Review Article

Cholesterol efflux from sperm: approaches and applications

Zahid NASEER, Ejaz AHMAD, Melih AKSOY*

Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Adnan Menderes University, Aydın, Turkey

Abstract: Following ejaculation, sperm appear morphologically mature, but functionally they are inactive. To attain functional capability or capacitation in vivo, sperm must reside in the female reproductive tract for a certain period of time before fertilization. In the female reproductive tract, cholesterol efflux from the sperm plasma membrane is considered vital for the initiation of capacitation. A number of in vitro studies have used biological and nonphysiological means to understand the phenomenon of cholesterol efflux and capacitation. In this review, we present a short overview of the role of cholesterol efflux in regulating sperm capacitation using various agents. This review provides insight to researchers designing new procedures for sperm preparation to be used in assisted reproductive technology.

Key words: Cholesterol efflux, sperm, capacitation, in vitro fertilization

1. Introduction

Sperm are very peculiar cells because they undergo several essential maturational changes throughout their life span to accomplish specific functions. The rates of these changes or modifications are highly variable at different subcellular regions (acrosome, post acrosome, midpiece, tail, etc.) of sperm. In the epididymis, epithelial secretions and the constituents of luminal fluids provide an environment for sperm to attain motility and fertilizing ability. Though freshly ejaculated sperm are motile and have fertilizing ability, they are unable to fuse with the oocyte immediately after ejaculation. During their migration through the female reproductive tract, sperm undergo a series of controlled biochemical and membranous changes known as capacitation (1). During capacitation, sperm experience a number of events such as an increase in respiration and motility patterns (2,3), removal of cholesterol from the plasma membrane (4), destabilization of membranes (5,6), an increase in intracellular pH and calcium (7,8), activation of second messenger systems (9), and increased tyrosine phosphorylation of sperm proteins (10). Subsequently, capacitated sperm undergo the acrosome reaction, which is a morphological alteration consisting of a series of point fusions between the outer acrosomal membrane and the overlying plasma membrane (11,12). These functional changes are not one event but are a combination of sequential and concomitant processes involving modifications at the molecular level in both the head (i.e. preparation for the acrosome reaction) and the tail (i.e. motility changes).

Existing research suggests that cholesterol depletion from the acrosomal region is a prerequisite for the acrosome reaction and sperm–egg binding. Similarly, changes in the cholesterol concentration in the plasma membrane over the midpiece and tail region increase lateral mobility of the membrane components, which in turn support the hyperactive motility of sperm (13). The presence of several cholesterol acceptors such as albumin and high-density lipoprotein (HDL) in the follicular and oviductal fluid mediates cholesterol efflux from the sperm membrane in vivo (14,15). However, in vitro, cholesterol efflux from the sperm plasma membranes has been facilitated by cholesterol acceptors such as bovine serum albumin (BSA) (6,16–18), HDL (14,19), and β-cyclodextrin (20). Likewise, seminal proteins and Percoll gradient centrifugation have been used to induce cholesterol efflux from sperm to promote the acrosome reaction (21–23). This review summarizes studies conducted on the approaches used for cholesterol efflux and their application in sperm capacitation, acrosome reaction, and fertilization ability.

2. The role of cholesterol in sperm membranes

The cellular membranes of sperm consist of phospholipids and sphingolipids in large proportions, along with sterols, which have a role in membrane composition for different regulatory functions (24). Cholesterol is the major essential sterol required for viability and cell proliferation. In addition, cholesterol stabilizes the membrane, reduces membrane permeability, facilitates morphological mem-

brane characteristics and enables cell-to-cell interactions, influences membrane phase transition, provides suitable microenvironments for membrane-associated proteins, and serves as a membrane antioxidant (25). The interaction of cholesterol with membrane phospholipids and sphingolipids influences membrane fluidity. The cholesterol/phospholipid ratio is an important determinant of membrane fluidity and stability. The distribution of cholesterol, which is incorporated during sperm maturation in the male genital tract, is variable at different regions of the sperm. Usually, the sperm head has a high amount of cholesterol, which regulates the membrane fluidity and plays an important role during the capacitation process (26).

