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Comparative aspects of sperm membrane fatty acid composition in silver (*Vulpes vulpes*) and blue (*Alopex lagopus*) foxes, and their relationship to cell cryopreservation ☆

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Abstract

Cryogenic protocols have been developed for the storage of farmed silver fox (*Vulpes vulpes)* spermatozoa. However, these same protocols and modifications of these protocols have failed to satisfactorily preserve spermatozoa collected from farmed blue foxes (*Alopex lagopus*). Because cryogenic success has been linked to membrane composition, the plasma membrane lipid composition of farmed blue fox and silver fox spermatozoa was studied. Silver fox spermatozoal membranes have significantly higher levels of docosapentaenoic acid (DPA; 22:5, *n*-6) compared to blue fox spermatozoa, and blue fox spermatozoal membranes have significantly higher levels of stearic acid (18:0). Silver fox spermatozoal membranes not only have a higher ratio of unsaturated/saturated membrane fatty acids, but also higher levels of membrane desmosterol and cholesterol.

Keywords: Silver fox; *Vulpes vulpes*; Blue fox; Arctic fox; *Alopex lagopus*; Spermatozoa; Membranes; Lipids

The membrane lipid composition of spermatozoa in relationship to cold-shock and cryogenic behavior has been and continues to be an area of active research. Differences in spermatozoal membrane composition have been recognized [2,6,32,33] and implications with regard to temperature-induced membrane responses have been reported [7,18,19,42]. The susceptibility of sperm to rapid cold shock has been associated with a high ratio of unsaturated/saturated membrane fatty acids and with low levels of cholesterol within the spermatozoal membrane. Meanwhile, spermatozoa

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that possess a low ratio of unsaturated/saturated membrane fatty acids, or have high levels of cholesterol within spermatozoal membranes, seem to resist cold shock injury [44]. It has also been suggested that the proportion of unsaturated fatty acids may influence the physical properties of the sperm membrane, including membrane "fluidity" [22], non-electrolyte permeability [25], and the temperature at which a phase transition may occur within membrane phospholipids [24].

While White [44] has argued that a high ratio of unsaturated/saturated membrane fatty acids and low levels of cholesterol within the spermatozoal membrane are associated with cold-shock injury, other reports seem to indicate a need for polyunsaturated fatty acids (PUFAs) within spermatozoal membranes. In humans, sperm motility strongly correlates with sperm membrane docosahexaenoic acid (DHA;22:6, *n*-3) levels ($r = 0.82$, $p < 0.001$) [17], and sperm collected from asthenozoospermic males have less membrane DHA compared to normal males [3,4]. The addition of tuna oil to the diets of boars promoted increased levels of DHA (22:6, *n*-3) and decreased levels of docosapentaeneoic acid (DPA; 22:5, *n*-6) within spermatozoal membranes and the increased levels of DHA within boar spermatozoal membranes was associated with increased motility and reduced proportions of spermatozoa with abnormal morphologies [36].

Since PUFAs can influence membrane fluidity [5], it is not surprising to see that membrane fluidity is a predictor of cryogenic success in humans [16]. The link between spermatozoal membrane DHA levels and cryogenic success has been observed in other systems. Bull spermatozoa [34,35] and African elephant spermatozoa [40] have been successfully cryopreserved and have high levels of DHA. Meanwhile, boar spermatozoa [34,35] and Asian elephant spermatozoa [40] have relatively low levels of DHA and their sperm are difficult to freeze.

To study whether or not spermatozoal membrane lipid composition is related to cryogenic behavior, we have analyzed the spermatozoal membrane lipid composition of farmed blue fox (*Alopex lagopus*) and silver fox (*Vulpes vulpes*). The wild blue, also known as the artic fox (*Alopex lagopus*), is a canid species that has declining population densities within the northernmost regions of North America and Eurasia [10,14]. The causes of the population decline of the wild blue (or artic) fox within artic areas, particularly in Fennoscandia, have been attributed to urban expansion into the countryside and growing competition with the closely related and widely abundant red fox (*Vulpes vulpes*) [10]. As a result of the economic importance in the pelting industry, attempts aimed at the conservation of the blue fox and the silver fox (a color mutant of the red fox) have increased [10]. One solution has been the use of artificial reproduction technologies (ART) within captive populations, including the use of artificial insemination and in vitro fertilization [9–14]. To establish gene banks, the preservation of non-germ-line cells, such as fibroblasts or blood cells, would also be desirable, especially when deceased numbers of specimens are found in the wild. While the use of fresh semen in ART protocols has been a successful breeding technique for both farmed blue and silver foxes [10], the use of thawed cryogenically preserved samples has been problematic. While spermatozoa collected from the silver fox can be frozen [9,11–13], these same protocols and permutations of these protocols have failed to satisfactorily preserve blue fox spermatozoa [9,11–14]. Thus, the aim of this study was to characterize the membrane lipid composition of spermatozoa isolated from farmed blue fox and silver foxes. The possible impact of the EDTA and Tris extenders was also studied because the EDTA extender is used for short-term preservation of fresh fox spermatozoa [14], and the Tris–fructose–citrate extender with 20% egg yolk (v/v) (Tris-EY) is used for long-term cryogenic preservation of silver fox spermatozoa [9,11–13].

Materials and methods

Cryogenic extenders and buffers

Artificial insemination and in vitro fertilization studies using fresh semen from blue fox and silver fox are routinely performed using a EDTA buffer system, pH 6.6, which contains 54.6 g/L anhydrous