[Frontiers in Bioscience 1, d103-117, July 1, 1996]

MEMBRANE LIPID DYNAMICS DURING HUMAN SPERM CAPACITATION

Paz Martínez[1] and Antoni Morros[2,3]

[1] *Instituto de Biología Fundamental. Unidad de Inmunología, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona) Spain.* [2] *Unitat de Biofísica. Departament de Bioquímica i de Biologia Molecular,Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona) Spain*

TABLE OF CONTENTS

1. ABSTRACT

Sperm membranes have an unusual lipidic composition which is distinct from those of mammalian somatic cells. They have high levels of plasmalogens, a kind of ether-linked lipids, and a high content of polyunsaturated fatty acyl groups. Plasmalogens may form non-diffusible membrane regions or domains, whereas polyunsaturated ethanolamine plasmalogens are known to destabilize the lipidic bilayer. During transit of sperm through the female reproductive tract, sperm-coating proteins bind to heparin-like glycosaminoglycans. An essential feature of capacitation is the removal of cholesterol from the acrosomal membrane of sperm. Albumin and high-density lipoproteins present in the uterine and follicular fluid act as cholesterol acceptors. Plasma membrane of sperm organize in large non-diffusible lipid domains. This regionalization affects the distribution of both lipids and proteins. A barrier to lateral diffusion of lipids and proteins in the equatorial segment has been reported and contributes to the formation of macrodomains. Lateral separation into cholesterol-rich and cholesterol-depleted micro-domains could also be created. Cone- shaped phospholipids induce the formation of non-bilayer phases and might facilitate membrane fusion.This review will discuss the removal of coating proteins, cholesterol efflux, domain organization, relocalization of lipids and proteins and the role of fusogenic lipids during capacitation.

Received 05/06/96; Accepted 06/18/96

[3] To whom correspondence should be addressed, at Unitat de Biofísica, Department de Bioquímica i de Biologia Molecular, Facultat de Medicina, Universitat Autònoma de Barcelona 08193, Bellaterra, Barcelona, Spain. Fax #: 34-3-581 2011 Email: amorros@cc.uab.es

2. INTRODUCTION

The highly differentiated and polarized human spermatozoa need to fulfill various requirements before they fertilize the oocyte: 1) protection and stability for their travelling through the female reproductive tract. 2) hyperactivated motility to swim across the various regions of the female genital conducts. 3) high fusogenicity of sperm membranes at the right time and position for acrosome reaction to occur. Then, spermatozoa have full fertilizing ability to penetrate the oocyte and fuse with its membrane. The preparative events that render the spermatozoa competent for fertilization are collectivelly known as capacitation. Capacitation is a process that takes place *in vivo* after the spermatozoa have been present in the female tract for a period of time or *in vitro* under certain conditions and media. This has been widely reviewed by several authors (1-6).

There are several structural and functional aspects of sperm membranes that may contribute to the

flexibility of this type of cell to become well adapted to the different environments that it will encounter. It should be emphasized that drastic changes occur in the plasma membranes of sperm from their epididymal maturation to their capacitation in the female reproductive tract. Major substances responsible for these functional changes are lipids. Sperm membranes have a very distinctive lipid composition: highly unsaturated plasmalogens that may contribute to the formation of non-diffusible membrane regions or domains capable of regionalizing both lipids and proteins. The high amount of non-bilayer forming lipids in the sperm plasma membrane seems to induce unstable membrane domains that have been described as playing a major role in membrane fusions during the acrosome reaction (7-10). The plasma membrane of the sperm head has high amounts of cholesterol. Cholesterol is known to regulate the fluidity of membrane lipid bilayers and to play an important role in capacitation. As spermatozoa pass through the female genital tract, cholesterol is removed from sperm membranes and is picked up by albumin and high density lipoproteins (11-12).

During the transit of sperm through the female reproductive tract, which contains high concentrations of glycosaminoglycans, coating proteins previously bound to sperm surface are trapped by heparin-like glycosaminoglycans: removal of proteins occurs in a time-dependent manner and this is considered to constitute the first step towards capacitation. The role of these external coating-proteins is to protect sperm on its long path to the oocyte and to prevent early development of acrosome reaction (13-18).

