Osmotic tolerance of feline epididymal spermatozoa
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During the cryopreservation process, spermatozoa are exposed to solutions which are hypertonic and contain high concentrations of cryoprotectant. However, addition and removal of cryoprotectant subjects the spermatozoa to a substantial osmotic stress. Spermatozoa from different species and different degree of maturation may have different susceptibility for osmotic stress depending on the composition of the cell membrane and this will affect their tolerance for the freezing-thawing stress. The aim of this study was to evaluate the effect of osmotic stress on the feline epididymal spermatozoa from different regions of the epididymis. Epididymal spermatozoa from three regions (caput, corpus and cauda) were exposed to different osmolality (75, 300, 600, 900, 1200 mOsm) in a single step. Percentage of motile spermatozoa was measured subjectively and membrane integrity (SyBR-14 positive cells) was evaluated prior to and after exposure to different osmolality solutions for 10 min and returned to 300 mOsm. Percentage of sperm with permeable membrane (SNARF-1 positive cells) and high mitochondria membrane potential (JC1) were evaluated using flow cytometer and compared among epididymal regions (caput, corpus and cauda). All the parameters were compared using General Linear Model. The percentage of motile epididymal spermatozoa decreased significantly when spermatozoa were exposed to osmolality ≤ 75 mOsm and ≥ 600 mOsm. Sperm membrane of corpus and cauda spermatozoa showed signs of damage when exposed to ≥ 900 mOsm and returned to isotonic condition (P< 0.05), decreased from 66% in corpus and 69% in cauda to 47% and 34% respectively, while caput spermatozoa appeared to be highly resistant to the hypotonic and hypertonic conditions as showed no regression in number of spermatozoa with intact membrane (59%, single exposed and 46%, return to isotonic). Moreover caput spermatozoa showed a highest percentage of membranes permeability. A high mitochondrial potential was observed in cauda spermatozoa compared with the other two regions. In conclusion, the membrane of caput spermatozoa is more resistant to a deviation in osmolality than the membrane of corpus and cauda spermatozoa. However, spermatozoa from corpus and cauda regions have high mitochondrial activity and have higher percentage of motility than caput spermatozoa. The sperm motility seems to be the critical parameter which is more sensitive to the deviation in osmolality. Exposure of epididymal spermatozoa to hypo- or hypertonic stress and return to isotonic condition, affects epididymal sperm motility more than a single exposure without returning to isotonic condition.