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The Effect of Cold Shock on Function and Morphology of Dog Epididymal Spermatozoa

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Dog spermatozoa were recovered from the caudae epididymides of 23 domestic dogs which were 11 pure breed and 12 mix-breed dogs ranging in age from 0.6 to 3 years. The experimental designs were as follows: 1) the effect of chilling to 0, 10 or 37℃, 2) the kinetics of chilling injury at 0 or 4℃, and 3) the effect of sugars at 0℃. Viable spermatozoa were recovered by percoll gradient separation and adjusted to 5×10^7 spermatozoa/mL. In experiment 1, spermatozoa were diluted with 0.33 M glucose supplemented with 3% BSA (G-BSA) at 1:2 dilution. Spermatozoa were loaded into straws and exposed at 0, 10 or 37℃ for 30 min. In experiment 2, spermatozoa were prepared as the experiment 1 and exposed for 0.5, 5, 15, or 30 min at 0 or 4℃. In experiment 3, spermatozoa were diluted with different sugars (0.33 M galactose, glucose, fructose, mannitol, lactose, sucrose, raffinose) and cooled to 0℃ for 30 min. Sperm membrane integrity, motility and acrosome integrity were assayed after rewarming at 37℃ for 5 min. Sperm motility and membrane integrity abruptly decreased with decreasing temperature but acrosome integrity gradually decreased ($p<0.05$). Sperm motility was more sensitive to cold shock than membrane integrity and acrosome integrity. Spermatozoa cooled to 0℃ were more damaged than those at 4℃. Sperm motility was not different among exposed times at both 0 and 4℃. However, membrane integrity of spermatozoa exposed for 30 min at both 0 and 4℃ was significantly lower ($p<0.05$). Sperma-

tozoa diluted in 0.33 M fructose or galactose showed lower motility and higher morphological abnormality with coiled tail at 0℃. These sperm characteristics were strongly related. These results indicate that dog epi-didymal spermatozoa are relatively sensitive to rapid cooling and higher morphological abnormality at 0℃ was shown in spermatozoa diluted in fructose and galactose.

Key words) *Dog Epididymal spermatozoa, Cold shock, Sugars, Sperm membrane Integrity, Sperm motility*