3. LIPID COMPOSITION OF MATURE SPERM

The lipid composition of plasma membrane of mammalian spermatozoa is markedly different from those of mammalian somatic cells. The major lipid composition of human spermatozoa presented in Table 1 is based on data reported by Alvarez and Storey (19) and Mack *et al.*. (20) which are in accordance with the composition given by Poulos and White (21). Human spermatozoa have unusually high levels of ether-linked lipids and a high content of unsaturated fatty acyl groups such as docosahexaenoyl (22:6 chains). Sphingomyelin is also a major lipid constituent of the plasma membranes of sperm. Although these molecules do not have an unusual amount of glycolipids, they contain a structure that is unique to sperm: sulfogalactosylglycerolipid or seminolipid. The ratio cholesterol/phospholipid is around 1; this high cholesterol content seems to play an important role in capacitation.

Ether-linked lipids are shown to be very abundant in several mammalian sperm plasma membranes (22, 23). Ether lipids are glycerophospholipids that contain either one alkenylether group at position *sn*-1 of glycerol (named plasmalogens), or one (at position *sn*-1) (Figure 1) or two alkylether groups. Plasmalogens are the most abundant ether lipids in human sperm and in animal cells. Choline and ethanolamine plasmalogens have specific molecular conformation (24), probably responsible for a more densely packed structure as compared with diacylglycerophospholipids. A special behavior of polyunsaturated ethanolamine plasmalogens has been described in artificial and biological membranes and they may facilitate membrane fusion (24, 25) as will be described in detail. Choline plasmalogens may contribute to form non-diffusible membrane regions, that confer stability on the membranes (10, 24).

Mammalian spermatozoa undergo important changes during their passage through the epididymis that affect their lipid composition. The lipid pattern of cauda epididymis spermatozoa (mature cells), however, is similar to that in ejaculated spermatozoa. During epididymal maturation, a decrease in the amount of sperm lipid has been reported in the sperm of boar, rat, bull and ram (26-28). However, one of the relevant changes associated with the maturation of rat sperm is that the ratio plasmenyl/phosphatidyl increases from 0.33 in caput epididymis to 0.95 in cauda epididymis. As a consequence of these changes, the plasmalogens become a major phospholipid component in the cauda and plasmenylcholine becomes the most abundant phospholipid (28). Similar data were obtained from the analysis of bovine spermatozoa in which choline plasmalogens were the major phospholipid (35% of total lipid phosphorous) (29). Since ether linkages are not easily cleaved by the action of lipases (24), ether lipids might confer stability on membrane during sperm maturation.

Very high amounts of polyunsaturated fatty acid chains, especially docosahexaenoic (22:6 chains), are found in the plasma membrane of human sperm (table 1). These chains are concentrated in the ether-containing phospholipids, as proposed for the plasma membrane of ram sperm (22, 30). The other dominant fatty acid chains are saturated, predominantly 16:0. A striking feature of ether lipids, described in the bovine sperm, is the composition of their acyl chains: almost exclusively (97%) a 16:0 moiety at carbon *sn*-1 and either a 22:5 (20%) or 22:6 (75%) moiety at carbon *sn*-2 (29). A diagram of this plasmalogen molecule is presented in Figure 2; the configuration of the polyunsaturated acyl chain (22:6) is based on the model presented by Stubbs and Smith (31). An approximately helical conformation is proposed, with a shortening of the chain. The motional freedom of the chain is severely limited by the lack of rotation at the six double bonds, highly restricting changes in

