A176 Physiology of Reproduction in Male and Semen Technology

Immunodetection of angiotensin converting enzyme in Nelore sperm

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Testicular form of angiotensin-converting enzyme (tACE) is a ectoenzyme anchored to periacrossomal region of the sperm. This enzyme is able to release the extracellular portion of proteins anchored by glycosylphosphatidylinositol (GPI) that are essential for oocyte fertilization. The study was designed to perform immunodetection in spermatozoa and seminal plasma, and immunolocalization in spermatozoa of tACE before and after freezing semen of Nelore bulls. Semen samples from 10 sexually mature bulls were used. After collection with eletroejaculator, half of the ejaculate was frozen and the other half processed in natura. Immediately after collection or thawing, the semen was centrifuged twice and the pellet resuspended with TALPH. Samples were standardized to a concentration of 100x106 spermatozoa in 100 µl and submitted to SDS-PAGE and immunocytochemistry using anti-ACE antibody (Costa and Thundathil, 2012. Anim Reprod Sci 133: 35- 42). The monoclonal antibody used was able to recognize a single protein band at 100 kDa in sperm suspension of the 10 Nellore as well as seminal plasma of these animals. The protein bands were very well identified, clearly demonstrating the presence of ACE in sperm cells and seminal plasma. The ACE immunodetection in sperm was characterized by intense staining observed on all periacrossomal region showing the location of this enzyme in the sperm. After thawing, it is clearly perceived that the cryopreservation process reduced the intensity of protein bands, suggesting that this enzyme was lost during the protocol used. Corroborating with the western blot data, we also note that the cryopreservation process reduced the intensity of the fluorescent dye in the immunocytochemistry technique, suggesting that this enzyme is lost during the cooling / freezing. However, there was no change in the enzyme's location according to the freezing protocol used. We conclude that the cryopreservation process reduces the amount of TACE in the Nelore bulls sperm.

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