ABSTRACTS

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The influence of seminal plasma and two antioxidants: Catalase and N-acetyl-L-cysteine on the quality of chilled dog semen

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OBJECTIVES AND METHODS: The data on the antioxidative status in the sperm cells and seminal plasma in dogs is scarce. The results regarding the effects of the addition of some antioxidants to extenders on the quality of the dog semen are inconsistent (1,2,3). Moreover, the role of seminal plasma in the antioxidative defence is not well understood in canidae (4). Generally, it is assumed that removal of plasma before semen preservation is beneficial. On the other hand, there are some data revealing advantageous effect of plasma addition to preserved semen before artificial insemination (5).

The aim of the present experiment was to investigate the quality of the chilled dog semen incubated in the presence of antioxidants: catalase or N-acetyl-L-cysteine, and preserved with or without seminal plasma. The investigation was carried out on 60 ejaculates collected from 6 healthy, fertile dogs, aged 2-6 years. Sperm-rich fractions of ejaculates were collected from each dog once a week for 10 weeks by masturbation. Each time the semen collected from 6 dogs was pooled and divided into two samples: sample A (no centrifugation, presence of plasma) and sample B (centrifugation 500g, 5 min-plasma removal and Tris buffer supplementation). Samples containing plasma (sample A) and with no plasma (sample B) were subdivided and extended with volume ratio 1:2 in Tris-based extender (subsample A1, B1), in Tris-based extender containing catalase (subsample A2, B2) and in Tris-based extender containing N-acetyl-L-cysteine (subsample A3, B3). The extended semen was cooled to 5°C over 1.5 hrs and incubated in a refrigerator. Motility was assessed at Day 0, Day 5 and Day 10 of semen incubation. IVOS system ver. 12.2L (Hamilton Thorne Biosciences, MA, USA) was used for motility evaluation. Motility parameters assessed were: total motility MOT, progressive motility (PMOT), average path velocity (VAP), curvilinear velocity (VCL), straight line velocity (VSL), beat cross frequency (BCF), amplitude of lateral head displacement (ALH), straightness (STR), linearity (LIN), subpopulation of rapid cells (RAPID). Flow cytometer FACSCalibure (Becton Dickinson, CA, USA) was used to assess semen quality at Day 0 and Day 5. SYBR-14/PI fluorescent probes were used to assess plasma membrane integrity. PNA-Alexa Fluor/PI were used to assess acrosome integrity. Annexin-V/PI were used to assess apoptotic changes. JC-1 stain was used to estimate mitochondrial activity. C11-fluorescent probes were used to assess plasma membrane integrity. PNA-Alexa Fluor/PI were used to assess acrosome integrity. BODIPY581/59 stain was used to assess lipid peroxidation within spermatozal membranes.

RESULTS: The percentages of motile spermatozoa, progressively motile spermatozoa and percentage of rapid cells in groups A and B were 89.40±5.3, 84.90±7.7, 66.30±12.9, 52.30±16.5 (P<0.05); 74.10±14.0, 57.70±18.7 (P<0.05), respectively. On the contrary the percentage of motile spermatozoa at Day 5 of incubation was significantly higher in group B1 vs. A1 (77.9±10.1 vs. 62.5±17.4, p<0.05). No significant differences of motility parameters between samples treated and non-treated with antioxidants within group A and group B were noted. The percentage of live spermatozoa at Day 0 in group A and B were 90.12±3.7 and 85.73±6.9 (P<0.05) and the percentage of live spermatozoa with intact acrosome were 86.34±3.1, 83.93±2.9 in sample A and B, respectively. There were no significant differences in plasma membrane integrity, acrosome integrity and lipid peroxidation intensity, between samples treated or not treated with the use of antioxidants at day 5 of incubation. The percentage of cells with highly active mitochondria at Day 5 was the lowest in group A1, whereas the highest values were observed in groups A2 and B2 (catalase addition).

CONCLUSIONS: Our results confirmed the beneficial influence of the removal of seminal plasma on the preservation of spermatozoal structure and its function in chilled semen. It should also be noted the transient decrease of motility parameters at the day of dilution in the semen depleted of plasma. The addition of antioxidants did not change the motility pattern nor the membrane integrity, acrosome integrity and lipid peroxidation status. However, it was demonstrated that the addition of catalase improves the mitochondrial activity of the dog semen chilled and incubated in 5°C.

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(5) Nöthling JO, Shuttleworth R, de Haas KM, Thompson PN. Homologous prostatic fluid added to frozen-thawed dog spermatozoa prior to intravaginal insemination of bitches resulted in better fertility than albumin-free TALP. Theriogenology 2005; 64, 975-91.