Table 1. Major lipids in human spermatozoa

Component	nmol/10^8 cells
Phospholipid[a]	
Choline diacylglycerophospholipid	37.0
Ethanolamine diacylglycerophospholipid	31.5
Choline plasmalogen	12.5
Ethanolamine plasmalogen	20.0
Phosphatidylserine	
8.5	
Phosphatidylinositol	6.1
Phospahtidylglycerol	0.6
Sphingomyelin	20.0
Cardiolipin	2.1
Total phospholipid	138.3
Fatty acids[a] (Chain length: number of double bonds)	
Saturated fatty acids	
Hexadecanoic (palmitic) (16:0)	105.5
Octadecanoic (stearic) (18:0)	35.9
Unsaturated fatty acids	
Octadecenoic (oleic) (18:1)	32.6
Octadecadienoic (linoleic) (18:2)	23.2
Icosatrienoic (20:3)	14.9
Icosatetraenoic (arachidonic) (20:4)	20.1
Docosahexaenoic (22:6)	108.0
Sterols[a]	
Cholesterol	133.0
Desmosterol	78.5
Total sterols	211.5
Glycolipids[b]	6.4

[a] Adapted from Alvarez and Storey, 1995 (19)

[b] Adapted from Mack *et al.*, 1987 (20)

conformation (31). Epididymal maturation results in significant alterations in the proportions of major fatty acid chains. In several species an increase of 22:4 and 22:5 fatty acid chains is observed (28, 32). This is probably linked to the increase of plasmalogens already discussed.

One of the important components of the plasma membrane of sperm is sphingomyelin, which shows maximal level of saturated fatty acids (80-97%) in goat and boar sperm (26, 27). This lipid is known to exert a rigidifying effect on biomembranes, that is, it tends to condense the bilayer,perhaps due to hydrogen bonding between the amide bonds or via the free

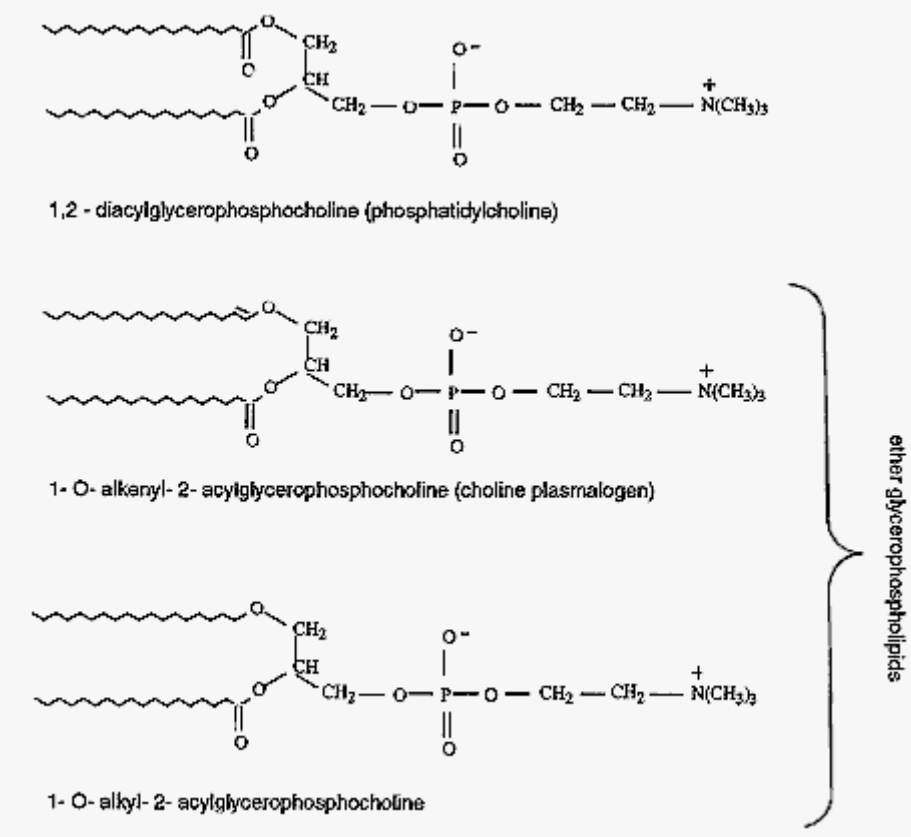

Figure 1 - Comparison of phosphatidylcholine with ether-linked choline phospholipids

hydroxyl group (33, 34). It has been proposed that sphingomyelin preferentially interacts with cholesterol and determines the sterol content in the plasma membrane (35, 36).

