Animal Reproduction Science 133 (2012) 109–116

Contents lists available at SciVerse ScienceDirect

Animal Reproduction Science

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

Effect of different monosaccharides and disaccharides on boar sperm quality after cryopreservation

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ARTICLE INFO

Article history:
Received 22 September 2011
Received in revised form 14 March 2012
Accepted 15 June 2012
Available online 21 June 2012

Keywords:
Disaccharide
Monosaccharide
Extender
Cryopreservation
Boar sperm

ABSTRACT

The aim of the present study was to evaluate the cryoprotectant effect of different non-permeating sugars for boar sperm. Pooled semen from three boars was used for the experiments. In the first experiment, the sperm quality of boar sperm cryopreserved with an egg-yolk based extender supplemented with different monosaccharides (glucose, galactose or fructose) was compared to a control cryopreserved in lactose–egg yolk extender. In the second experiment, the effect of five disaccharides (lactose, sucrose, lactulose, trehalose or melibiose) on boar sperm cryosurvival was studied. Several sperm quality parameters were assessed by flow cytometry in samples incubated for 30 and 150 min at 37 °C after thawing: percentages of sperm with intact plasma membrane (SIPM), sperm presenting high plasma membrane fluidity (HPMF), sperm with intracellular reactive oxygen substances production (IROSP) and apoptotic sperm (AS). In addition, the percentages of total motile (TMS) and progressively motile sperm (PMS) were assessed at the same incubation times with a computer-assisted sperm analysis system. Freezing extenders supplemented with each of the monosaccharide presented smaller cryoprotective effect than the control extender supplemented with lactose ($P < 0.05$). However, from the three monosaccharides tested, glucose provided the best sperm quality after freezing–thawing. With respect to the disaccharides studied, samples frozen with the extender supplemented with lactulose exhibited in general the lowest sperm quality, except for the percentage of capacitated sperm, which was highest ($P < 0.05$) in the samples cryopreserved with the trehalose extender. Our results suggest that disaccharides have higher cryoprotective effect than monosaccharides, although the monosaccharide composition of the disaccharides is also important, since the best results were obtained with those disaccharides presenting glucose in their composition.

1. Introduction

Recently it has achieved great advances in the use of frozen–thawed semen in artificial insemination of swine (Okazaki et al., 2009; Casas et al., 2010; García et al., 2010). But there are still many weaknesses in the sperm cryopreservation process that should be improved before frozen–thawed boar semen can be applied with the same efficiency of liquid semen in routine AI programmes in commercial pig farms. One important improvement should be adjustments in the composition of the freezing extenders. The basic ingredients of current sperm freezing

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http://dx.doi.org/10.1016/j.anireprosci.2012.06.010

extenders are the same as those used 35 years ago, including the cryoprotectant agents glycerol, egg yolk and sugars (Johnson et al., 2000; Barbas and Mascarenhas, 2009).

The beneficial effect that sugars have on boar sperm freezing has been reported in several works (Pursel et al., 1972; Yi et al., 2002; De Los Reyes et al., 2002; Corcuera et al., 2007; Malo et al., 2010). Nevertheless, the cryoprotective effect of these compounds is not fully defined. Sugars are not able to diffuse across the plasma membrane, creating an osmotic pressure that induces cell dehydration and a lower incidence of intracellular ice formation (Fuller, 2004), and some authors declare that the cryoprotective effects of the sugars on sperm cells may differ according to the molecular weight of the sugars (Anchordoguy et al., 1987; Molinia et al., 1994), and the sugars having higher molecular weights exhibited a greater cryoprotective effect (Pursel et al., 1972).

Nowadays lactose is the sugar most commonly used for boar sperm cryopreservation, due to the good results it provides (Roca et al., 2004; Fraser et al., 2007; Sancho et al., 2007; De Mercado et al., 2010; Buranaamnuay et al., 2011). However, recent studies showed that the sugars, like trehalose, with the same molecular weight than lactose but with different composition in monosaccharides, had different protective effect against the freezing process (Hu et al., 2008, 2009; Gutiérrez-Pérez et al., 2009; Malo et al., 2010). These results suggest that the molecular weight, the composition or structure of these sugars could influence their cryoprotectant ability.