3. Why is cholesterol efflux necessary for sperm capacitation?

Mammalian sperm are unable to fertilize an egg until they undergo capacitation, either during their passage through the female reproductive tract or in a suitable in vitro medium. During capacitation, spermatozoa become hyperactive and their acrosome becomes destabilized to initiate the acrosome reaction. The removal of seminal plasma depletes some sterols from the plasma membrane and capacitates the sperm. Cholesterol depletion from the acrosomal and postacrosomal regions is vital to capacitation and promotes the acrosome reaction and the sperm–egg binding process. The acrosomal region has a very tight membrane that is composed of sulfoconjugates of cholesterol (27). The presence of large charged and hydrated polar sulfate groups of these cholesterol sulfates provides stability and prevents disruption of the nonbilayer organization of the acrosomal membrane compared to other regions of sperm (28). Cholesterol efflux, after desulfation, is thought to increase membrane fluidity and allow greater lateral movements in integral membrane proteins. The increase in membrane fluidity enhances calcium influx in order to mediate the acrosome reaction (29). Similarly, changes in cholesterol concentrations in the plasma membrane over the midpiece and tail may increase lateral mobility of the membrane components, supporting hyperactivated motility for penetrating an egg.

4. Agents used for cholesterol efflux

Cholesterol depletion is a time-dependent process and can be accomplished in vitro by using biological and nonphysiological cholesterol acceptors (30). A wide variety of such cholesterol acceptors are used to modify the membrane cholesterol content in sperm and somatic cells. Following are some physiological and nonphysiological cholesterol acceptors that have been tested in vitro.

4.1. Bovine serum albumin

Serum albumin is a protein derived from human and bovine serum and is widely used as a carrier molecule for a variety of substances (e.g., cholesterol, hormones, ions, fatty acids, amino acids). It also serves as a pH buffer, an osmotic pressure regulator, and a heavy metal ion chelator in different biological processes (31,32). In 1971 Toyoda et al. (33) reported successful in vitro fertilization in mice by using a chemically defined medium supplemented with BSA. Later, the role of BSA in capacitation media was tested for mouse, rat, and human sperm capacitation (7,16–19,22,34). Likewise, BSA has been used in most media to maintain sperm motility and to promote membrane cholesterol efflux (18–19,34). The mechanism of cholesterol removal by albumin is not clear, but BSA presumably solubilizes or hydrolyzes a small amount of cholesterol from the cell surface because it does not have specific binding sites for cholesterol (2). Removing cholesterol from the sperm plasma membrane markedly increased concentrations of the phospholipids when sperm were incubated in medium supplemented with BSA. The increase in phospholipids leads to increased membrane fluidity, which is the first landmark for the initiation of capacitation (35).

The role of BSA as a cholesterol acceptor has been compared with other compounds such as beta-cyclodextrin and HDL in in vitro studies. The detailed results of those studies regarding the influence of BSA on sperm capacitation and fertilization are presented in the Table. A considerable decrease (20%–60%) in cholesterol concentration occurred when rat or mouse epididymal sperm were incubated with different concentrations (4–20 mg/mL) of BSA (16,18). Similar observations were reported for human sperm incubated in the presence of BSA for cholesterol efflux and induction of the acrosome reaction (29,36). However, the effect of BSA on cholesterol efflux, the acrosome reaction, and the fertilization rate has not yet been investigated in many species, such as cattle, sheep, goats, and rabbits. Collectively these data offer persuasive evidence for a possible cholesterol acceptor role for BSA in other mammalian sperm capacitation. This is an area ripe for investigation.

4.2. β-Cyclodextrin

Cyclodextrins are nonbiological molecules found in the female reproductive tract or in oocyte envelopes, but these have been used as highly efficient cholesterol acceptors for cholesterol release as an early event of in vitro sperm capacitation and acrosome reaction. Cyclodextrins are cyclic oligosaccharides consisting of α-(1–4)-linked D-glycopyranose units, which are primary degradation products of starch. These compounds act as potent carriers for hydrophobic drugs because of their high water solubility. Cyclodextrins are usually found as hexamers (α), heptamers (β), or octomers (γ). The β-cyclodextrins as compared to α- and γ-cyclodextrins have the highest affinity for inclusion of cholesterol due to their differences in size and hydrophobicity (37,38). Earlier β-cyclodextrins were employed

Table. Effect of different cholesterol acceptors on sperm cholesterol efflux, capacitation, and fertilization rates in different species.

