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Review Article

Role of Membrane Lipid Fatty Acids in Sperm Cryopreservation

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Lipid is an important constituent of cell membrane. Membrane lipid composition of spermatozoa has been correlated to different function. Many researchers have related membrane lipid with survival success after cryopreservation or cold shock. Sperm maturation and acrosome reactions are natural phenomenon, but cryopreservation or cold shock is not. Therefore, sperm cells are not programmed for such change and undergo stress. So the change in membrane lipid composition due to cold shock or cryopreservation may be looked upon as response of spermatozoa to a certain stressed condition. A significant body of research worked on the relationship between membrane lipid and fatty acid composition and ability of cell to tolerate adverse change in temperature. However, as the approach of different research groups was different, it is very difficult to compare the changes. Studies have been done with different species, ejaculated/seminal or epididymal sperm. Lipid analyses have been done with whole cell membrane isolated by different methods. Fatty acids estimated were from whole cell, plasma membrane, head membrane, or phospholipids. The cryopreservation condition, media composition, and diluents/cryoprotectants were also different. At this onset a comprehensive review is needed to cover changes of sperm membrane lipid composition of different species under different cryopreservation conditions.

1. Introduction

Sperm cell is unique in many respects including structure and function. It is capable of fertilizing egg; it functions in a body different from its origin and gender. Its plasma membrane is also different from most other cell membranes in lipid composition. It contains high amount of polyunsaturated fatty acids (PUFA), especially diPUFA (phospholipids esterified with two PUFA), which is found only in sperm, retina, and certain brain areas [1, 2]. In particular, PUFA are known to contribute to membrane fluidity and flexibility [3–5]. Membrane lipid composition has been related to their specific functions, because it promotes the creation of microdomains with different fluidity, fusogenicity, and permeability characteristics [2], required for reaching and fusing with the oocyte.

Phospholipids are the most representative lipid fraction of the sperm cell membranes, of which phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin are the major components [6]. Lipid and fatty acid composition of sperm cells differ not only for different animals [7, 8] but also for different species [9–12], even for fertile and subfertile population of same species [13–15].

The definite lipid pattern of ejaculated spermatozoa is reached only after epididymal maturation. Plasma membrane lipids of the goat [16], ram [17], and boar [18] spermatozoa have been shown to undergo marked changes during epididymal maturation. The lipid content of whole sperm decreases during epididymal maturation in boar, bull, ram, and rat [19–26], and the cholesterol content decreases in ram, rat, and hamster sperm [27–29]. The cholesterol : phospholipid ratio and concentration of phosphatidylserine, phosphatidylethanolamine, cardiolipin, and ethanolamine plasmalogen decrease in whole ram sperm [20, 27]. However, increases occur in the amount of the sulphoconjugated sterols in whole hamster and human sperm [28–30] and in unsaturated fatty acids in whole ram sperm [27]. Studies using plasma membrane isolated from boar spermatozoa confirm earlier results with whole sperm that the amount of lipid decreases during epididymal maturation [18]. Although there is a decrease in cholesterol, no significant change is seen in the cholesterol : phospholipid ratio.

There are also decreases in phosphatidyl ethanolamine and phosphatidyl inositol as well as increases in dermosterol, cholesterol sulphate, phosphatidylcholine, and polyphosphoinositides. There is a decrease in the level of fatty acids and an increase in diacylglycerol but no change in the degree of saturation of fatty acids. Plasma membrane from the anterior head region of ram sperm is particularly rich in ethanolamine and choline phosphoglycerides [17]. The amount of dermosterol and ethanolamine in this region of the plasma membrane decreases whereas the cholesterol : phospholipid ratio increases, during epididymal maturation. The goat sperm plasma membrane is particularly rich in ether lipids phosphatidylcholine and phosphatidylethanolamine. Of all the membrane phospholipids, diacyl phosphatidylethanolamine decreases most strikingly (approx. 65%) during epididymal maturation of sperm [16].

Changes in the amount and composition of lipids in the plasma membrane of sperm during maturation are thought to explain why ejaculated sperm is more sensitive to cold shock than is testicular sperm [31, 32]. These changes may also account for the maturation-dependent decrease in charge density at the phospholipid-water interface of ram spermatozoa, detected by electron spin resonance [31] and the decrease in membrane fluidity of bull spermatozoa, seen by fluorescence polarization spectroscopy [33]. Analysis of testicular and ejaculated ram spermatozoa by fluorescence recovery after photobleaching (FRAP) indicates that there are regional differences in the decrease of plasma membrane fluidity [34]. During maturation, the diffusion rate of fluorescent lipid analogue increases in all regions of the sperm except the midpiece.

