Bulletin UASVM, Veterinary Medicine 67(1)/2010
ISSN 1843-5270; Electronic ISSN 1843-5378

Evaluation of Temporal Development of the Acrosome Reaction *In Vitro* in Spanish Black Berrendo Breed Bulls by Epifluorescence Methods.

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SUMMARY

Black Berrendo cattle breed belongs to the Andalusian heritage bovine livestock and it is a endangered one, protected by the EU programs of cattle conservation. There are only seminal frozen doses from 10 bulls of this breed. It is important to know their ability to be capacitated in vitro for later use in IVF as a technique for recovery of their populations.

The aim of this study was to evaluate the evolution of the acrosome reaction in vitro through the time in a defined culture medium by epifluorescence microscopy methods in seminal frozen samples of Spanish Black Berrendo bulls.

Samples (0.25 ml.) of frozen semen from 5 Berrendo Black breed bulls were thawed and incubated with Sp-TALP culture medium enriched with 100 µgr/ml of sodium heparin as capacitating agent. Aliquots of 100 µl were taken at 0, 60 and 120 minutes. These were stained with Propidium Iodide and FITC-PNA following a modified protocol in our lab and extended on slides. Slides were mounted and observed in a fluorescence microscope with excitation filter BP 450-490 nm and a emission filter of BP 515-560nm. Acrosome reaction was positive in those sperm cells in which a green color in the acrosomal region and a faint red coloration in the rest of the head were found and negative one in those in which only the faint red coloration appeared. The capacitating percentage was evaluated on individual bulls in different times of capacitating analyzing 200 cells three times. The results showed some significant differences at 0.05% for different times. After 60 minutes of incubation the capacitating percentage is the adequate to use for IVF. The values obtained in this Andalusian bovine breed were consistent with previous studies in other breeds. In the study 50% of frozen sperm samples of this breed were suitable to use for IVF. This technique proves to be quick and effective way to evaluate bovine semen capacitated in vitro.