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Acrosin activity is a good predictor of boar sperm freezability

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ABSTRACT

The main aim of this study was to determine whether acrosin activity could predict boar sperm freezability. For this purpose, we characterized the changes in sperm quality and acrosin activity throughout the cryopreservation procedure of sperm samples from 30 Pietrain boars by analyzing four critical steps: step 1 (extended sperm at 15 °C), step 2 (cooled sperm at 5 °C), step 3 (30 minutes postthaw), and step 4 (240 minutes postthaw). Freezability ejaculate groups were set on the basis of sperm motility and membrane integrity after freeze–thawing. Results obtained highlighted the low predictive value in terms of freezability of sperm motility and kinematics and sperm membrane integrity, as no differences between good and poor freezability ejaculates were seen before cryopreservation. Significant differences ($P < 0.05$) between ejaculate groups were observed in the cooling step at 5 °C for sperm kinetic parameters, and after thawing for sperm motility and membrane integrity. In contrast, acrosin activity appeared as an indicator of boar sperm freezability because the differences ($P < 0.05$) between good and poor freezability ejaculates manifested yet in extended samples at 15 °C. On the other hand, we also found that variations in sperm kinematics, membrane lipid disorder, intracellular calcium content, acrosome integrity, and acrosin activity throughout the cryopreservation procedure were indicative of a significant damage in spermatozoa during the cooling step in both ejaculate groups. In conclusion, the main finding of our study is that acrosin activity can be used as a reliable predictor of boar sperm freezability because it differs significantly between good and poor freezability ejaculates yet before freeze–thawing procedures took place, i.e., in the refrigeration step at 15 °C.

1. Introduction

Sperm cryopreservation is currently the most efficient method for long-term storage of boar spermatozoa [1,2]. However, freeze–thawing protocols are known to affect boar sperm function and survival, as well as to decrease sperm fertilizing ability, as a result of alterations in plasma membrane, acrosome and nucleus integrity, and of reduced sperm motility [1,3–5].

In boars, extensive damage of sperm plasma membrane during cooling and thawing has been attributed to the low content of cholesterol and saturated phospholipids, as compared with other species [6]. This species-specific lípid composition results in a decreased sperm viability after thawing and also in a shortened life span of surviving spermatozoa [3,4]. Both limitations require artificial insemination (AI) to be carried out using large numbers of spermatozoa and closely timed to the moment of ovulation [6,7].

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Moreover, the sperm ability to sustain cryopreservation procedures, i.e., freezability, differs among boars and also among ejaculates coming from the same boar [8–11]. Therefore, ejaculates can be classified according to their resistance to withstand cryopreservation procedures into "good freezability ejaculates" (GFEs) and "poor freezability ejaculates" (PFEs) [1–3].

Prediction of boar ejaculate freezability is usually based on the evaluation of several sperm parameters after thawing, such as membrane integrity and sperm motility [3,7,10,12]. Besides, recent research has been focused on the identification of molecular markers that could be used as reliable predictors of boar ejaculate freezability [1–4,13]. In spite of this, the predictive value of sperm quality parameters before cryopreservation remains controversial, whereas molecular approaches are complex and time consuming [4,13], and thus they do not have a real application in routinary practice.

Against this background, the main objective of the present approach is to determine the predictive value of acrosin activity in terms of boar sperm freezability. For this purpose, we have characterized the changes in acrosin activity, together with sperm quality parameters, throughout the entire cryopreservation procedure by analyzing four critical steps: step 1 (extended sperm at 15 °C), step 2 (cooled sperm at 5 °C), step 3 (30 minutes postthaw), and step 4 (240 minutes postthaw). Analysis of sperm parameters 240 minutes postthaw gives an estimation about the survival of frozen-thawed (FT) sperm within the insemination-to-ovulation interval recommended for cryopreserved doses [4,13].

2. Materials and methods

2.1. Material

All chemicals were obtained from Sigma–Aldrich Química, S.A. (Madrid, Spain) unless otherwise indicated.

