ABSTRACTS
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OBJECTIVES AND METHODS: This study aimed to compare the spermatic and antioxidant profile of canine sperm collected from epididymidis or by digital manipulation (ejaculated sperm). Each semen fraction (I, II and III) was collected separately from five sexually mature dogs of distinct breeds, aged from 2 to 7 years. After one week, testicles were surgically removed and epididymidis were stored at 5°C for 19 hours prior to processing. Epididymal sperm was retrieved through incisions in each segment (head, body and cauda). Ejaculated and epididymal sperm were evaluated for sperm motility (%), forward progressive velocity (arbitrary scale from 0 to 5) and specific patterns of sperm motility (computerized motility analysis-CASA, Hamilton-Thorne Ceros 12.1). Sperm membrane status was assessed by eosin/nigrosin stain in order to characterize live and dead cells. In addition, the enzymes catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPX) were determined in each semen fraction, as well as in the fluid collected from epididymal head, body and cauda. Data were compared by ANOVA and LSD test (p ≤ 0.05). The present experiment was approved by the Bioethics Committee of the Faculty of Veterinary Medicine - USP.

RESULTS: Sperm-rich fraction motility was statistically higher (82%±6) compared to sperm collected from epididymal head (0.2%±0.13), body (37%±4.0) and cauda (63%±5.1). Regarding sperm velocity, no statistical difference was verified between the ejaculate (3.6±0.2) and cauda epididymal sperm (3.3±0.3), whereas head epididymal sperm (0±0) presented no velocity. Epididymal samples showed lower membrane integrity (head: 55.5%±7.6; body: 59.4%±6.8 and cauda: 69.3%±4.7) in relation to the sperm-rich fraction (90.2%±5.9). Ejaculated and cauda epididymal sperm presented the highest total motility and percentage of fast spermatozoa evaluated by CASA. Furthermore, progressive motility was statistically higher in sperm-rich fraction (55.6%±5.3) and decreased progressively in epididymal samples (cauda: 33.7%±2.1; body: 8.7%±1.3 and head: 0±0). Body and head epididymal sperm showed higher percentage of static sperm (56.7%±4.8 and 94.8%±2.6, respectively), which was statistically different from the ejaculated (10%±5.5) and caudal sperm (23.2%±2.3). These results suggest that semen samples recovered from cauda epididymidis is considered more mature, whereas body and head epididymal sperm presented alterations possibly related to immaturity, as these segments are responsible for the acquisition of sperm motility (1). Regarding antioxidant assay, no difference among sperm origins was verified for SOD. Although at low concentration, catalase level was higher in cauda epididymal sperm (0.4 UI/mL±0.2) in relation to other samples (head: 0.09 UI/mL±0.05 and body: 0±0). Comparing semen fractions, catalase concentrations presented no statistical difference. For GPX assay, sperm-rich fraction and cauda epididymal sperm showed statistical higher concentrations (46.6 UI/mL±1.7 and 85.9 UI/mL±12.0, respectively) in comparison to other fractions (fraction I: 15.5 UI/mL±2.2 and fraction III: 8.7 UI/mL±1.7) and epididymal samples (head: 36.4 UI/mL±9.0 and body: 47.4 UI/mL±9.6). Cauda epididymis is the most active segment for protein synthesis as sperm maturation progresses (2), thereby justifying the increased glutathione concentration. In addition, we can infer that the cauda epididymis is the main source of GPX production of canine semen samples. Tail epididymal sperm presented a significant positive correlation between glutathione concentration and motility (r=0.8; p<0.002), the same occurred with SOD and fast sperm cells (r=0.7; p<0.015). The samples of epididymis body had also a positive correlation between glutathione and motility (r=0.9; p<0.001), as well as between SOD and intact sperm membrane (r=0.9; p<0.0002).

CONCLUSION: Semen retrieved from epididymis cauda showed similar characteristics to sperm-rich fraction, allowing for suitable use in canine semen biotechnologies. Catalase concentration was low in all samples, while SOD was detected consistently. There is an increased production of glutathione in the epididymal cauda in relation to other segments, as well as in sperm-rich-fraction. Results suggest that GPX is the main canine sperm antioxidant, thus inferring its use as supplement compound for cryopreservation.