrosome, midpiece length, tail length and total sperm length. Sperm evaluation was expanded to include acrosome coverage, i.e. the surface area of the acrosome as a percentage of the total area of the sperm head, and a characterization of the tail, particularly the midpiece. Tygerberg strict criteria were used to evaluate sperm morphology. The data show that the staining method and the chemical reagents applied have a substantial effect on the dimensions and shape of the stallion sperm. The literature on the subject draws attention to the fact that a staining technique should be established or developed which would enable precise and unambiguous analysis of the morphology and morphometry of human and animal spermatozoa. Moreover, a standard should be developed for preparing specimens for morphological analysis. This would enable comparison of results between laboratories, thus enhancing the value of morphological analyses of sperm in predicting and evaluating fertility.

Contents

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1. Introduction

The most important parameters in semen evaluation are the concentration, motility and morphology of sperm. Morphology is considered to be reliable indicator of fertility, and the fertilization capacity of an individual is positively correlated with the percentage of sperm cells with normal morphological structure (Phetudomsinsuk et al., 2008; Vlasiu et al., 2008). Despite the fact that the importance of sperm morphology is well known, due to a lack of standardization of techniques for preparing, evaluating and staining it is highly likely that the potential of this parameter has not yet been fully exploited. The lack of standardization in staining techniques used for evaluation may contribute to the discrepancies noted in many comparative studies (Henkel et al., 2008; Maree et al., 2010). The fundamental problem is that the use of different staining techniques for a particular material or type of analysis may affect the number of morphologically normal sperm observed and cause discrepancies in their dimensions. Under these circumstances a male may be classified as an individual with normal sperm morphology according to one laboratory and as having anomalies according to another (Gago et al., 1998). This especially creates difficulties for doctors and veterinarians comparing sperm analyses from laboratories that use different techniques (McAlister, 2010). Differences in staining evaluation, which may be slight, are particularly problematic in the evaluation of fertility disorders in cases in which the morphology parameters are within reference values (Kruger et al., 1987).

According to guidelines by the Society for Theriogenology (SFT), evaluation of stallion sperm morphology should be performed on fresh, unstained samples using a microscope with phase contrast (Kenney et al., 1995). Most andrology and veterinary laboratories do not have access to this type of microscope and perform staining of stallion sperm using various techniques, often recommended for other species. Examples include eosin-nigrosin complex, recommended by SFT for evaluating bull sperm, or the Papanicolaou staining technique, recommended by the World Health Organization (WHO) (2010) for evaluating human sperm. The aim of the study was to determine the effect of two techniques for staining stallion sperm on the morphology and morphometric dimensions of sperm cells and the formation of artefacts.

2. Material and methods

The subject of the study was the sperm of Polish Half Bred stallions, isolated post mortem from the tail of the epididymis. Ten individuals aged about 3–4 years were selected for the study. The sperm cells were isolated according to a technique described by Evans et al. (1964), modified by Andraszek and Smalec (2011). The prepared specimens were stained using two techniques:

2.1. Silver nitrate staining

Staining with silver nitrate is a routine staining technique used to identify acidic chromatin proteins and chromatin rDNA and RNA. In the case of the silver nitrate staining the head and tail of the sperm cells were stained differently and the midpiece was clearly visible. A 50% $AgNO_3$ solution and a gelatin colloid solution were applied to the specimens in a proportion of 1:2. The smears were covered with a cover slip and incubated for 15–20 min at 60 °C in saturated humidity (Andraszek and Smalec, 2011). When the samples took on a brown colour the chemical reaction was stopped by washing the specimen several times with distilled water. The specimens were dried at room temperature without access to light.

2.2. Staining with eosin + gentian violet complex

Specimens fixed in 96% ethyl alcohol were washed in distilled water and then lightly stained with 10% aqueous eosin blue solution for 60 s. The specimens were then washed with distilled water and stained with gentian violet for 3–5 min. After staining the specimens were washed and dried at room temperature (Kondracki et al., 2005).

The sperm cells were evaluated under an Olympus BX50 microscope at 100× magnification. The smears were evaluated and analysed using the MultiScan image analysis system and measurement software from Computer Scanning Systems. The Olympus BX50 microscope is a high quality research microscope with phase objective lenses and the capability of analysing fluorescence slides. The microscope works in conjunction with the MultiScan image analysis system by Computer Scanning Systems. The MultiScan system has an option for brightness correction enabling optimal adjustment of brightness for maximum contrast. The system is intended for the visualization, acquisition, management, storage, processing and analysis of images, with particular focus on measurement functions. The source of the image for the MultiScan software is the subprogram Multiplier, which uses a frame grabber card. An image from a video camera is displayed by the card in real time on the monitor. MultiScan is an image analysis program and is not used to improve the quality of photographs of slides but to extract important information from them.

From each individual 60 randomly selected well-formed sperm cells were analysed (30 stained with silver nitrate and 30 with eosin + gentian violet complex). Morphometric measurements were made of the following: length, width, perimeter and surface area of the sperm head, surface area of the acrosome, midpiece length, tail length and total sperm length. Sperm evaluation was expanded to include acrosome coverage, i.e. the surface area of the acrosome as a percentage of the total area of the sperm head, and a characterization of the tail, particularly the midpiece. Tygerberg strict criteria were used to evaluate sperm morphology (Kruger et al., 2004). These criteria specify four additional indices characterizing