ABSTRACTS

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RELATIONSHIP BETWEEN MOTILITY AND MITOCHONDRIAL FUNCTIONAL STATUS IN CANINE SPERMATOZOA

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Introduction - In mammalian spermatozoa, motility is one of the most important parameters for evaluating the quality of the semen. Nowadays computer assisted sperm analyzer (CASA) allows to observe numerous parameters of sperm motility and provides repeatable and accurate results with standardized settings [1,5]. Motility is strongly related to the ability of spermatozoa to manage its energy status. However the role of the mitochondria in sperm function is controversial. Previous studies supposed that mitochondrial respiration was the predominant source of ATP required for sperm flagellar movements that represent the greatest energy consumption for the sperm cell. Sperm motility should indirectly reflect the ability of midpiece mitochondria to propel the sperm and could represent a way to detect the ATP production. Therefore changes in mitochondrial membrane potential could be a good indicator of motility and the functional status of spermatozoa. However recent advances on mouse sperm suggest that glycolysis has the major role in providing the ATP, required for sperm motility throughout the entire length of the flagellum, suggesting that mitochondrial oxidative phosphorylation is not essential for male fertility [4]. The aim of this study was to associate the evaluation of the most important motility parameters assessed by Hamilton Thorn computer aided semen analyzer (IVOS-12) to the viability and the mitochondrial functional status examined by fluorescent staining markers on fresh ejaculated canine semen collected from 10 dogs of unknown fertility.

Material and Methods - Ten adult dogs aged 16 months to 10 years were used. Semen was collected by manual manipulation [2]. Sperm-rich fraction was collected, taking care to avoid contamination with pre-spermatic and prostatic fraction. Each ejaculate was diluted in PBS to a final concentration of 70 x 10^6 ml^-1. A sperm drop was mounted on a Leja® chamber at 38°C and seven different randomly microscopic fields were scanned by IVOS-12. Each sample was measured for percentage of motile spermatozoa, velocity average pathway (VAP), velocity straight line (VSL), curvilinear velocity (VCL), amplitude lateral head (ALH), straightness (STR), linearity (LIN) and percentage of rapid cells. Sperm viability was assessed with triple staining using the PI/SYBR-14 Sperm Viability Kit and JC-1 (Molecular Probes). SYBR-14/PI is a combination of the green membrane-permeant DNA-fluorescent dye SYBR-14, that is deacylated and entrapped in living cells, and the red membrane-impermeant fluorescent dye PI, which can only permeate damaged membranes [5,6]. JC-1 is a cationic dye that possesses the unique ability to differentially label inner mitochondrial membrane (IMM) with high or low potential. In mitochondria with high membrane potential, JC-1 forms multimers emit fluorescence in the orange wavelength while in mitochondria with low membrane potential JC-1 forms monomers emit fluorescence in the green range. The heads of viable spermatozoa showed bright green fluorescence (SYBR-14) while sperm cells with damaged membranes were stained red (PI). Aerobic functional midpiece showed orange JC-1 fluorescence while midpiece with low activity of mitochondria showed green JC-1 fluorescence. The percentages of live/dead spermatozoa and of midpiece with high/low IMM potential were determined by evaluating at least 200 cells for each sample by an epifluorescence microscope.

Results - Sperm motility assessed by IVOS-12 ranged from 29 to 98%. Motility parameters and concentration were substantially lower in old dogs than in the young ones. Viability and mitochondrial status of spermatozoa, evaluated using the triple staining, showed important
variations ranging from 74 and 99% (viability) and from 53 to 87% (IMM high potential). Ejaculates with a high percentage of live cells showed a major number of spermatozoa with orange fluorescence in the midpiece for the presence of mitochondria with high IMM potential. Sperm cells with orange JC-1 fluorescence were obviously alive, whereas sperm cells with green JC-1 fluorescence were dead or alive. Almost all living spermatozoa with cytoplasmic droplets or tails anomalies showed orange JC-1 fluorescence. Dead spermatozoa showed green JC-1 fluorescence.

**Discussion** - The functional integrity of dog spermatozoa mitochondria seem to be more correlated to sperm viability than to sperm motility. Ejaculates with a low number of motile spermatozoa showed an unexpected high number of mitochondria with orange JC-1 fluorescence. Even sperm cells with tail anomalies have shown a high IMM potential although they were not motile. This report represents the first preliminary study about canine sperm mitochondrial functional status and confirms recent advances that have reconsidered the importance of mitochondria for sperm motility [3,4]. As observed in mouse, even mature canine sperm could produce almost entirely ATP by anaerobic glycolysis in the fibrous sheath of the tail that contains glycolytic enzymes. Presence of a high IMM potential in non motile spermatozoa suggests that ATP efficiently produced by mitochondrial respiration may be important for sperm survival in the female genital tract but could not be sufficient to support sperm motility.

**References**