Author	Species	Conc. of agent used	Decrease in cholesterol (%)	Acrosome reaction (%)	Fertilization rate (%)
Bovine serum albumin					
Davis et al., 1979 (16)	Rat	4 mg/mL	~12% decrease	-	-
Go and Wolf, 1985 (18)	Mouse	20 mg/mL	~60% decrease	-	-
Osheroff et al., 1999 (36)	Human	5 mg/mL	~30% decrease	48%	-
Zarintash and Cross, 1996 (63)	Human	26 mg/mL	~26% decrease	-	-
β-Cyclodextrin					
Choi and Toyoda, 1998 (41)	Mouse	0.25 mM	----	45%	53%
		0.75 mM	~50% decrease	-	21%
Visconti et al., 1999 (30)	Mouse	M-β-CD (3 mM)	Sperm = $3 \pm 0.6^{\blacklozenge}$ Media = $410 \pm 8^{\blacklozenge}$	-	-
		2-OH-p-β-CD (3 mM)	Sperm = $120 \pm 3^{\blacklozenge}$ Media = $309 \pm 5^{\blacklozenge}$	75%	-
		Control	Sperm = $431 \pm 4^{\blacklozenge}$ Media = $11 \pm 1^{\blacklozenge}$	-	-
Iborra et al., 2000 (42)	Goat	8 mM	~50%–60% decrease	35%	-
Funahashi, 2002 (64)	Boar	MbCD (0.125–2 mM)	~20%–45% dose-dependent	-	-
		Control	Less than 20%	-	-
Osheroff et al., 1999 (36)	Human	M-β-CD (3 mM)	Sperm = $157 \pm 2^{\blacklozenge}$ Media = $295.7 \pm 5^{\blacklozenge}$	-	-
		2-OH-p-β-CD (10 mM)	Sperm = $157 \pm 2^{\blacklozenge}$ Media = $295 \pm 5^{\blacklozenge}$	-	-
		Control	Sperm = $459 \pm 4.6^{\blacklozenge}$ Media = $11.3 \pm 0.3^{\blacklozenge}$	-	-
Cross, 1999 (43)	Human	2.5–10 mM	89%	26%	
Bovine seminal plasma					
Thérien et al., 1998 (15)	Mouse	120 µg/mL	~51%	38%	-
		HDL (100 µg/mL)	~39%	15	-
Percoll gradient centrifugation					
Tanphaichitr et al., 1996 (22)	Mouse		Control = $0.235 \pm 0.04^{*}$ PGC = $0.203 \pm 0.024^{*}$	-	-
Furimsky et al., 2005 (23)	Mouse		Control = $0.338 \pm 0.068^{*}$ PGC = $0.120 \pm 0.017^{*}$	Control ~90%–92% PGC ~95%–100%	Control = 57 ± 1 PGC = 74 ± 6
Miscellaneous					
Huneau et al., 1994 (60)	Ram	20% estrous sheep serum	Treatment ~60% Control ~14%	-	Treatment ~85% Control = 0.7%
Langlais et al., 1988 (19)	Human	5% human female serum or follicular fluid	Treatment ~50% Control ~14%	-	-
Sugkraroek et al., 1991 (61)	Human	3.5% HSA medium	5%	-	-
Benoff et al., 1993 (6)	Human	30 mg/mL HSA	15%		
Ehrenwald et al., 1988 (62)	Human	Incubated protein-free medium	31%	AR was induced by LPC	-

*: nmol/mg sperm DNA; $\blacklozenge$: ng steroid/10^6 sperm ± SEM.

to extract cholesterol from erythrocyte and model membranes (37–39). Similarly, derivatives of β-cyclodextrin (methyl-β-cyclodextrins and 2-hydroxyl-β-cyclodextrins) have been used efficiently for cholesterol efflux in cultured cells (40).

Numerous experiments have been conducted to evaluate the effect of cyclodextrins on cholesterol removal and sperm functions in different species (Table). Choi and Toyoda (41) found that cyclodextrins induced capacitation by removing cholesterol from mouse sperm in vitro. Visconti et al. (30) and Iborra et al. (42) concluded that β-cyclodextrin derivatives are equally effective cholesterol-binding heptasaccharides, which mediate cholesterol efflux from mouse and goat sperm in a concentration-dependent manner. Cross (43) observed that high cholesterol efflux achieved by using β-cyclodextrins or their derivatives is essential for the acrosome reaction in human sperm. These previous reports indicated that cyclodextrin possesses the ability to bind sperm membrane for cholesterol efflux; it could be a better choice than the other biological hydrophobic compounds for in vitro sperm preparation.

