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FLOW CYTOMETRIC, COMPUTER ASSISTED AND TRADITIONAL SPERM ANALYSIS IN FERTILE AND SUBFERTILE DOGS

Introduction and aim. The evaluation of semen in dogs with fertility disorders is routinely performed by using traditional methods including subjective assessment of motility, determination of sperm concentration and morphology. The data on the results of computer assisted (CASA) and flow cytometric (FC) analysis of semen of subfertile dogs are scarce. The aim of present study was to compare sperm characteristics of fertile and subfertile dogs by using traditional methods, CASA and FC assessment of sperm cells stained with fluorescent probes.

Materials and methods. The study was carried out on 38 dogs of different breeds, aged 2.5 to 8 years. The dogs were divided into two groups. Group A (fertile males, 22 dogs, >60% of successful breeding) and Group B (subfertile males, 16 dogs, <40% of successful breeding). Semen evaluation included macroscopic examination, subjective motility and morphology assessment as well as CASA and FC analysis. The parameters of motility were evaluated using Hamilton-Thorne Sperm Analyser IVOS. The motility parameters were: VAP (average path velocity, μm/s), VSL (straight line velocity, μm/s), VCL (curvilinear line velocity, μm/s), ALH (amplitude of lateral head displacement, μm), BCF (beat cross frequency, Hz), LIN (linearity, %), MOT (total motility, %), PMOT (progressive motility, %), subpopulation of RAPID (%) cells. Evaluation of spermatozoal characteristics such as sperm membrane integrity (SYBR-14/PI), acrosomal damages (PNA Alexa Fluor® 488), mitochondrial potential (JC-1) and the spermatozoa DNA integrity by sperm chromatin structure assay (SCSA) was performed on a FACSCalibur (Becton Dickinson, San Jose, CA, USA) flow cytometer.

Results. The volume of sperm-rich fraction of ejaculate (mL), subjectively assessed motility (%), concentration (10^6/mL), total number of spermatozoa (10^6), morphologically normal spermatozoa (%) in group A vs. B were 2.8 ±0.8 vs. 2.7 ±0,9 ($P>0.05$), 84.8 ±7.3 vs. 44.4 ±25.3 ($P<0.001$), 191.0 ±122.5 vs. 74.6 ±102.5 ($P<0.05$), 515.9 ±298.6 vs. 143.1 ±144.3 ($P<0.001$), 72.9 ±19.0 vs. 45.5 ±17.8 ($P<0.001$), respectively. Predominant morphological defects in group A and B were: abnormalities of acrosome 2.8 ±2.5 and 6.5 ±2.7 ($P<0.001$), midpiece abnormalities 7.2 ±5.3 and 15.9 ±4.0 ($P<0.05$), proximal cytoplasmatic droplets 2.5 ±4.2 and 10.2 ±18.5 ($P<0.001$), coiled tail 2.4 ±3.9 and 6.5 ±7.3 ($P<0.05$), respectively. MOT, PMOT assessed by CASA in group A and B were 87.5 ±17.2 and 47.3 ±28.8 ($P<0.001$), and 58.6 ±18.9 and 22.6 ±18.5 ($P<0.001$). VAP, VSL, VCL, LIN and RAPID were significantly higher in group A. The percentages of live (SYBR+ PI-) and dead (SYBR- PI+) sperm cells in group A vs. B were 82.6 ±10.0 vs. 48.9 ±19.4 ($P<0.001$) and 9.4 ±6.7 vs. 24.0 ±13.7 ($P<0.001$). Percentage of live spermatozoa with intact acrosome (PNA- PI-) was 83.2 ±8.2 and 60.0

± 24.8 (P<0.001), and the percentage of live sperm cells with ruptured acrosome (PNA+ PI-) was 3.3 ±2.1 and 15.6 ±22.0 (P<0.001) in group A and B, respectively. In SCSA test DNA Fragmentation Index (%DFI) was 5.9 ±4.2 and 13.2 ±9.0 (P<0.001), and High DNA Stainability (%HDS) was 3.6 ±5.2 and 5.3 ±2.7 (P>0.05) in group A and B, respectively. The percentages of spermatozoa with high, medium and low mitochondrial activity in group A and B were as follows: 79.3 ±30.6 and 40.7 ±28.5 (P<0.05), 16.1 ±29.4 and 29.5 ±27.5 (P<0.05), 3.4 ±2.5 and 22.6 ±23.5 (P<0.001).

Conclusions. 1. Motility, concentration and morphology of sperm cells are impaired in subfertile dogs as a result of anomalies of spermatogenesis and sperm development. 2. The changes of sperm motion characteristics in subfertile dogs may be described as decrease of velocity, linearity and reduction of population of rapid cells. 3. Disintegration of acrosome and plasma membrane as well as decrease of mitochondrial potential characterize sperm cells of subfertile dogs. 4. The structure of chromatin of sperm cells of subfertile dogs is fragmented.