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The effect of the addition of seminal plasma and antioxidants to frozen thawed ram semen

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Intoduction The structural changes in ram spermatozoa after cryopreservation are a fact assumed by several authors (1). This fact can be explained by a combination of factors that include a low level of membrane phospholipids and also by the oxidation process that generate the reactive oxygen species (ROS) which, when in high levels, are toxic to spermatic cells (2). The seminal plasma (SP) is a complex mixture of components as proteins, sugars and antioxidants. Evidence of high levels of pregnancy after cervical insemination in ewes with frozen thawed semen after a swim-up procedure supplemented with SP indicates a way to improve fertility using this method for artificial insemination in sheep (3). The aim of this study was to evaluate the effect of the addition of SP and the antioxidants catalase (CAT) and Trolox (TRO) on the structural and kinetics parameters on frozen thawed ram semen, independent of individual characteristics of cryopreservation resistance.
Materials and Methods With this purpose four groups were established, CO (semen sample + 200µL PBS), CAT (12,5mg/mL+ PBS = 200µL), TRO (100µMol/100 x 10^6 sptz + PBS = 200µL) and SP (60% of seminal plasma diluted in PBS) added to semen samples in the proportion of 1:1. The SP was obtained from tree different rams, after semen collection all ejaculates were pooled, centrifuged and filtered as described by Mortimer & Maxwell (4). Semen samples were obtained from 13 rams and after dilution for a final concentration of 100 x 10^6 sptz/0,25mL were frozen. One sample from with ram per group were thawed and immediately mixed on the solutions groups for posterior analyses after 5 minutes of incubation. The kinematics parameters, as total motility, progressive motility, average path velocity, curvilinear velocity and straight-line velocity were analyzed using the computer assisted sperm analyzer (CASA). The membrane integrity (MI) were determined by by the combination of fluorescent probes Propidium Iodide and Carboxyflorescein, and on the capacitation status analyzed by the assay using Chortetracycline (CTC) (4).
Results and Discution There were no statistic differences (P>0.05) between groups on the kinematics parameters, on the membrane integrity, and on the capacitation status analyzed by the assay using Chortetracycline (CTC). Probably in our experimental conditions the oxidative stress generated wasn't high enough to permit the effectiveness of the antioxidants.

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Semen quality (SQ) and scrotal circumference (SC), in Nelore bulls, from 2 to 6 years old

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Introduction SC and SQ are important parameters for selecting bulls with adequated reproductive efficiency during the first 21 days of breeding season. However, there are still some doubts related to bull age in relation to semen quality, as far as reproductive efficiency is concerned (Feliciano Silva et al., 1993). The aim of this study was to evaluate the evolution curve of SC and total semen defects (TD) from 2 to 6 year-old Nelore bulls, elucidating doubts concerning SQ as the animal ages.
Material and Methods Semen samples from 163 Nelore bulls, aging from 2 to 6 years, were collected by electro ejaculation and evaluated according to Brazilian College of Animal Reproduction (1998). Models of linear and quadratic regression were estimated (Sampaio, 2002), verifying the evolution of TD and SC according to age.
Results A linear model (Y=0.59-0.53X; R^2=0.92, p<0.01) was estimated for TD from 2 to 6 years, showing that TD decreased linearly in animals from 2 to 6 year olds, indicating that Nelore bulls did not reach their full semen maturity by six years of age. A quadratic model (Y=0.29+0.27X-0.22X^2; R^2=0.97, p<0.05) showed that in Nelore bulls, SC growth stabilized around 4 years of age.
Conclusion Nelore bulls raised on pasture from 2 to 6 years of age did not reach their reproductive plenitude, based on SQ, eventhough reaching full SC development by 4 years of age, showing the importance of evaluating SQ besides SC, when selecting for reproductive efficiency.

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Older breeding bulls are exposed to oxidative stress and decreased welfare condition

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Often breeding bulls are affected by pathologies caused by ageing and suboptimal feeding and housing conditions. In human, oxidative stress induced by reactive oxygen species is known to be involved in the process of ageing and in many age related pathologies. Moreover, that condition could also compromise the reproductive function. The aim of this research is to evaluate the pattern of semen production, some oxidative stress markers and cortisol blood levels in bulls of different age used as sires. Animals (n = 81) come all from the same artificial insemination centre and were of the same breed (Holstein Fnesian). The age of bulls range from 1 to 9 years and the animals were maintained in individual pens, and subjected to the same breeding and feeding management. Semen were collected twice per week and blood samples were withdrawals once a month for four months. Semen were evaluated for motility by computer assisted semen analysis system and number of seminal doses produced were also recorded. Welfare and oxidative stress conditions were evaluated from blood's analysis of glutathione (GSH) and cortisol levels, and glutathione peroxidases (GPx) and superoxide dismutase (SOD) activity. Statistical analysis of the data were performed subdividing the subjects for age into 3 groups (group 1 = <2 years, group 2 = fro 2 to 6 years, and group 3 = >6 years old). The GSH levels were significantly higher (P<0.001) in groups 2 and 3 compared to group 1 (537.4 ± 17.4, 863.1 ± 25.1 and M for groups 1, 2 and 3, respectively). The bloodµ937.5 ± 28.0 cortisol concentrations were also higher (P< 0.001) for older animals (1.4 ± 0.4, 5.1 ± 0.5 and 4.6 ± 0.4 ng/mL for groups 1, 2 and 3, respectively). The GPx activity decreases (P<0.01) with age (203.8 ± 5.6, 177.1 ± 8.3 and 174.1 ± 9.2 U/mg Hemoglobin, for groups 1, 2 and 3, respectively). The SOD activity also decreased with age but the differences were not significant (1515.9 ± 85.5, 1343.4 ± 126.9 and 1152.6 ± 140.1 U/mg Hemoglobin, for groups 1, 2 and 3, respectively). Semen doses production for each collection day were also significantly influenced by age with higher production for bull of group 2 (740.7 ± 35.6) compared to groups 1 (252.9 ± 37.6) and 3 (572.3 ± 53.6), while no differences emerged from spermatozoal motility analysis. In conclusion, the oldest animals have been more sensible to oxidative stress as they have lower GPx and SOD activity and higher GSH levels. Older bulls seem also more stressed than the younger based on higher blood cortisol concentrations and strategies to improve welfare are needed.