2. Changes in Lipid Associated with Cryopreservation

Cryopreservation affects sperm membrane integrity [35, 36]. Differences in fatty acid composition and lipid class ratios in spermatozoa among species are important factors in the freezability of the male gametes [37] (the different animals/species and cryopreservation methods are summarized in Table 1). According to Pettitt and Buhr [38] freezing and thawing results in lipid modifications and domains of sperm head plasma membrane react differently to cryopreservation. Furthermore, some studies [39, 40] investigated the ability of sperm cells to take up lipid components or fatty acids from the surrounding environment during incubation in vitro [41].

The major problem associated with cryopreservation of sperm cells is the loss of viability using freeze thaw process [36, 42–54]. Loss of viability is related to membrane leakiness which is induced by sperm phospholipids peroxidation [55, 56]. Different workers have assessed the effect of cryopreservation on sperm membrane fatty acid composition in different species including human.

2.1. Human System. Human sperm cryopreservation is considered to be an important therapeutic option with several practical applications [57, 58]. Analyses of the fatty acid pattern of membrane phospholipids and plasmalogen of human spermatozoa have demonstrated significant levels of PUFAs [59]. The major damage caused during cryopreservation is peroxidation of lipids especially phospholipid bound polyunsaturated fatty acids (PUFAs) [60, 61]. But it is also established that membrane stress rather than lipid peroxidation is related to sublethal cryodamage [62]. The lipid composition of whole spermatozoa is well documented [7, 59].

According to Schiller et al. [63], significant changes in lipid composition are associated with freezing/thawing. They suggested that matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectra revealed clear differences between the spermatozoa and the seminal plasma as well as between the native and the cryopreserved spermatozoa. The concentration of 1-stearoyl and 1-palmitoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine shows rapid decrease following cryopreservation. Alvarez and Storey reported a reduction of some fatty acids in human spermatozoa after freezing, with a reduction in PUFA C18:2, C20:3, C20:4, and docosahexaenoic acid (C22:6 n-3; DHA) and an increase of the saturated fatty acid (SFA) C: 16 and SFA C: 18 after freezing [64]. Martínez-Soto et al. [65] confirmed a strong correlation between the fatty acid composition of the human spermatozoa or seminal plasma and the sperm parameters of the samples after thawing. They described a significant correlation between the fatty acid composition of the human spermatozoa or seminal plasma and the sperm parameters of the samples after thawing. PUFA, n-3, and specially DHA are directly correlated with sperm motility and viability after freezing/thawing, and monounsaturated fatty acids (MUFA) were inversely correlated. They also suggested that in the future the fatty acid composition could be used as a predictor of the capacity of cryopreservation of a seminal sample. The susceptibility of spermatozoa to rapid cold shock has been associated with a high ratio of membrane PUFA : SFA and with low levels of cholesterol within the sperm membrane [8]. Lipid diffusion which is reflected in the dynamics of the recovery of the lipid reporter probe ODAF [5-(N-octadecanoyl)aminofluorescein] during FRAP is significantly reduced after thawing in all regions of spermatozoa [66]. Correlation between fluidity and postthaw recoveries of motile and viable spermatozoa showed that there is marked variation between membrane anisotropy values, which were significantly high in cryopreserved samples compared to fresh samples. Furthermore, recovery of motile and viable spermatozoa is strongly correlated to anisotropy of fresh spermatozoa. The higher the membrane fluidity before freezing, the better the response of spermatozoa to cryopreservation [67]. As PUFAs can influence membrane fluidity, it is not surprising to see that membrane fluidity is a predictor of cryogenic success in humans.

2.2. Other Systems. Apart from human spermatozoa other animal systems were also subjected to cryopreservation. The changes associated with lipid composition were studied thoroughly in animal spermatozoa like boar, bovine, marsupials, goat, ram, fowl, and so forth. The ability to cryopreserve spermatozoa from all of the domestic species is challenging. Even

Table 1: Different animals/species and cryopreservation methods.

