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Alterations of Domains in the Plasmatic Membrane Due to Damages of the Perinuclear Theca of Pig Preserved Spermatozoa

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Abstract: Samples of semen from 12 pigs, three from Yorkshire, Landrace, Duroc and Mexican Hairless each where obtained to study cryopreservation methods. Three stages of boar semen cryopreservation were evaluated: none (fresh stage), cooling at 5°C and freezing at -196°C then thawing to 56°C for 12 sec. Perinuclear theca damage and domain alterations were selected as indices of seminal quality, as measured by electronic and fluorescence microcopy, respectively according to two lineal models considering by separately the effect of semen preservation and breed. Integrity and absence of perinuclear theca significantly ($p<0.001$) decreased and increased, respectively according to a decrease in temperature of cryopreservation, from 87.4 to 58.8% and from 0.8 to 26.2%, respectively. This same significant ($p<0.001$) effect was found for acrosomal and post-acrosomal membrane distribution of domains, from 92.1 to 76.8% and from 3.1 to 13.1% in this same order. Slight but highly significant ($p<0.001$) differences were observed when theca integrity was evaluated as affected by breed, with highest and lowest values for Yorkshire and Pelón Mexicano pigs, respectively. No breed effect was encountered for presence of acrosomal domains. A strong interdependence was found between perinuclear theca damage and domain distribution. In this connection, a highly significant ($p<0.001$) positive, interdependence was observed between the theca damage and acrosomal domain ($r = 0.87$), while this same relationship was although highly significant ($p<0.001$), negative in nature for equatorial and post-acrosonal domains ($r = -0.77$ and -0.85, respectively). This experiment confirmed that cryopreservation methods may severely affect semen quality of pigs and that genotype may further influence these same indices. More research is needed for improving methods of preservation of pig semen quality, from the point of view of perinuclear theca and domain characteristics of spermatozoa.

Key words: Pig, semen quality, perinuclear theca, spermatozoa, breed

INTRODUCTION

The use of frozen pig semen is far from being placed in a relevant position (Gilian *et al*., 2004; Wongtawan *et al*., 2006) and it is at least partially due to the spermatozoid susceptibility to damage provoked by the freezing-thawing process (Wevar *et al*., 1997; Johnson *et al*., 2000; Guthrie and Welch, 2005; Roca *et al*., 2006). This fact leads to the use of higher cell concentrations per dosage during insemination (Cerolini *et al*., 2001). However, freezing of pig semen shows interesting possibilities for applying not only in breeding or commercial pig herd improving, but in programs of local breed conservation and research in reproduction (Curry, 2000; Holt, 2000).

The real problem that cryopreservation experiences is not the ability degree of the spermatic cell to be kept viable during storage at -196°C, but it is in fact the combination of negative effects that freezing-thawing process determine on sperm physiology and morphology (Watson, 2000; Córdova and Gutierrez, 2002). Overall, cell damage is reflected in a decrease in motility and ultrastructural membrane damage (Johnson *et al*., 2000; Tienthai *et al*., 2004). In this connection, membranes of pig spermatozoid are more sensible to damage due to freezing than those of other domestic species, due to its lipid composition (Cerolini *et al*., 2001). This membrane is considered as a puzzle of domains where every one does have specific characteristics. The domains are established

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