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Effect of butylated hidroxytoluene (BHT) on goat sperm cryopreservation in egg yolk and soybean lecithin based-extenders

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Our aim was to study the effect of BHT as antioxidant, added in different concentrations to egg yolk or soybean lecithin-based extenders. Briefly, fresh ejaculates from six Blanca de Rasquera bucks (2 year old) were collected and immediately mixed in equal quantities. Pooled semen was split into two samples. One sample was centrifuged twice at 600× *g* for 10'. Then the pellet was split into four equal aliquots and re-suspended in a Tris-based extender containing 15% (v/v) powdered egg yolk and 0, 0.6, 2 or 5 mM of BHT with 5% glycerol. The other sample was directly split into four equal aliquots and re-suspended in an extender containing 1% (w/v) of soybean lecithin with 0, 0.6, 2 or 5 mM of BHT and 5% glycerol. Sperm viability determined by eosine-nigrosine stain and total motility analysed by a CASA system (ISAS®) were assessed (mean ± SE, n = 6) after 4 h at 5°C and after thawing, showing no differences among treatments on refrigerated sperm samples. Likewise post-thaw viability was similar among samples preserved without or with 0.6, 2 or 5 mM of BHT in soybean lecithin (45.5 + 3.6; 46.7 + 2.8; 39.2 + 2.3; 43.9 + 4.6) or powdered egg-yolk based media (45.4 + 4.6; 49.9 + 2.6; 47.6 + 5.1; 54.1 + 4.2) respectively. However, total motility was different ($p < 0.01$) among powdered egg yolk (21.6 + 2.4; 31.8 + 3.7; 20.8 + 3.0; 37.2 + 5.2) and soybean lecithin preserved samples (22.9 + 2.7; 32.6 + 2.5; 31.9 + 2.5; 31.5 + 4.3) suggesting that more analysis should be done in order to understand these results. Supported by INIA (RZ2009-00008-00-00), Generalitat de Catalunya (2009SGR0621).

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Specific nano-targeting of Y-chromosome bearing spermatozoa

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Flow cytometric detection and separation of X- or Y-chromosome bearing sperm populations based on DNA quantity is a commercially available method for sex pre-selection. This study aims to develop a novel protocol for sorting bovine spermatozoa based on the qualitative difference between the sperm populations using distinct nanomarker-labelled gene sequences. With a self-developed software the bovine Y-chromosome as published in NCBI was searched for sequences capable of triplex formation. Promising sequences were blasted and additionally tested by southern blotting for specifity. For successful candidates a melting curve was established using locked nucleic acids (LNA) as triplex forming oligonucleotides (TFO) and synthetic duplex-DNA. Sequences shown to form stable triplexes were DIG-labelled, conjugated to gold nanoparticles (AuNP) and subsequently co-incubated with condensed spermatozoa nuclear matrix in a phosphate buffer solution varying pH, MgCl2 concentration and temperature. The results indicate that specific triplex hybridisation in the condensed nuclear matrix of bovine spermatozoa is possible at pH 7, 10 and 20 mM MgCl2 at room temperature. In conclusion, AuNP labelled TFO have the potential to became novel markers for sperm DNA sequences facilitating quantitative sorting. More studies are needed to understand conditions, ranking and kinetics of AuNP hybridisation on entire spermatozoa.

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Sperm quality and oxidative stress in cryopreserved semen from Nelore bulls of different ages

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The aim of this study was to assess motility, plasma membrane integrity and susceptibility to lipid peroxidation of frozen-thawed semen of different ages bulls. Forty animals were allocated to three groups: young 1.8–2 years old (n = 9), adult 3.5–7 years old (n = 19) and aged bulls 8–14.3 years old (n = 12), including three ejaculates per animal. Motility was evaluated by microscopy and plasma membrane integrity was assessed by flow cytometry using the using propidium iodide fluorescent probe. The samples were incubated at 37°C for 90 min with ferrous sulphate (4 mM) and sodium ascorbate (20 mM) to induce lipid peroxidation and evaluated by TBARS assay (thiobarbituric acid reactive substances) analyzed under a spectrophotometer. Spermatozoa from young and adult bulls showed higher motility (38.9 ± 10.9% and 38.2 ± 8.8%, respectively; mean ± SD; $p > 0.05$) and plasma membrane integrity values (54.7 ± 9.5% and 46.7 ± 10.1%, respectively; $p < 0.05$) compared to aged bulls (33.7 ± 6.1% motility and 37.5 ± 9.8% membrane integrity; $p < 0.05$). Considering the susceptibility to lipid peroxidation, adults were more susceptible than young bulls (341.83 and 191.53 ng × 10^6 spermatozoa; median; $p < 0.05$), whereas aged bulls did not show significant differences. The study showed that spermatozoa from young bulls are more resistant to oxidative stress and cryopreservation.
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Incubation of thawed canine sperm increases DNA fragmentation

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Currently, sperm DNA fragmentation (sDF) is an important cause in the rapid decline of sperm quality after thawing. The aim of this work was to assess the effect of incubation on canine frozen-thawed semen using the SCDt simulating the conditions experienced by spermatozoa into the female reproductive tract. Semen was collected by digital manipulation. The sperm rich fraction of 12 ejaculates from three different dogs was pooled each time. All the pooled semen samples (n = 4) presented physiological values concerning to routine semen parameters. Then, semen samples were diluted to a final concentration of 100 × 10^6 sperm/ml in two steps with CaniPRO™Freeze. Sperm were frozen in liquid nitrogen vapours for 10 min and stored into a nitrogen tank. Straws were thawed in a water bath (30 s/37°C) and incubated for 24 h at 38°C before analysis. The sperm DNA fragmentation was assessed in frozen-thawed semen immediately assayed after thawing or after 24 h incubation at 38°C using the Sperm-Halomax® (Halotech DNA SL, Madrid, Spain). A total of 500 sperm per slide were counted using fluorescence microscopy. The results were compared by ANOVA and expressed as mean ± standard error of the percentages of DF positive cells. The results indicate a significant increase ($p < 0.05$) in DNA fragmentation when semen was stored after thawing compared to immediately controlled straws (2.73 ± 0.2 vs. 1.46 ± 0.1).