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Do different portions of the boar ejaculate vary in their ability to sustain cryopreservation?

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Abstract

Previous studies have shown sperm quality post-cryopreservation differs depending on the fraction of the seminal plasma boar spermatozoa are fortuitously contained in. As such, spermatozoa contained in the first 10 mL of the sperm-rich fraction (portion I) have better sustained handling procedures (extension, handling and freezing/thawing) than those contained in the ulterior part of a fractionated ejaculate (second portion of the sperm-rich fraction and the post-spermatic fraction, portion II). However, those studies were performed using pooled samples. In the present study, individual ejaculates were used. Split ejaculates (portions I and II) from five boars were frozen and thawed using a conventional freezing protocol, followed by computer-assisted motility and morphology analysis (CASA and ASMA, respectively), as well as an Annexin-V assay for spermatozoa from each boar and ejaculate portion. Significant differences between portions were observed in all ASMA-derived variables, except in one boar. Also significant differences were observed between boars and ejaculate portions in sperm quality post-thaw. We identified, however, boars showing best results of motility and sperm membrane integrity post-thaw in portion I, while in other boar the best results was observed in portion II. It is concluded that the identification of the ejaculate portion more suitable to sustain

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cryopreservation in each individual boar may be a readily applicable and easy technique to diminish variation in sperm freezability among boars.

Keywords: Boar; Cryopreservation; Ejaculate portions; Spermatozoa; CASA; ASMA; Annexin

1. Introduction

Cryopreservation of boar semen is associated with different insults to the spermatozoa, such as cold shock, osmotic stress and intracellular ice crystal formation during freezing and (again) during thawing (Mazur, 1984). Such insults lead to major injuries in a large number of spermatozoa and following thawing, a large number of spermatozoa are found dead or grossly damaged. As well, among the surviving spermatozoa, a subpopulation is affected, basically showing a shortened life span, both in vitro and in vivo (Bailey et al., 2000). Among these cells, a certain percentage show changes in behavior (motility patterns, redistribution of Ca^{2+}, etc.) collectively called "capacitation-like changes" for their resemblance with this process, and such the concept of "cryocapacitation" (Watson, 1995) was assumed as partially responsible for the reduced fertility the processed semen presented (Watson, 2000; Green and Watson, 2001). There is some evidence supporting the theory of the capacitation-like changes induced by cooling and rewarming (Fuller and Whittingham, 1997; Kaneko et al., 2003), but such changes have always been estimated using the chlortetracycline (CTC) assay, a method whose mechanism of action has not yet been clarified.

In any case, whether these changes really resemble capacitation or merely represent unspecific damage to the sperm plasma membrane, finally resulting in a shorter lifespan of the spermatozoa, remains to be determined. In view of these facts, the use of frozen-thawed boar semen, although valuable as a tool to transfer genetic material, has not achieved widespread acceptance for commercial breeding by artificial insemination (AI). Reasons for this lack of acceptance include the lower cost and good success of liquid semen AI providing no impetus for change. Also, the poor post-thaw survival of pig spermatozoa and the between boar variation in freezing success constrain fertility to AI with frozen semen. Various approaches have been used attempting the improvement of the quality of frozen-thawed boar semen, including novel packaging systems (Eriksson and Rodriguez-Martinez, 2000), changes in holding times before freezing (Eriksson et al., 2001), addition of various additives (Peña et al., 2003a, 2004; Roca et al., 2004, 2005), and new technologies to perform deep intrauterine inseminations (Vazquez et al., 2005). However, the existence of great variability among boars to sustain cryopreservation, and even differences between ejaculate portions (Peña et al., 2003a,b), suggests that an important approach to improve the current freezing technologies for boar semen is the identification of those boars and/or ejaculate fractions that will better sustain this technology. Previous works (Peña et al., 2003a,b, 2004) indicate that spermatozoa present in the first 10 mL of the sperm-rich fraction (portion I) better sustain cooling and freezing-thawing compare to those fortuitously present in the bulk ejaculate. The ejaculate portion had proven a significant effect on sperm membrane integrity, motility patterns and capacitation-like changes, including the use of an Annexin-V assay