2.2. Semen collection

The study was performed using 30 ejaculates obtained from 30 sexually mature Piétrain boars (one ejaculate per boar) from the same genetic line (Riudarenes, Girona, Spain). All boars were two years old and were included in an AI program because they produced ejaculates of high sperm quality, with more than 80% of spermatozoa without morphoabnormalities, 80% total motility, 50% progressive motility, 80% sperm viability, and 80% nonexocytosed acrosomes (data not shown). Before and throughout the experimental period, the boars were housed in the same climate-controlled pens, fed under standard protocols and provided with water *ad libitum,* and subjected to a semen collection rhythm of twice per week, following the guidelines established by the Animal Welfare Directive of the Regional Government of Catalonia.

All ejaculates were obtained within 4 weeks by the same expert technician by mounting of the males on a dummy sow and the gloved-hand technique. The barn containing the dummy was placed in the same building in which the boars were lodged, so there was not any effect of seasonality in the collection procedure. The sperm-rich fraction of each ejaculate was collected in a plastic insulated flask with a gauze fitted over to remove the gel and prediluted 2:1 (v:v) in a long-term extender (Duragen, Magapor SL, Zaragoza, Spain). The extended semen samples were cooled and maintained at 15 °C for shipment to the laboratory of TechnoSperm in the University of Girona. Shipped semen samples were sent in an insulating recipient at 15 °C on the same day of extraction. Once in the laboratory, sperm quality analyses of semen samples were performed to ensure that minimal standards were fulfilled before cryopreservation.

Analyses of sperm quality were also performed in the four following steps: (1) before starting the cryopreservation procedure (i.e., at 15 °C), (2) at the end of the cooling step (i.e., after sperm being cooled at 5 °C in lactose–egg yolk [LEY] extender for 120 minutes), (3) 30 minutes after thawing (FT 30 minutes), and (4) 240 minutes after thawing (FT 240 minutes). Sperm parameters analyzed were sperm membrane integrity, sperm motility, acrosin activity, intracellular calcium levels, and sperm membrane lipid disorder.

2.3. Sperm cryopreservation and thawing

Semen samples were cryopreserved following the procedure described by Carvajal et al. [14] and adapted in our laboratory [3,4,7]. After a holding time of 24 hours at 15 °C (step 1: extended) [5], the semen samples were aliquoted and centrifuged at 15 °C and 640× *g* for 3 minutes and the pellets obtained from each boar were pooled and diluted to 1.5×10^9 spermatozoa/mL into a freezing medium containing LEY. After cooling to 5 °C for 120 minutes (step 2: cooled), semen samples were rediluted to 1×10^9 spermatozoa/mL in another freezing medium (LEYGO) composed by LEY supplemented with 6% glycerol and 1.5% Orvus Es Paste (Equex STM; Nova Chemical Sales Inc., Scituate, MA, USA). Semen samples were packed into 0.5-mL plastic straws (Minitub Ibérica SL, La Selva del Camp, Spain) using an automatic filling system (MPP Quattro; Minitub Ibérica SL). The straws were transferred into a programmable freezer (IceCube 14S-B with SY-LAB software; Minitub Ibérica SL) and cooled for 5 minutes and 13 seconds at the following rates: −6 °C/min from 5 to −5 °C (1 minute 40 seconds), −39.82 °C/min from −5 to −80 °C (1 minute 53 seconds), held for 30 seconds at −80 °C and −60 °C/min from −80 °C to −150 °C (1 minute 10 seconds). The straws were finally plunged into liquid nitrogen (−196 °C) for storage.

After samples had been stored for at least 2 weeks in liquid nitrogen, four straws per boar were thawed for 20 seconds in water bath at 37 °C and diluted to 1:3 (v:v) in Beltsville Thawing Solution at the same temperature [15]. Frozen-thawed spermatozoa were analyzed at 30 (step 3: FT 30 minutes) and 240 minutes (step 4: FT 240 minutes) postthaw.

2.4. Analyses of sperm motility

To assess the sperm motility, an aliquot per ejaculate was taken in each of the previously defined steps of the