REPRODUCTIVE BIOLOGY

ORIGINAL RESEARCH

Characteristics of spermatozoa of whole ejaculate and sperm-rich fraction of dog semen following exposure to media varying in osmolality

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SUMMARY

This study aimed to analyze the effects of different osmolalities on the characteristics of spermatozoa originating from whole ejaculates (WE; including the prostatic fluid) and the sperm-rich fractions (SRF). Ejaculates, collected from four mixed-breed dogs, were exposed for 10 min at room temperature to Tris-fructose-citrate (TFC) solution with osmolality ranging from 150 to 1100 mOsm. After treatment spermatozoa were evaluated by microscopic analysis of motility and fluorescent assessments of plasma membrane integrity (carboxyfluorescein diacetate and propidium iodide, CFDA/PI) and mitochondrial function (rhodamine 123, R123). Irrespective of the sperm source, there was a complete loss of motility when spermatozoa were exposed to TFC solution with 1100 mOsm. There were no marked differences in the sperm characteristics between media with 300 and 350 mOsm, regardless of the ejaculate collection procedure. However, a marked reduction in motility of spermatozoa retrieved either from the WE or SRF

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was observed after exposure to different anisosmotic conditions (150, 550 and 800 mOsm). In all dogs, spermatozoa from the WE exhibited greater osmotolerance in terms of plasma membrane integrity and mitochondrial function when exposed to anisosmotic conditions (150, 550, 800 and 1100 mOsm). There were inter-dog variations in response to various osmotic conditions. The findings of this study indicated that spermatozoa from the WE tolerated exposure to a wider range of osmolality than those from the SRF. It seemed that the presence of prostatic fluid of dog semen rendered the sperm membrane structures less susceptible to osmotic stress. *Reproductive Biology 2009* ***9*** *2: 113-126.*
Key words: dog, spermatozoa, prostatic fluid, osmotic pressure

INTRODUCTION

Sperm cells are progressively exposed to major osmotic challenges during cryopreservation; they become dehydrated and shrink due to local hypertonicity [12, 13]. All these factors may profoundly affect the sperm membrane architecture. In many species the osmotic pressure of the epididymal fluid is hyperosmotic, whereas it is closely isotonic for the seminal plasma and uterine fluid [18]. Spermatozoa are exposed to hypotonic osmotic stress during their migration in the female reproductive tract [2].

Seminal plasma, a complex mixture of secretions originating from the testes, epididymis and accessory sex glands, contains factors that modulate the fertilizing ability of spermatozoa [11, 27]. The ejaculate of the dog consists of three fractions, the second being rich in spermatozoa and the first and third fractions consisting of prostatic fluid [5]. It should be noted that prostatic fluid accounts for more than 95% of the volume of dog ejaculate [9]. There have been some conflicting reports concerning the effect of the prostatic fluid on sperm function. Some authors demonstrated that prostatic fluids, when present in significant amounts in the semen sample, could severely compromise post-thaw sperm function [6, 20, 22, 25]. In contrast, the results of other studies showed that prostatic fluid enhanced the fertility of frozen-thawed dog semen [15, 16]. Owing to the influence of components