The high cholesterol/phospholipid ratio is originated in the epididymis, which displays a high rate of cholesterol synthesis, and it is transferred into the plasma membrane of maturing spermatozoa (2). A 2-fold increase in the cholesterol/phospholipid molar ratio is observed in ram spermatozoa during sperm maturation (22). Stabilization of the membrane by cholesterol may be beneficial to spermatozoa that must travel through the female genital tract.

Cholesterol sulfate is a normal constituent of the plasma membrane of sperm and it is also present in seminal plasma (37). During epididymal transit, an 18-fold increase in the amount of sterol sulfate of the plasma membrane of sperm has been described in hamster (38, 39). This anionic lipid is sequestered into the plasma membrane of the head and of the midpiece of the sperm (40). It has been postulated that cholesterol sulfate could regulate the fluidity of the sperm membrane during epididymal maturation and later during capacitation and acrosome reaction in the female genital tract (26). Interestingly, cholesterol sulfate contributes to the net negative charge of the external surface of sperm (40).

Epididymal maturation induces a significant increase in the negative charge of the sperm membrane due to the uptake of sialoglycoproteins (41) and anionic lipids such as cholesterol sulfate (40), sulfogalactolipids (42) and cardiolipin (28).

4. LIPID CHANGES DURING CAPACITATION

4.1. Cholesterol efflux from sperm membrane

Plasma membranes of ejaculated sperm are considered to be destabilized by loss of cholesterol from the membrane during the course of capacitation (12,43). Cholesterol is known to regulate the fluidity of membrane lipid bilayers and the permeability of membrane (44, 45) and to modulate the lateral mobility of integral proteins and functional receptors within the membrane. The "flip-flop" of phospholipids, that is to say, their passage from one leaflet of the bilayer to the other, is markedly restricted by the increasing rigidity and order conferred on lipid bilayers by cholesterol (11). Davis was the first to report that removal of membrane

cholesterol, with a consequent decrease in the cholesterol/phospholipid ratio, constitutes an important step in sperm capacitation (46). Hoshi *et al.*. reported the existence of a close relationship between human sperm capacitation and the cholesterol/ phospholipid ratio: lower ratios correspond to faster sperm capacitation, as indicated by the sperm penetration assay (43).

Cross reported that human spermatozoa in the continued presence of seminal plasma do not become acrosomally responsive. The purified inhibitory activity in seminal plasma was identified as that of cholesterol on the basis of its solubility in organic solvent, its chromatographic behavior and its mass spectrum (47). This cholesterol from plasma membrane is thought to be transferred to high density lipoproteins present in the oviductal fluid in the female genital tract, thus promoting capacitation.

Part of the cholesterol in the plasma membrane is present in the form of sulfoconjugates. The cholesterol sulfate accounts for only 2% of the total sterol present in human sperm; however, as it is concentrated in the membranes overlying the acrosome, it probably accounts for as much as 20% of the sperm head surface and is likely to contribute to membrane stability in this region (37, 40).

Cholesterol sulfates inhibit the ability of capacitated rabbit sperm to penetrate the zona pellucida, because of their membrane-stabilizing properties (48). The difference between cholesterol sulfate and unesterified cholesterol may be due in part to the different polar headgroups of these two molecules. Cholesterol has a relatively small polar group: a poorly hydrated hydroxyl group; in contrast, cholesterol sulfate has a large charged and hydrated polar sulfate group. Consequently, cholesterol sulfate stabilizes the membrane bilayer, thus avoiding disruption to a non-bilayer organization (37, 49). The action of the cumulus steroid sulfatases on steroid or cholesterol sulfate has been postulated to be part of the mechanism of capacitation (11). Cholesterol removal, after desulfation, is thought to increase membrane fluidity and to allow greater lateral movements of integral membrane proteins. This is associated with a greater permeability to calcium, which is apparently the key trigger in the acrosome reaction (48). Alternatively, factors that serve to neutralize the charge of sulfate groups, such as high ionic strength or binding of divalent cations appear to reduce the bilayer stabilizing capacity of cholesterol sulfate (49).