Animal	Cryopreservation methods	Reference number
Human		
	Semen was diluted using human sperm preservation medium, TEST-Yolk buffer, or glycerol, subjected to slow manual cooling in liquid nitrogen (LN_2) vapour, and stored in LN_2.	[47]
	The procedure is based on the program of cooling speed doubling: from 20 to 5°C at 0.5°C/min; from 5 to 4°C at 1°C/min; from 4 to 3°C at 2°C/min; from 3 to 2°C at 4°C/min; from 2 to 1°C at 8°C/min; from 1 to −80°C at 10°C/min. After being held 10 minutes at the final temperature, −80°C then were transferred to LN_2.	[62]
	Normozoospermic, oligozoospermic, asthenozoospermic, and oligoasthenozoospermic semen samples were frozen in pellets on the surface of dry ice using a glycerol-based cryoprotectant with egg yolk.	[65]
	Sperm cells were frozen or cold-shocked by lowering the temp. rapidly from 37 to 0°C on melting ice.	[66]
	Sperm cells were frozen using the programme: from 20 to −4°C at 5°C/min; from −4 to −30°C at 10°C/min; from −30 to −140°C at 20°C/min and then were transferred for storage to LN_2.	[67]
Ram and goat		
Merino rams	Ejaculates were obtained by electrical stimulation. Semen was also collected from the caput and cauda epididymis. Spermatozoa were cold-shocked by placing tubes containing the semen held at 37°C into a bath at 0°C for 10 min.	[20]
Zandi rams	The diluted semen was cooled at 4 to 5°C for 2 h. Then the samples were placed into the LN_2 vapor at a height of 4 cm above the liquid for 8 minutes, and then the straws were plunged into LN_2.	[80]
Goat	Epididymal sperms were cryopreserved using programmable freezer: from 30 ± 2° to 5°C at 0.25°C min, from 5 to −20°C at 5°C min, and from −20 to −100°C at 20°C min then transferred to LN_2.	[81]
Goat	Semen was frozen in pellet form on dry ice, and then plunged into LN_2.	[82]
Mahabadi bucks	Ejaculated semen samples were diluted and equilibrated at 5°C for 150 min. Samples were frozen in LN_2 vapor, 4 cm above the L, for 7 min; subsequently the straws were plunged into the LN_2 for storage.	[83]
Blanca-Celtiberica buck	Diluted semen was cooled to 5°C for 2 h, diluted further, and held at 5°C for 2 h; another sample was cooled to 5°C for 4 h; both samples were frozen over N_2 vapour for 10 min, 4 cm above N_2 level, plunged, and stored in LN_2.	[84]
San Clemente bucks and Tennessee Myotonic buck	Semen aliquots were cooled on ice for 1 h before transfer to a 5°C refrigerator and kept for total of 1-2 h; then extender was added. Fully extended semen was equilibrated at 5°C for 2 h. Semen was frozen by suspending straws in N_2 vapour for 10 min, then plunging into LN_2.	[85]
Saanen bucks	Semen samples were cryopreserved using programmable freezer using a fast freezing curve (from 25°C to 5°C at 0.25°C/min and from 5°C to −120°C at 20°C/min) that started at 28°C. After reaching a temperature of 5°C (~80 min), the straws were subjected to an equilibration time for 120 min. The freezing curve was implemented immediately after the equilibration time and was sustained until the temperature reached −120°C, then were placed in LN_2.	[87]
Spanish ibex (*Capra pyrenaica*)	The diluted sperm suspension was cooled to 13°C in a water bath, further cooled to 5°C over 1 h, kept for 2 more h, and frozen by placing in N_2 vapour 5 cm above the surface of LN_2 for 10 min before plunging into the LN_2.	[102]
Bull		
Friesian bull	Semen was cooled to 5°C over 30 min, equilibrated for 6 h at 5°C, and frozen above the surface of LN_2 (a temperature of −120°C was attained in 7 min).	[73]
Holstein bulls	Semen was cooled to 5°C in 1 h; after 4 h it was pellet-frozen on solid CO_2.	[74]
Holstein, Jersey, and Guernsey	The semen was cooled immediately after collection to 15 to 20°C and held at this temperature for one-half to one hour; then diluted semen was cooled slowly to a storage temperature between 4 and 7°C.	[76]
Prim Holstein	Electroejaculated semen samples were cooled from 34 to 4°C in 1.5 h, held for 2 h, and then descended in LN_2. Rate: 4 to −10°C at 0. 7°C/10 s; −10 to −150°C at 7°C/10 s.	[77]
Holstein	Fresh sperm samples were cooled to 4°C and held for 1 h (addition of diluent). Cryostraws were placed ~5 cm above the LN_2 surface for 10 min and then put directly into the LN_2 for storage	[78]

Table 1: Continued.

