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Review article

The effect of selected staining techniques on bull sperm morphometry

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ABSTRACT

Sperm morphometry has some value as an indicator of reproductive capacity in males. In laboratory practice a variety of slide-staining methods are used during morphological evaluation of semen to predict male fertility. The aim of this study was to determine the effect of staining of semen using four different techniques on the morphometry of the bull sperm cell. The material for the study consisted of semen collected from test bulls of the Black-and-White variety of Holstein-Friesians. The results obtained in the study indicate differences in the dimensions of bull sperm heads when different slide staining techniques were used. The most similar results for sperm head dimensions were obtained in the case of SpermBlue® and eosin + gentian violet complex, although statistically significant differences were found between all the staining techniques. Extreme values were noted for the other staining techniques – lowest for the Papanicolaou and highest for silver nitrate, which may indicate more interference in the cell by the reagents used in the staining process. However, silver nitrate staining was best at identifying the structures of the sperm cell. Hence it is difficult to determine which of the staining methods most faithfully reveals the dimensions and shape of the bull sperm.

Contents

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1. Introduction

Sperm morphology continues to be an important parameter in predicting fertility in both humans and animals (Keel et al., 2002; Maree et al., 2010; Lasiene et al., 2013). One solution in evaluation of sperm morphology is computer-assisted sperm analysis (CASA), which reduces the subjectivism of morphological analysis (Gago et al., 1998), but these systems are relatively expensive and also not free of errors resulting from automation of the analysis process. The concept of morphology is closely linked to sperm cell dimensions falling within the norms for a given species. Hence many authors have observed relationships between sperm morphometry and male fertility (Casey et al., 1997; Hirai et al., 2001; Esteso et al., 2006; Núñez-Martínez et al., 2007).

Sperm morphometry is so an important indicator of reproductive capacity in the male (Gosz et al., 2010). According to a study by Katz et al. (1986), the sperm cells of infertile men were larger in terms of the long and short axis of the sperm head than in fertile men, and the length-to-width ratio of the head was greater as well. This has also been confirmed by other studies (Klimowicz et al., 2005; Niżański and Klimowicz, 2005). The results of experiments on human sperm correspond to data obtained in veterinary medicine. A pronounced difference in sperm head size in fertile and infertile males has been discovered in stallions, boars and dogs (Gravance et al., 1996; Núñez-Martínez et al., 2007; Banaszewska et al., 2011). Animals with smaller sperm head dimensions have been found to be more fertile. Research has shown that not only the size of the sperm affects fertilization capacity, but also the size of the tail and midpiece. Sperm cells with longer tails have greater fertilization capacity due to their greater motility. Data concerning morphometric dimensions enhance knowledge of the actual *in vitro* and *in vivo* fertilization capability of sperm, and also make it possible to determine the suitability of sperm for freezing prior to fertilization (Hirano et al., 2001), while morphological evaluation can to a certain extent indicate the functional capabilities of sperm in terms of acrosome function. Based on evaluation of the state of the acrosome during morphological analysis of the sperm cell we can draw conclusions about the acrosome reaction and the release of proteolytic enzymes during penetration of the zona pellucida (Nikolettos et al., 1999), which in consequence allows us to better predict fertilization capacity (McAlister, 2010; Menkveld et al., 2011).

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. In laboratory practice a variety of slide-staining methods are used during morphological evaluation of semen to predict male fertility. SpermBlue® is a stain that is often preferred for evaluation of human and animal sperm (Van der Horst and Maree, 2009). The Papanicolaou method is used for analysis of human semen (Menkveld et al., 1990; WHO, 1999). For evaluation specifically of bull semen, a staining technique using a complex of eosin and gentian violet is recommended (Blom, 1981; Kondracki et al., 2012). As an experimental method, staining with silver nitrate, which enables more precise analysis of individual structures of the sperm cell, may be used as well (Andraszek and Smalec, 2011; Andraszek et al., 2014a,b). Each of these methods, due to the chemical composition of the reagents used during the staining process, has a different effect on the stained cells. Hence the aim of this study was to determine the effect of staining of semen using four different techniques on the morphometry of the bull sperm cell.

2. Material and methods

The material for the study consisted of semen collected from test bulls of the Black-and-White variety of Holstein-Friesians. A total of 20 individuals at the age of one and a half years were selected for the study.

At least one ejaculate from each bull were collected and assessed. The ejaculates were taken by means of the artificial vagina technique. The semen was maintained at room temperature until needed for slide preparation for morphology and morphometry. Slides were prepared within 15 min of collection. Sperm morphology was evaluated using: Papanicolau, SpermBlue®, eosin + gentian violet complex and silver nitrate. Firstly, the routine sperm smear was made and allowed to air dry. For Papanicolau staining method the air-dried slides were placed into 96% ethanol for fixation for 15 min and then stained using the routine protocol recommended by WHO (WHO, 2010) (reagents from Sigma Chemical Co., St. Luis, MO, USA). At the end of the procedure the slides were dehydrated with equal parts of absolute ethanol and xylene, then cleared with xylene alone for 1 min and mounted with DPX medium. The SpermBlue® staining method was carried out as previously described using commercially available kit (Microptic SL, Barcelona, Spain) (Van der Horst and Maree, 2009). Briefly, the slides were placed horizontally on a staining tray and covered with 1 ml of SpermBlue® fixative for 10 min. Then the fixative was gently removed. Immediately, without washing or drying the slides, 0.5 ml of SpermBlue® stain was put onto each fixed sperm smear for 12–15 min. The care was taken to displace stain equally across the smear surface. After removal of the stain by gently running off, the slides were slowly dipped into distilled water (only one or two dips lasting for 3 s). Then the slides were left in an upright position to air dry. Finally, the slides were mounted with DPX medium. All chemicals in this study were purchased from Sigma Chemical Company. For the eosin + gentian violet complex staining method, smears were prepared by carefully dragging a drop of the fresh sperm across a degreased microscopic slide heated to 37 °C (Kondracki et al., 2005). The slides were allowed to air dry for a minimum of 2 h, and were then prepared and preserved in a 96% ethanol solution during a 5-min exposure. After 30 min, the preserved slides were washed in distilled water, and then coloured with a 10% aqueous solution of eosin during a 20- to 60-s exposure. The coloured slides were washed in distilled water and coloured with gentian pigment during a 3- to 5-min exposure. After colouring, the slides were washed and dried. The slides were gently rinsed with distilled water for 2 min to remove debris. This procedure led