A question arises concerning the regions of sperm membrane from which cholesterol is released. Electron microscopic evaluation of freeze-fractured filipin-treated guinea pig spermatozoa cultured under capacitating conditions revealed a loss of cholesterol

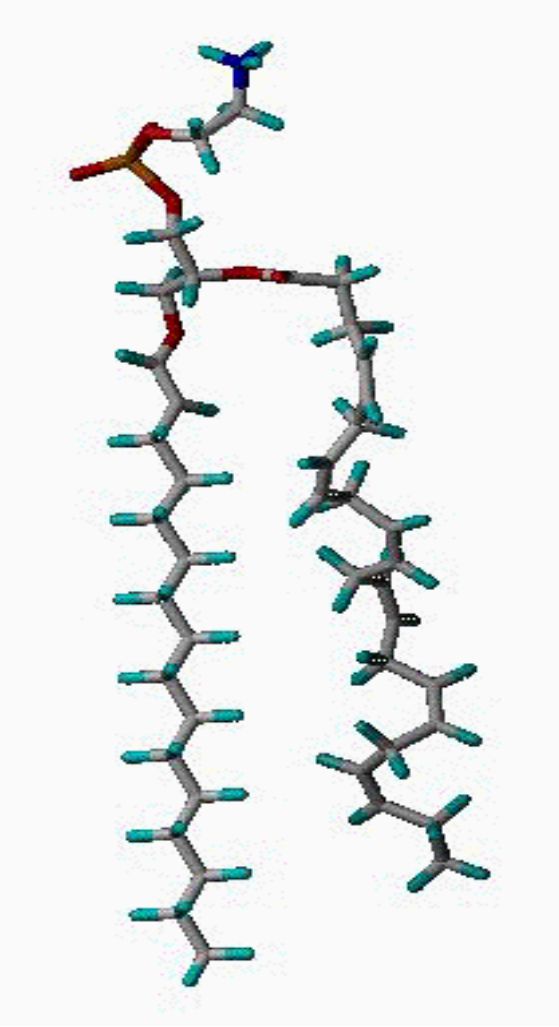

Figure 2 - Diagrammatic representation of an ethanolamine plasmalogen (1-O-hexadecyl-2-docosahexa-enoylphospha-tidylethanolamine) (16:0/22:6), drawn using the computer program Sybyl 6.2.

from fusogenic areas of the plasma membrane overlying the acrosome (8, 50). Cholesterol in both the anterior and the equatorial regions of human sperm head was also detected by Tesarík and Fléchon by examinig filipin-cholesterol complexes. Acrosomal plasma membrane is probably prevented from fusion with the acrosomal outer membrane by its high concentration in anti-fusogenic sterols. During *in vitro* capacitation, cholesterol-free patches are developed, these patches becoming the only sites favorable to the initiation of membrane fusion (51). The periacrosomal plasma membrane, which is rich in cholesterol (51, 52), is the region where membrane fusion takes place during the acrosome reaction. It is from this region that cholesterol is preferentially removed (53).

4.2. Cholesterol acceptors

Albumin and high-density lipoproteins present in uterine fluid, follicular fluid, or an *in-vitro* medium containing serum albumin act as sterol acceptors and induce cholesterol efflux from sperm membrane (46, 54). Human sperm cholesterol efflux after 4 hours of capacitation in human follicular fluid or serum is about 50% of the total sperm-bound cholesterol (54). On the other hand, cholesterol-enriched albumins, whose sterol binding sites are saturated, inhibit fertilization in a sterol concentration-dependent manner (55, 56). Following a 60 minute incubation with bovine serum albumin (BSA), mouse spermatozoa become depleted (29%-50%) of sterol in a time-dependent manner (55).