Animal	Cryopreservation methods	Reference number
Swiss brown bull	Diluted semen was cooled to 4-5°C over 2 h and then frozen by being placed into the LN_2 vapor at a height of 4 cm above the liquid for 8 min; after that, the straws were plunged into LN_2.	[79]
Boar		
	The semen was cooled to 22°C over 2 h and further cooled to 5°C over 3 h by placing in a cold room. Samples were then frozen in LN_2 vapors at 30°C/min from 5°C a final temperature of −70°C. Then straws were plunged directly into LN_2.	[38]
Norwegian Landrace and Duroc	The diluted semen was cooled at 15°C over 3 h and then cooled to 4°C over 2 h period. Semen was frozen in a controlled rate freezer for 9 min. The freezing chamber was precooled to −100°C. Immediately after the straws were transferred to the chamber it was warmed at 10°C/min to −70°C and held for 1 min before lowering the temperature to −120°C at 50°C /min. The straws were held at −120°C for 4 min before transferring to LN_2.	[11]
Landrace, Large White, and commercial hybrids	Semen was cooled at 15°C for 3 h and was further cooled in a programmable freezer to 5°C over 90 min; samples were cryopreserved using programmable freezer. The freezing chamber was precooled to −110°C. Immediately after loading the straws it was warmed at 45°C/min to −60°C and held for 1 min before lowering the temperature to −130°C at 20°C/min. The straws were plunged into LN_2.	[41, 90]
Marsupial		
Eastern grey kangaroo (*Macropus giganteus*) Koala (*Phascolarctos cinereus*) Common wombat (*Vombatus ursinus*)	Epididymal sperms were subjected to cold shock by rapid cooling.	[91]
Canine samples		
Blue fox (*Alopex lagopus*)	Semen was cryopreserved in different extenders; FA and sterols of plasma membrane were analysed.	[10]
Red/silver fox (*Vulpes vulpes*)	Cooling at a moderate rate (2–5°C/min from 4-5°C to below the freezing point, i.e., from −7 to −15 or −20°C) and freezing at a rapid rate from −20 to −50 or −70°C.	[92, 94, 95]
Dog	Cooling at a moderate rate (2–5°C/min from 4-5°C to below the freezing point, i.e., from −7 to −15 or −20°C) and freezing at a rapid rate from −20 to −50 or −70°C.	[94]
Beagle and Golden Retriever	The samples were maintained at a temperature of 4°C for total 1 h 30 min. Then the canine semen was frozen in LN_2 vapours (4 cm above the level of the LN_2) at −110°C for 10 min then immersed vertically in LN_2 for storage at −196°C.	[99]
Elephant		
African Elephant (*Loxodonta Africana*) Asian Elephant (*Elephas maximus*)	Ejaculated semen samples were stored at −70°C or cryopreserved in LN_2.	[9]
Stallion		
Andalusian stallions	The spermatozoa were slowly cooled to 4°C within 1 h and frozen horizontally in racks placed 4 cm above the surface of LN_2 for 10 min, after which they were directly plunged in LN_2.	[100, 101]
Bats/flying fox		
Indian flying-fox (*Pteropus giganteus*) Variable flying-fox (*Pteropus hypomelanus*) Grey-headed flying-fox (*Pteropus poliocephalus*) Rodrigues flying-fox (*Pteropus rodricensis*) Large flying-fox (*Pteropus vampyrus*)	Electroejaculated semen samples were cooled at 50°C/min to 4°C.	[12]

though all of the cells must endure similar physical stresses associated with the cryopreservation processes, sperm from the different species is very different in size, shape, and lipid composition, all of which affect cryosurvival. Thus, when a cryopreservation protocol has been optimized for sperm of one species, it may not be ideal for sperm of the other [68].