Inducing cholesterol in sperm plasma membrane with cyclodextrin molecules (CLCs) (44) enhanced the cryotolerance in rams (45), bulls (46–48), bucks (49), elephants (50), and stallions (51). This might be due to the fact that increased cholesterol in the sperm cell membrane enhanced the osmotic tolerance and inhibited the acrosome reaction (52). On the other hand, fertility after use of CLC-treated frozen-thawed semen is questionable. Although removal of cholesterol from the sperm membrane is essential for capacitation, further studies are needed regarding the effect of cholesterol removal from CLC-treated sperm after cryopreservation and its impact on fertilization or fertility.

4.3. Bovine seminal plasma
Bovine seminal plasma (BSP) contains a family of closely related major proteins designated as BSP-A1, BSP-A2, BSPA3, and BSP-30-kDa. These BSP proteins are secretory products of seminal vesicles; they bind to sperm upon ejaculation and play a role in capacitation (53). Upon ejaculation, the BSP proteins interact with the choline phospholipids on the sperm membrane (54) and bind to heparin in the uterine environment for capacitation (55). Therien et al. (15,21) described how BSP proteins accelerate capacitation of bovine epididymal sperm in the presence of heparin and HDL. Thérien et al. (15) also showed that the BSP proteins and HDL play an important role in the efflux of cholesterol from the plasma membrane of sperm during capacitation. Therefore, sperm capacitation by BSP could be effective in in vitro fertilization.

4.4. Percoll gradient centrifugation
Centrifugation through a gradient of Percoll has been used for more than 2 decades to prepare motile sperm from for

intrauterine insemination and in vitro fertilization (56,57). It is well known that Percoll gradient centrifugation (PGC)-capacitated mouse sperm has a higher fertilizing ability than washed capacitated sperm or whole populations of sperm (58,59). Tanphaichitr et al. (22) described how washing or PGC had no effect on the cholesterol and phospholipids content of mouse spermatozoa. In contrast, Furimsky et al. (23) reported that PGC lowered the cholesterol content in capacitated mouse sperm, which showed good fertilizing ability compared to the control. However, to the authors' knowledge, the effect of PGC on cholesterol removal and capacitation in other mammalian species has not been studied.

4.5. Miscellaneous agents
Several authors have reported significant cholesterol removal through the addition of various biological components to the incubation medium or through the use of biological fluids. Female biological fluids have also been used for cholesterol efflux from sperm. Huneau et al. (60) used 20% estrous sheep serum in an incubation medium for cholesterol efflux and reported higher cholesterol efflux (60% vs. 14%) and fertility (85% vs. 0.7%) in the treated ram sperm compared to the control. In another study, Langlais et al. (19) incubated human spermatozoa in a medium supplemented with 5% human female serum or follicular fluid. Their results showed increased cholesterol efflux (50%) in the presence of 5% human female serum or follicular fluid, whereas low cholesterol efflux (14%) was observed in spermatozoa incubated with protein-free medium. In contrast, Davis et al. (17) found that cholesterol efflux from sperm membranes was not statistically significant using comparable concentrations of serum albumin. Sugkraroek et al. (61) found that using 3.5% human serum albumin (HSA) medium only caused a 5% cholesterol removal from fertile donors' sperm. Similarly, Benoff et al. (6) reported 15% cholesterol efflux in human sperm when incubated with 30 mg/mL HSA in a medium. Ehrenwald et al. (62) did not detect any acrosome-reacted spermatozoa after 31% cholesterol efflux in 90 min. They obtained acrosome responsiveness only after the addition of lysophosphatidylcholine. These authors concluded that biological agents (estrous sheep serum, HAS, follicular fluid, etc.) might be useful for cholesterol efflux and capacitation in sperm.

5. Conclusion
In summary, the outcome of these studies (Table) provides evidence that BSA and β-cyclodextrins are the best choices for cholesterol efflux and sperm capacitation for in vitro fertilization. Other biological substances had little influence on sperm cholesterol efflux, but their effect on cholesterol efflux, capacitation, and fertility should be explored across more species.

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