For *in-vitro* capacitation, cholesterol-free liposomes have also been used. Ehrenwald *et al*. (53) showed 28%-31% cholesterol removal from bovine spermatozoa by incubating them for 90 minutes in the presence of cholesterol-free liposomes. Incubation of those spermatozoa with lysophosphatidylcholine resulted in acrosome reaction in 40% of cells as compared with a 14% acrosome reaction in the control spermatozoa whose cholesterol was not removed. Ehrenwald *et al*.(57) also demonstrated that the capacity of bovine sperm to penetrate both zona-free hamster oocytes and *in vitro* matured bovine oocytes was markedly increased by a reduction in cholesterol of sperm membrane. These results suggested that cholesterol efflux may be an early step in bovine sperm capacitation.

Although it is well established that albumin itself can function as a sterol acceptor from living mammalian cells (58), Ravnick *et al*. (59) presented evidence that the lipid transfer protein-1 (LTP-1) is responsible for this activity. They suggested that the variable ability of albumin preparations to support *in-vitro* sperm capacitation is largely attributable to the presence of the contaminating LTP-1. They proposed that this protein, which is present in human follicular fluid, may contribute to the capacitation of sperm (60).

5. LIPID DOMAINS AND MEMBRANE FLUIDITY

5.1. Macrodomains and microdomains

When describing membranes of specialized cells with highly regionalized functions, macrodomains are defined; they correspond to morphologically distinguishable large membrane areas. Extending from the submicron level to the molecular scale, microdomains based on different lipid properties are also detected in cell membranes (61-64). Spermatozoa are a prime example of regionalized cells with specific functions localized in distinct regions: acrosome reaction takes place in the anterior region of the head, and sperm-oocyte plasma membrane fusion occurs in the equatorial region of the head. Motion energy from mitochondria occurs in the midpiece, while motility is exhibited in the tail. This regionalization affects the distribution of both plasma membrane lipids and proteins.

Lipid composition and lipid diffusibility in plasma membrane appears to be different for each of these distinct morphological regions of spermatozoa. Two macrodomains of different composition are observed in the head of spermatozoa from epididymis by means of freeze-fracture: the acrosomal cap and the post-acrosomal segment. The acrosomal cap portion of the plasma membrane is highly fusogenic and during acrosome reaction it fuses with the underlying acrosomal membrane. On the contrary, the post-acrosomal segment of the plasma membrane is non-fusogenic under the same conditions (8). Evidence of barriers to membrane protein and lipid lateral diffusion has been found at the equatorial region (65-67).

During capacitation and acrosome reaction, proteins are relocated. The migration of some proteins, such as PH-20, during acrosome reaction may partially result from an alteration of the barriers mentioned above (68, 69). SP-10 protein, a testis-specific acrosomal protein, is redistributed in capacitating sperm (70); SP-10 is detected in the equatorial segment and in the inner acrosomal membrane after acrosome reaction. Protein reorganization and clustering during capacitation has been shown in the acrosomal region of boar sperm by immunoelectron microscopy (71). A 78 kDa sperm antigen becomes clustered over the principal segment of the acrosome and it is localized in the postacrosomal and equatorial region after the acrosome reaction. During capacitation, a rat epididymal protein of 37 kD which is associated with sperm surface during maturation, migrates to the equatorial segment (72).

5.2. Biophysical, biochemical and cytochemical techniques for the analysis of lipid regionalization in the plasma membrane of sperm

In electron microscopy studies of freeze-fracture preparations, the polyene filipin has been widely employed as a probe of membrane structure because of its selective affinity for cholesterol and its sensitivity to the membrane-sidedness of sterol. Friend (8) has applied this technique to the study of guinea pig sperm membrane and he concluded that cholesterol is more abundant (4-fold) over the acrosomal cap region than over the post-acrosomal segment. Moreover, the inner fusogenic leaflet of the plasma membrane over the acrosome is relatively rich in free sterols (8).