2.2.1. Cattle System. Increased concentrations of free cholesterol, free fatty acids, triacylglycerol, and cholesterol ester are associated with decreased sperm motility and fertility [69]. It is also elucidated from the works of bull semen that increased age of bulls has an inverse correlation with the fatty acid composition, especially PUFA and DHA. It is suggested by Argov-Argaman et al. [70] that such alterations might affect the semen's capacity to successfully undergo the cryopreservation procedures, which are widely used in intensive reproduction management.

Egg yolk is widely used and generally accepted as an essential ingredient in diluents employed for the freezing of bovine spermatozoa for use in artificial insemination (AI). It is known to be an efficient protectant of spermatozoa from cooling/thawing [71]. Different forms of lipid extracts confer cryoprotection to frozen spermatozoa [72]. Foulkes and Stewart [73] have demonstrated the maintenance of fertility in spermatozoa frozen in an egg yolk lipoprotein. Irreversible binding of egg yolk lipoprotein and its role in cryopreservation are well elucidated through immunological investigations [72]. Some authors are reluctant in using the whole egg yolk as an extender for cryopreservation as it diminishes the respiration and motility of spermatozoa [74–76]. Thus easy techniques for extraction of the essential part, low density lipoprotein (LDL), needed for cryopreservation have been suggested and it is also commented that LDL extenders have improved ability in retaining sperm viability and motility compared to the commercial extenders of egg yolk [77]. A comparative study of egg yolk from five avian species as cryoprotectant showed that pigeon egg yolk has the highest success after thawing in bull sperm [78]. Addition of bull semen extender with n-3 fatty acid and α-tocopherol enhanced postthaw sperm characteristics. Before semen freezing, percent DHA was higher in fatty acid treatment than that in the group without fatty acid and decreased significantly in both groups after thawing. A plausible reason for this decrease could be excessive lipid peroxidation during cryopreservation of sperm. This is due to excessive peroxidation as suggested earlier. But addition of α-tocopherol is effective and can reverse the effect [79]. Similar works in ram semen also showed similar result with FA and α-tocopherol [80]. According to Chakrabarty et al. [81], total lipid and its components, that is, neutral lipids, glycolipids, and phospholipids, decreased significantly after cryopreservation in case of goat semen. Among neutral lipids sterols, steryl esters and 1-O-alkyl-2,3-diacyl glycerol decreased appreciably, while, among phospholipids, major loss was observed for phosphatidylcholine and phosphatidylethanolamine. Unsaturated fatty acids bound to the phospholipids diminished while the percentage of saturated acids increased. Addition of egg yolk plays a major role during the freezing step of goat cryopreservation and the addition of trehalose significantly improved its cryoprotectant activity. Furthermore, neither glycerol nor egg yolk alone could reduce the intact acrosome percentage; however, combination of these two protectants significantly reduces the percentage of intact acrosome spermatozoa [82]. Salmani et al. [83] have suggested the use of soyabean-lecithin which is a suitable plant based cryoprotectant for caprine sperms. The beneficial effects of soyabean-lecithin as a substitute for egg yolk during cryopreservation of goat sperm are established in the works of Jiménez-Rabadán et al., Roof et al., Salmani et al., and Vidal et al. [84–87].

2.2.2. Boar System. The fatty acid composition of boar spermatozoa is interesting, since they contain some 25% docosapentaenoic acid (C22:5 n-6; DPA) and 30% DHA [88, 89]. Maldjian et al. [90] have reported a decrease in long chain PUFA and an increase in SFA taken up or passively bound to the spermatozoan membranes. Another distinct change in lipid content was that in the spermatozoan cholesterol level. A study on two different breeds (Norwegian Landrace and Duroc) of boar semen revealed no significant difference between breeds; however, there were significant male-to-male variations within breeds in postthaw percentages of live sperm. The most abundant fatty acids in the plasma membranes from both breeds were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1 n-9), DPA, and DHA. The ratio of $\sum$ DPA and DHA/ $\sum$ all other membrane fatty acids was significantly related to survival rate (plasma membrane integrity) of sperm for both Norwegian Landrace and Duroc boars. Thus Waterhouse et al. [11] concluded that male-to-male differences in sperm survival rate after freezing and thawing may be partly related to the amount of long-chain PUFA in the sperm plasma membranes.