Bearing these results in mind, the objective of this study was to compare the cryoprotective effects of different sugars on boar sperm, according to their composition.

2. Materials and methods

2.1. Reagents and media

All chemicals were of analytical grade. Unless otherwise stated, all media components were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and were made with purified water (18 MΩ cm; Automatic GR Wasserlab, Spain). Orvus ES Paste (OEP) is marketed as Equex STM (Nova Chemical Sales Inc., Scituate, MA, USA).

The basic medium used for semen extension was Beltsville Thawing Solution (BTS, composed of 205 mM glucose, 20.39 mM NaCl, 5.4 mM KCl, 15.01 mM $NaHCO_3$, and 3.35 mM EDTA; pH 7.2 and 310 ± 5 mOsm/kg; Johnson et al., 1988), containing kanamycin sulphate (50 mg/mL).

For evaluating the presence of apoptotic sperm, Annexin-binding-buffer was used (composed of 10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$; Peña et al., 2003).

The control medium used for sperm freezing was lactose–egg yolk extender (LEY) composed of 80% (v:v) β-lactose solution (310 mM in water), 20% (v:v) egg yolk and 100 μg/mL kanamycin sulphate (pH 6.2 and 330 ± 5 mOsm/kg; Westendorf et al., 1975). The other diluents tested were made by substituting the lactose in the diluent by each of the other saccharides at the same concentration (80% of sugar solution (310 mM in water)).

2.2. Source, handling, evaluation and processing of ejaculates

Three sexually mature and fertile crossbreed boars, with ages ranging from 1.5 to 2 years, were used in this study. Boars were housed at a commercial insemination station (Prosepor S.A., Segovia, Spain), in climate-controlled individual pens (15–25 °C). They had ad libitum access to water and were fed a commercial diet according to the nutritional requirements for adult boars. The protocols used fulfilled the European regulations for the care and use of animals for scientific purposes (EC Directive 2010/63/EU).

Sperm-rich fractions were collected using the gloved-hand method, extended in BTS (1:1 [vol/vol]) and evaluated for conventional semen characteristics using standard laboratory techniques. Only ejaculates presenting ≥200×10^6 sperm/mL, ≥85% sperm with normal morphology, ≥75% of motile sperm and ≥80% of viable sperm (sperm with an intact plasmatic membrane), were selected for cryopreservation.

The extended semen was transferred to 50 mL plastic tubes, cooled to 15 °C, packaged in insulated containers and sent (in less than 2 h) to the laboratory (Pig Research Centre, Instituto Tecnológico Agrario de Castilla y León, Spain) for cryopreservation.

2.3. Freezing–thawing procedure

Spermatozoa were cryopreserved using the straw-freezing procedure described by Westendorf et al. (1975) and subsequently modified by Thurston et al. (2001) and Carvajal et al. (2004).

At the laboratory, extended sperm-rich fractions were pooled and centrifuged at 2400 × *g* for 3 min at 15 °C (Universal 320 R, Hettich Zentrifugen, Germany), and the pellets were diluted with their correspondant freezing extender to a concentration of 1.5×10^9 cells/mL. After further cooling to 5 °C within 120 min, the extended sperm were diluted (2:1; v:v) in egg yolk sugar–Glycerol–Orvus Es Paste extender (92.5% egg yolk sugar extender + 6% Glycerol, v:v + 1.5% OEP) to yield a final concentration of 1×10^9 cells/mL and were then packed into 0.5 mL PVC-French straws (Minitüb, Tiefenbach, Germany). Straws were frozen using a controlled-rate freezer (IceCube 14S; Minitüb), as follows: from 5 to −5 °C at a rate of 6 °C/min, from −5 to −80 °C at 40 °C/min, held for 30 s at −80 °C, then cooled at 70 °C/min to −150 °C and plunged into liquid nitrogen (LN_2). The straws remained in the LN_2 tank for at least two weeks before thawing.

Straws were thawed in a circulating water bath at 37 °C for 20 s. Thawed sperm samples were extended in BTS (1:1, v/v; 37 °C) and incubated in a water bath at 37 °C for 150 min. Sperm analyses were performed 30 and 150 min after thawing.

2.4. Evaluation of sperm characteristics

Each frozen–thawed sample was assessed for motility, viability (plasma membrane integrity), reactive oxygen