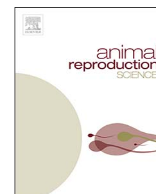
Animal Reproduction Science 184 (2017) 218–227

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci



Integrity of head and tail plasmalemma is associated with different kinetic variables in boar sperm

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ARTICLE INFO

Keywords:
Plasma membrane
Sperm lifespan
Sperm velocity
Sperm viability
Semen storage
Pig

ABSTRACT

An intact and functional sperm plasmalemma has a major role in sperm motility and fertilizing capacity. Several techniques have been developed to evaluate the integrity of the sperm plasma membrane, but there are still some inconsistencies concerning the methods that are more closely associated with sperm function. In this study, the aim was to: i) evaluate the integrity of the boar sperm plasmalemma during 72 h of semen storage at 17 °C using four techniques: eosin/nigrosin (E/N), propidium iodide/carboxyfluorescein diacetate (PI/CFDA), hypo-osmotic swelling test (HOST), and combined HOST with eosin staining (HOST/E), ii) assess the correlations and the limits of consistency among these techniques, iii) and estimate the relationships with the acrosomal status and sperm kinetics. Results indicate that the integrity of the sperm plasmalemma decreases during 72 h of storage, although significant differences were found only using the HOST and HOST/E techniques. Moreover, use of E/N and PI/CFDA results in greater values relative to the undamaged sperm membrane than use of HOST and HOST/E at any incubation time. Overall, results using all techniques were consistent and correlate except for findings with PI/CFDA and HOST, which was slightly below 95%. Moreover, values using the techniques for the evaluation of the integrity of the sperm head and tail membranes are positively associated with the acrosomal status and different kinetic variables with the tail integrity being related to rapid linear trajectories and the head integrity to rapid curvilinear trajectories. The results of this study provide new insights into the relevance of evaluating the boar sperm plasmalemma in the routine spermiogram.

1. Introduction

In artificial breeding systems, the main goal of the sperm analysis is to accurately estimate the male fertility potential. In boars, the integrity of the sperm plasmalemma has a major role in male fertility (Gadea et al., 1998; Juonala et al., 1998; Pérez-Llano et al., 2001). Two main characteristics of the sperm plasmalemma are commonly assessed in the standard spermiogram: structural and functional integrity. The first characteristic is commonly assessed by vital staining (e.g. Brito et al., 2003), whereas the second characteristic is evaluated using hypo- (Jeyendran et al., 1984) or hyper- (Quintero-Moreno et al., 2004) osmotic solutions. Because these assessments evaluate a single component of the sperm cell (i.e., head compared with tail), a more exhaustive evaluation of the sperm membrane integrity can be provided by combining both assessments.

For the simultaneous assessment of the sperm head and tail membranes, the hypo-osmotic swelling test (HOST) has been combined with vital staining for bright-field (eosin: Zhu and Liu 2000; eosin/nigrosin, E/N: Přinosilová et al., 2014) or *epi*-fluorescence

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http://dx.doi.org/10.1016/j.anireprosci.2017.07.020
Received 27 March 2017; Received in revised form 16 July 2017; Accepted 27 July 2017
Available online 29 July 2017
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(Hoechst: Lechniak et al., 2002; propidium iodide/carboxyfluorescein diacetate, PI/CFDA: Pérez-Llano et al., 2009) microscopy. The combined HOST with eosin staining (HOST/E) has provided evidence for four different sperm subpopulations, which are accurate predictors of male fertility (man: Chan et al., 1991; Ramirez et al., 1992; bull: Tartaglione and Ritta 2004; Quintero-Moreno et al., 2011). In boars, the HOST has been combined with E/N (Přinosilová et al., 2014), Hoechst (Lechniak et al., 2002), and PI/CFDA (Pérez-Llano et al., 2009) staining, but there is still limited knowledge concerning the correlation and agreement between this assessment and the other techniques in this species.