2.2.3. Marsupial System. Marsupial spermatozoa isolated from cauda epididymis had higher levels of long chain PUFA in membrane as compared to spermatozoa isolated from caput epididymis. A study conducted in Kangaroo, Koala, and Wombat confirmed the presence of high PUFA and a high ratio of unsaturated/saturated fatty acids, whereas cholesterol levels are found to be very low in all three species. According to White [8], cold shock resistance is related to high levels of membrane sterols and a low ratio of unsaturated/saturated membrane fatty acids. But the spermatozoa of the koala, common wombat, and Eastern grey kangaroo had very low levels of membrane sterols and very high ratios of unsaturated/saturated membrane fatty acids, but are still resistant to cold shock injury [91].

2.2.4. Other Mammalian Systems. The wild blue or arctic fox (*Alopex lagopus*), is a canid species that has declining population densities within the northernmost regions of North America and Eurasia [92, 93]. The causes of the population decline of the wild blue 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*).

Thus artificial insemination (AI) is an efficient tool for increasing the population. While spermatozoa collected from the silver fox can be frozen [94, 95], these same protocols and permutations of these protocols have failed to satisfactorily preserve blue fox spermatozoa [94, 95]. Miller et al. [10] carried out experiments in farmed blue fox and silver fox (a colour mutant of red fox) to study the lipid composition in the sperm membrane. It revealed higher ratio of unsaturated/saturated fatty acids and cholesterol in silver fox than blue fox. This may be considered as a key component for the difference in success rates of semen samples after thawing.

The continued loss of natural habitats and the limited ability of wildlife parks and zoos to manage and breed wildlife have created a need for new and improved management and breeding strategies [96]. In case of elephants cryogenic, protocols have successfully preserved spermatozoa from African Elephants (*Loxodonta africana*), but these protocols and their modified forms failed to preserve spermatozoa of Asian Elephants (*Elephas maximus*). During cryogenic attempts, the spermatozoa of Asian elephants experience extensive acrosomal damage and thereby become useless for future breeding attempts [97, 98]. In Asian and African elephants, the most abundant fatty acid in spermatozoa membranes was DHA, along with lauric acid (C12:0), myristic acid (C14:0), palmitic acid, stearic acid, and oleic acid [9]. It is been concluded that higher levels of DHA in postthaw samples of African elephants may be beneficial for cryopreservation in comparison to Asian elephant. The differences in fatty acid composition can also be due to nutritional difference and genetic difference in fatty acid metabolism between African and Asian elephants [9]. Similarly bull spermatozoa have been successfully cryopreserved and have high levels of DHA; meanwhile boar spermatozoa with low levels of DHA are difficult to freeze [7, 22].

When canine samples were preserved separately with LDL and egg yolk, recovery percentage of sperm motility was higher in samples incubated with LDL. LDL medium also resulted in an improved preservation of spermatozoa during the freezing process in terms of acrosomal integrity, flagellar plasma membrane integrity, and DNA integrity [99].

García et al. [100] also examined the fatty acid and plasmalogen of the phospholipids of the stallion spermatozoa to analyze its relationship with sperm quality after thawing. One of the major drawbacks for the use of frozen thawed semen in equine breeding is the large variation in freezability among stallions. This variability can be explained through susceptibility to lipid peroxidation [101]. As the antioxidant enzymes do not vary considerably among stallions, it is obvious that PUFA and plasmalogens may play a role in such variability.

A study of lipid profile of flying fox (*Pteropus*) also revealed that stearic acid (C18:0) was the predominant saturated fatty acid and oleic acid (C18:1 n-9) was the predominant unsaturated fatty acid in both acrosomal and plasma membranes. Higher levels of PUFA in the acrosome can attribute to cryogenic success [12]. Chicken egg yolk is the most widely used extender in sperm cryopreservation. But Santiago-Moreno et al. [102] have used Quail egg yolk as an alternative to chicken egg yolk for threatened wild ruminant species, the Spanish ibex. Results showed that this alternative is not a good extender for chicken egg yolk as it provided lower motility and viability.

3. Scope of Future Work

Though a good amount of work has been done on the subject discussed above, lack of uniformity provides scope of lot more research work in this field. Change in phospholipids or cholesterol due to cryopreservation drew attention of many researchers as their addition in cryopreservation media provided beneficial effect. Number of works involving change in fatty acids is comparatively very small. Information is wanted with endangered species or animals under captive breeding.

Conflict of Interests

The authors declare that there is no conflict of interests.

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