EFFECT OF DISTINCT ANTIBIOTICS ON POST-THAWING VIABILITY OF RAM SPERM

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Sperm cryopreservation would be a suitable alternative for the preservation of ovine breeds at risk of extinction, such as the Crioula Lanada breed in Brazil. However, that strategy may be limited by the usually reduced post-thawing viability of ram sperm. Among the many additives present in extenders for frozen ram sperm, antibiotics are commonly added to prevent disease transmission through semen without jeopardizing its viability. The objective of this study was to evaluate the effects of distinct antibiotics commonly included in extenders for frozen semen on the viability of ram sperm after thawing. Ejaculates were collected twice a week during six weeks from five rams from the Crioula Lanada breed (n = 12), using an artificial vagina. At the time of collection, ejaculates were diluted 1:1 in a TRIS-egg yolk-based extender. Ejaculates were pooled with a fixed spermatozoa concentration of $50x10_6$ sperm/straws. Thirty minutes lates, glycerol was added to the extender. The pool was split in 5 treatments: T1, including no antibiotics (control); T2 (gentamicin, tylosin, spectiomicine and lincomycin); T3 (penicillin and streptomycin); T4 (Sodium ceftiofur); and T5 (enrofloxacin). Sperm was packed in 0.25 mL straws, cooled up to 5°C, frozen in vapor of liquid nitrogen and stored. After thawing, sperm motility was determined with optic microscope and integrity of sperm membrane, acrosome and DNA were determined with an epifluorescent microscope using, respectively, the following fluorescent probes: prodium iodide and carboxyfluorescein, prodium iodide and PNA-FICT and acridine orange in Carnoy. Sperm motility was lower ($P < 0.05$) for T5 (17.1%) than for both T1 (40.9%) and T2 (25.4), but no further differences in sperm motility were observed among treatments. Sperm membrane integrity did not differ ($P > 0.05$) for T1 (20.1%), T2 (18.5%), T3 (19.1%), T4 (20.1%) and T5 (13.3%). There were also no differences among treatments with regard to acrosome integrity (T1 = 83.2%, T2 = 82.0%, T3 = 85.3%, T4 = 82.0% and T5 = 80.0%). Sperm DNA integrity was lower ($P < 0.05$) for T2 (84.1%) than for T3 (92.0%), with no further differences observed among treatments. Therefore, the use of enrofloxacin would not be recommended due to its negative effect on post-thawing sperm motility. Also, the association of penicillin and streptomycin was associated with reduction in the sperm DNA integrity.

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A008 MALE REPRODUCTIVE PHYSIOLOGY AND SEMEN TECHNOLOGY

RECOMMENDED ANTIBIOTIC FOR RAM SEMEN CRYOPRESERVATION EXTENDER

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Antibiotics used in ram semen cryopreservation were only tested in bovines, without an assessment of effectiveness in controlling bacterial and the possible impact on ovine spermatozoa. In consequence, this study aimed to determine the bacterial control ability and the influence on sperm viability of different antibiotics for use in freezing extender. Treatments were established using a pool 20x109 sperm/mL obtained from the combination of the ejaculates of five Crioula lanada breed rams. In each routine (n = 5), microbiological evaluation and determination of sperm viability were performed before and after freezing. The base extender used was tris-egg-yolk-glycerol without antibiotics (control) or supplemented with antibiotics: gentamicin (500 µg/mL) + tylosin (100 µg/mL) + lincomycin (300 µg/mL) + spectinomycin (600 µg/ mL) = GTLS, penicillin (500 µg/mL) + streptomycin (100 µg/mL) = PENSTREP; sodic ceftiofur (50 µg / mL) = CEFT and enrofloxacin (1000 µg / mL) = ENRO in total dose or concentration 50 and 25% lower (-50 and -25) and higher (+25 and +50) to form the treatments (n = 21). The diluted semen was packaged in 0.25 mL straws, cooled and stabilized at 5° C, frozen in LN2 vapor and stored in a cryogenic container. Sperm viability was determined by evaluating the motility and morphology under phase microscopy and plasma membrane integrity, acrosome and DNA under epi-fluorescence microscopy. Semen samples from each treatment were plated by spreading on agar and brain heart infusion (BHI) and incubated at 37°C/48 h, for colony forming units (CFU) counting. Comparisons between means were made by Tukey test. Plasma membrane integrity, acrosome and sperm DNA, were not affected by treatment ($P > 0.05$), however, sperm motility was negatively impacted in a dose-dependent manner in ENRO group. The number of CFU in the control group (no antibiotics) was higher ($P < 0.05$) than in other treatments. The groups PENSTREP and CEFT (in all concentrations used) were less efficient ($P < 0.05$) than the groups GTLS and ENRO, especially in treatments GTLS+50, ENRO+25 and ENRO+50 which promoted a reduction in the number of CFU. Therefore it is concluded that, despite being effective in reducing the number of CFU, enrofloxacin is not recommended for use in ovine semen extender. The GTLS association, 25% more concentrated than the base, is highly recommended for ram semen extender since controlling bacterial growth without compromising viability.

Keywords: viability sperm, antibiotics, ram.