UAEM Universidad Autónoma del Estado de México

V CONGRESO AMERICANO DE CUNICULTURA, MÉXICO 2014
Facultad de Medicina Veterinaria y Zootecnia, Asociación Científica Mundial de Cunicultura – Rama Americana
Secretaría de Desarrollo Agropecuario del Gobierno del Estado de México, Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, Consejo Mexiquense de Ciencia y Tecnología

COMPARISION OF RABBIT SPERM VIABILTY EVALUATION USING EOSIN-NIGROSIN AND TRIPLE- STAINING

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ABSTRACT

Semen evaluation is a very important duty in any reproduction program. Different viability techniques are available for many species; however, not all can be applied to some species, since the phospholipid and protein composition of the sperm plasma membranes differ, allowing to obtained different patterns to evaluate and distinguish live from dead sperm, as well as the acrosome integrity. Viability is mostly important to evaluate when sperm is preserved for articial insemination. Therefore, the aim of the present study was to compare rabbit sperm viability using two different staining techniques. Semen from 3 reproductive active New Zealand rabbits was obtained and pooled. Right after obtaining the fresh semen samples, evaluation of viability was performed before and after dilution in a freezing diluent, right after the cooling period on one hour at 4°C, by staining the samples with eosin-nigrosin, as well as triple-staining. After the eosin-nigrosin slides were evaluated under an optic microscope (VELAB, VE-V1), incubation with 20%-giemsa was performed, and a new evaluation of the slide was done under a phase constrast microscope (Olympus, IX70). Countings of 100 sperm-cells was performed twice by each slide. Our results differed between techniques, while evaluating with eosin-nigrosin, viability was higher for all samples ($P<0.05$), when evaluation was done with the triple staining, viability results decreased drastically (40 to 80%, $P<0.05$). Therefore, no concordance on the viability results was found between both staining techniques. In the present study, evaluation of viability and acrosomal status using triple staining for rabbit sperm was very difficult, since patterns were not clear to differentiate live from dead sperm, neither the presence of the acrosomal vesicle. In conclusion, triple staining was not an appropriate technique for evaluation of rabbit semen.

Keywords: rabbit sperm viability, eosin-nigrosin, triple-staining, semen preservation.