Across the animal kingdom, sperm motility is a major factor involved in sperm fertilizing capacity. In boars, sperm motility is positively associated with the plasmalemma integrity of the sperm head (Zou and Yang, 2000), tail (Zou and Yang, 2000; Samardžija et al., 2008; Lima et al., 2015) or both (Pérez-Llano et al., 2009) components, even when sperm motility was subjectively evaluated. A more accurate and objective assessment of sperm velocity can be achieved by the Computer Assisted Sperm Analysis (CASA) which provides several kinetic variables and reduces the bias of subjective 'estimates' (Amann and Waberski, 2014). Using this approach, a comprehensive analysis of the relationships between boar sperm kinetics and the integrity of plasmalemma is still lacking and might be useful to define which variables are most closely associated with the sperm motility.

In this study, the aim was to: i) analyze the integrity of the boar sperm plasmalemma during 72 h of semen storage at 17 °C using four techniques for the evaluation of the head, tail, or both sperm cell components; ii) assess the correlation and the limits of agreement among the techniques; iii) evaluate the relationship between the integrity of sperm plasmalemma, acrosomal status, and sperm kinetics.

2. Materials and methods

2.1. Reagents

All reagents were purchased from Sigma-Aldrich (Prague, Czech Republic), unless otherwise stated.

2.2. Semen collection, transport, and dilution

Commercial sperm doses from 11 boars were provided by an Artificial Breeding Centre (Chovservis, a.s., Hradec Králové, Czech Republic). Semen was collected by the gloved hand method and filtered through gauze to remove gel particles. Samples were assessed for concentration using a Bürker chamber (sperm concentration = $44.30 \pm 6.03 \times 10^6$ sperm/mL; mean ± SD) and diluted to the final concentration of approximately 20×10^6 sperm/mL with Solusem® extender (AIM Worldwide, Vught, Netherlands).

Sperm samples were stored in a cooling box at 17 °C and analyzed at four different times of storage: 0, 24, 48, and 72 h. Sperm analyses were conducted using the Nikon Eclipse E600 (Nikon, Tokyo, Japan) microscope. Before any analysis, samples were incubated for 15 min in a water bath at 38 °C.

2.3. Sperm plasmalemma integrity

Sperm cells (n = 200) were evaluated per each analysis by the same trained observer.

2.3.1. Sperm head plasmalemma integrity by E/N

The test was performed using eosin/nigrosin staining (Minitube, Tiefenbach, Germany). After incubating 10 μL of sperm samples with 10 μL of eosin/nigrosin for 30 s at 38 °C, the cells were smeared and air-dried. Sperm evaluation was performed under bright-field microscopy (40× objective). Sperm having only a white stained head were considered as sperm with intact plasmalemma (Fig. 1A), whereas those having a partial or only a pink stained head were considered to have a damaged plasmalemma (Fig. 1B).

2.3.2. Sperm head plasmalemma integrity by PI/CFDA

The assessment was performed as described by Harrison and Vickers (1990), with minor modifications. Briefly, a 200 μL of sperm sample was added to the pre-warmed and freshly prepared staining medium containing: 750 μL PBS, 20 μL CFDA stock solution (0.46 mg/mL in DMSO), 20 μL PI stock solution (0.5 mg/mL in PBS), and 10 μL formaldehyde solution (3%). Samples were then incubated in the dark for 10 min at 37 °C. Subsequently, sperm cells were evaluated using an *epi*-fluorescence microscopy (40× objective, Nikon filter B-2A). Sperm with complete green fluorescence of the head were considered as sperm with an intact plasmalemma (Fig. 1C), whereas those having a partial or complete red fluorescence of the head were considered to have a damaged plasmalemma (Fig. 1D).

2.3.3. Sperm tail plasmalemma integrity by HOST

The assessment was performed using a hypo-osmotic solution consisting of 7.35 g/L sodium citrate and 13.51 g/L fructose (Jeyendran et al., 1984). Samples (200 μL) were diluted in 600 μL of pre-warmed HOST solution and incubated for 30 min at 38 °C. At the end of the incubation, samples were fixed using a formaldehyde solution (3%). Subsequently, sperm cells were evaluated using a phase-contrast microscopy (40× objective) and the percentage of sperm with swollen tails was considered as sperm with intact plasmalemma (Fig. 1E), whereas those with non-swollen tails were considered to have a damaged plasmalemma (Fig. 1F).

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