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Seminal plasma proteins interacting with sperm surface revert capacitation indicators in frozen-thawed ram sperm

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ABSTRACT

This study was conducted to evaluate the effects of interacting seminal plasma proteins (iSPP) obtained by AV or EE on frozen-thawed ram sperm in order to test the hypothesis whether this fraction could be sufficient to emulate the effect of complete seminal plasma (SP). Additionally, we evaluated whether these proteins have a differential effect between spermatozoa from high and low fertility rams and between breeding and non-breeding seasons. We assessed sperm motility, quality parameters (intracellular reactive oxygen species, membrane fluidity, plasma membrane permeability and mitochondrial activity) and capacitation status. The main findings from this work were: i) iSPP had no effect on sperm motility, whereas SP (AV or EE) addition produced the highest values of total motility (74.13 ± 2.99 and 72.27 ± 2.99 for AV and EE, respectively) and progressive motility (64.97 ± 2.64 and 63.73 ± 2.64 for AV and EE, respectively); ii) iSPP had no effect on sperm quality parameters ($p > 0.05$), but whole SP improved all parameters evaluated. Moreover, SP collected by AV yielded significantly higher viability (44.60 ± 2.87) and sperm with stable plasma membrane (44.56 ± 2.49) comparing with the addition of SP collected by EE (35.80 ± 2.47 and 36.67 ± 1.71, respectively); iii) iSPP and SP collected by EE, but not by AV, reverted molecular signals of capacitation as protein tyrosine phosphorylation caused by freezing temperatures; iv) there were no effects of fertility or season in sperm quality parameters evaluated. This study demonstrated that, although the iSPP have a clear decapacitating effect, including the ability to revert cryo-capacitation indicators, they are not sufficient to emulate the effects of complete SP regarding sperm functional parameters.

1. Introduction

The cryopreservation process, involving cooling, freezing and thawing produce serious detrimental changes in ram sperm function (Ari et al., 2011). Sperm cry-

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oinjury includes the reduction of viability, motility and mitochondrial membrane potential, chromatin damage, increase in the production of reactive oxygen species, activation of apoptosis (Said et al., 2010) and perhaps the most important cryoinjury, the premature induction of a capacitation-like status (Bailey et al., 2003). All of these alterations result in reduced longevity of the cryopreserved spermatozoa within the female reproductive tract, decreasing the likelihood of successfully interact with the oviduct or fertilize the ovum.

Seminal plasma (SP) is a mixed secretion from several glands of the male reproductive tract. Supplementation of frozen/thawed ram sperm with SP has been noted to improve sperm characteristics including motility, viability (Ollero et al., 1997; Domínguez-Rebolledo et al., 2007; Maxwell et al., 2007; Domínguez et al., 2008) and the ability to penetrate the cervical mucus (Graham, 1994; Maxwell and Johnson, 1999; El-hajj Ghaoui et al., 2007). Moreover, whole ram SP addition improved cryopreservation of goat semen (Ari and Daskin, 2010). Dott et al. (1979) observed that the effects of SP on sperm cells lasted even after the removal of SP.

The beneficial impact of SP on sperm has been attributed to its proteic components, since seminal plasma proteins (SPP) are able to increase sperm resistance against cold shock (Barrios et al., 2000; Pérez-Pé et al., 2001; Colás et al., 2009) and stimulate sperm function and fertilizing ability (Maxwell et al., 2007). It has already been shown that SPP support survival of ram spermatozoa acting not only at the plasma membrane level but also by inhibiting capacitation (Desnoyers and Manjunath 1992; Barrios et al., 2005) and apoptosis-like changes (Mendoza et al., 2013).

Bernardini et al. (2011) demonstrated that a fraction of SPP with affinity by the sperm membrane was able to repair ultrastructural damage and improve motility of frozen/thawed ram sperm. This fraction, enriched in RSVP14 and RSVP22 proteins, was called interacting SP proteins (iSPP). Previous work has demonstrated that these proteins were partially able to protect and repair ram sperm membrane against cold-shock damages and detrimental effects of cryopreservation process (Barrios et al., 2000 Pérez-Pé et al., 2001; Barrios et al., 2005; Ari and Daskin 2010) as well as to maintain fresh sperm in a decapacitated state (Mendoza et al., 2013). Domínguez et al. (2008) observed that the composition and protein concentration of SP varies according to season. Moreover, in a recent work we noted that ram iSPP varies according to the collection method applied and that the iSPP collected by electroejaculation (EE) has a greater concentration of low molecular weight proteins, such as those considerate as crioprotectans, than the same fraction obtained by artificial vagina (AV) (Ledesma et al., 2014a).

With this background, in this work we evaluated the effect of addition of iSPP obtained by AV or EE to thawed spermatozoa from ram with low or high fertility and tested the hypothesis that it could be sufficient to emulate the effect of complete SP. Additionally, we evaluated whether the iSPP had a differential effect on sperm quality between breeding and non-breading season.

2. Materials and methods

2.1. Reagents

Flow cytometry consumables (including the sheath fluid) were purchased from BD Biosciences (San Jose, CA, USA). The rest of the chemicals were acquired from Sigma. Chemicals were of the highest grade available. PBS was prepared in milli-Q water: 136.9 mM NaCl, 2.7 mM KCl, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, 7 mM Na_2HPO_4, 1.25 mM NaH_2PO_4, 1.5 mM KH_2PO_4 and 1 g/l PVA (290 mOsm/kg; pH 7.2).

2.2. Frozen semen

All experiments were performed using frozen spermatozoa from 10 adult Assaf rams divided in two groups of five rams each, accordingly to their fertility in low ($35.3 \pm 3.0\%$) and high ($60.4 \pm 1.5\%$) fertility. Frozen semen was supplied by OVIGEN (Centro de Selección y Mejora Genética de Ovino y Caprino de Castilla y León, Toro, Spain).

2.3. Animals

All animal procedures were in accordance with the Spanish Animal Protection Regulation RD 1201/2005, according to European Union Regulation 2003/65. Eight mature Assaf rams were used for obtaining SP and iSPP. Two experiments were carried out, one in autumn (breeding) and the other in spring (non-breeding season).

2.4. Seminal plasma and interacting seminal plasma proteins: collection and processing

SP and iSPP were obtained from all males by AV and EE according to Marco-Jiménez et al. (2005) separately by two days between methods. We performed one seminal collection during autumn (breeding season) and one seminal collection during spring (non-breeding season). Ejaculates with total motility ≥80% were pooled by collection method and then split in two parts. One of them was used to obtain SP and the other one was used to obtain the iSPP. Briefly, for obtaining SP, half of the pooled semen was centrifuged twice ($2000 \times g$ for 15 min at 4 °C). The clear supernatant (SP) was recovered, filtered (0.22 μm) and kept at −80 °C until use. The other half of the semen pool was used to recover iSPP. Ejaculates were washed 10 times with PBS to remove excess of unbound SP ($800 \times g$, 10 min). Sperm free from SP were incubated with 200 μL buffer 10 mM Glycine-HCl pH 3 (20 min, room temperature, with agitation). Finally, sperm were centrifuged and the supernatant was conserved and neutralized with 5 μL Tris-HCL 2 M pH 9.5. Protein concentration of SP and iSPP were assessed according to the method described by Bradford (1976), using BSA for the standard curve. The concentration of proteins eluted with low pH buffer from the sperm surface coming from a known volume of SP was calculated as mg/ml.