ABSTRACTS

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EFFECT OF DIFFERENT CONCENTRATIONS OF ASCORBIC ACID ON MOTILITY, MEMBRANE INTEGRITY AND CHROMATIN STATUS OF FROZEN-THAWED CANINE SPERMATOZOA WITHIN 6 HOURS OF STORAGE AT 37°C

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Objectives - The aim of this study was to examine whether supplementation of cryoextender with ascorbic acid (Asc) influences the quality of frozen-thawed canine semen.

Materials and methods - Pooled ejaculates (n=10) were assessed for cell morphology and quality with a computer assisted sperm analyser (CASA). After centrifugation, the semen was divided into four parts (A to D). Three parts were diluted with Uppsala I extender (v/v; 1) containing different concentrations of Asc (B: 1.2 mg/ml, C: 2.4 mg/ml, D: 4.8 mg/ml). One group (A) without Asc served as control. After dilution samples were equilibrated (4°C, 1h). Then the Uppsala II extender was added (v/v), a sample taken for quality control and the suspension filled in 0.5 ml straws for cryopreservation according to (2). After one month, samples were thawed in a water bath (70°C, 8 sec) and stored at 37°C without air and light. After one hour, a sample of each group was examined for quality (CASA, morphology, flow cytometry, sperm chromatin structure assay=SCSA; 4). Staining for flow cytometry was performed with FITC-PNA (Sigma-Aldrich, Vienna, A; L7381) and propidium iodide (PI; Sigma-Aldrich, Vienna, A; P4170) as described (3). Further examinations were performed after three and six hours. Because the SCSA is usually performed with a buffer designed for human sperm (TNE, pH 7.4, 279 mOsm/l), we additionally tested a Tris-fructose-citrate buffer (TFC, pH 6.76, 324 mOsm/l; 1).

Results - Within three hours after thawing, the parameters motility (%) and viability decreased significantly in all groups. However, in the supplemented groups, percentages of motile sperm were significantly lower than in the control group, at all times investigated (motility: A 1h: 62±5.8, 3h: 47±4.8, 6h: 37±6.7; D 1h: 48±11.8, 3h: 32.5±11.3, 6h: 18±11.8; p<0.01). Viability after 6 h did not differ between groups (p>0.05). The percentage of living (PI negative) cells, without acrosome reaction decreased significantly between the first and sixth hour (A: 1h: 60.5±7.7, 6h: 34.1±8.4; D: 1h: 48 ±17.1, 6h: 32±9; p<0.01), whereas the percentage of living cells with AR increased, in all groups; between groups, there was no difference (p>0.05). The SCSA parameters αT indicating DNA denaturation, the related SD indicating the extent of chromatin structure abnormality and compαT, indicating the percentage of cells outside the main population, were as follows: In αT, there was no difference between the control group (A) and the supplemented samples (B to D), neither with TNE nor with TFC (TCF-A: 336 ±130.7, TNE-A: 380.2±89.1; TCF-D:227.4±172.7, TNE-D: 338.1±137.4; p>0.01). Samples diluted with both diluents correlated concerning αT, but not compαT. When the averages were compared between TNE- and TFC-samples, there were no differences, neither for αT, nor for compαT (compαT: TCF-A: 2.5±1.7, TNE-A: 3.4±3.2; TCF-D: 2.5±2, TNE-D: 3.3±4.3; p>0.01). Similar results were obtained when the SD of αT were compared between groups and between diluents. Since the compαT values did not correlate between TNE- and TCF-samples, TCF should not be used for SCSA with dog sperm.

Conclusions – The addition of Asc to the cryoextender does not increase quality of frozen-thawed canine semen. This is in contrast to the finding that dietary supplementation with Asc...
has some membrane protecting effect. The results do not confirm toxic effects of Asc on sperm DNA that had been discussed previously (5).