Like the sterols, anionic phospholipids are demonstrable by polymyxin-B (50, 73) and by adriamycin (8). Different concentrations of acidic

lipids are found in adjacent domains of plasma membrane of guinea pig sperm, with higher concentration localized over the fusogenic acrosomal cap; the concentration of these lipids increases as the membrane becomes fusionally competent prior to the acrosome reaction (8, 50). Since sulfo-galactolipid seminolipid is the most prominent anionic lipid in the outer leaflet of mammalian sperm plasma membranes, it is likely that polymyxin B detects the distribution of seminolipid in the surface of the membrane (67). Negative charge density of the exterior phospholipid-water interface at the acrosomal region has also been detected by using surface-directed spin labels (74, 75).

Inner and outer leaflets are considered by some authors as two large and almost independent domains, but with the possibility of trans-bilayer lipid redistribution (61). The distribution of phospholipid in sperm membrane is asymmetric. In the ram sperm, choline containing phospholipids are situated mainly in the outer membrane monolayer, whereas cardiolipin (diphosphatidylglycerol) and phosphatidylserine are located predominantly in the inner layer (76).

Fluorescence recovery after photobleaching (FRP) measures the lateral diffusion of lipids in the plane of biological membranes. In most somatic cells, lipids are almost nearly free to diffuse laterally; in contrast, the plasma membrane of mammalian spermatozoa has large non-diffusing lipid fractions (77-79). These non-diffusing lipid areas increase as the spermatozoa differentiate during epididymal maturation (50% compared to 15% in most somatic cells) and also in capacitation. Immobile lipids are observed only in other polarized mammalian cells, such as epithelial cells, and may be due to lipid-lipid interactions (30).

At physiological temperature, most of the membrane lipids are organized in a fluid lamellar liquid-crystalline phase, ($L\alpha$ phase). As the temperature decreases, bilayer lipids undergo a transition to the gel phase ($L\beta$ phase), with more ordered fatty acids chains. In the gel-like areas, molecular motion is very slow and in the liquid-crystal-like microdomains, molecules move relatively freely. The phase transition temperature (T_m) depends on the lipidic composition of every membrane or even of each lipidic domain. Lipid phase transitions usually can be measured by differential scanning calorimetry (DSC) or in intact cells by fluorescence polarization and by Fourier transform infrared spectroscopy (FTIR). The phase behavior of egg yolk phosphatidylethanolamine as a function of temperature studied by DSC and FTIR is shown in Figure 3. Both curves show the phase transition $L\alpha$- $L\beta$ around 20°C. A second transition ($L\alpha$ - H_{II}), which will be discussed below, takes place around 50°C.

By using FTIR, Drobnis *et al*. (81) found a complex lipid phase behavior in the membranes of pig sperm that may represent multiple phase transitions, and which corresponds to various classes of lipids with different T_m. Alternatively, this may correspond to lipids located in the different membranes of the cell or in different domains.

Fluorescence polarization with lipid-like fluorescent probes gives a measure of membrane fluidity. Thus, a high fluorescence anisotropy corresponds to a more rigid membrane. By using this technique, when plasma membrane (PM) of sperm of rabbit was analyzed separately from the outer acrosomal membrane (OAM) and the inner acrosomal membrane (IAM), OAM was more rigid than the other two membranes. Since sphingomyelin is known to condense the lipid bilayer by forming lipid-lipid hydrogen bonds in the hydrophylic-hydrophobic interface, this could be partially attributed to the higher sphingomyelin/phosphatidylcholine ratio in OAM (82).

In the sperm of the bull and rabbit, differences in the fluidity of the two leaflets of plasma membrane were observed by fluorescence anisotropy. The inner leaflets show a significantly higher fluidity than the outer leaflets. The same differences between the two leaflets were observed in OAM and in IAM (82). This can be attributedto the asymmetric distribution of cholesterol using filipin as a probe (8).

Membrane fluidity in biological membranes may be ascertained by using merocyanine 540, a fluorescent dye that preferentially binds to fluid-phase domains. This probe reveals that the fusogenic portion of guinea pig acrosome has a greater degree of fluidity than the post-acrosomal segment. It is interesting to note that after the cells become capacitated, possibly due to migration of proteins towards the equatorial segment, the fluorescence extends to this region (50).

5. 3. Mechanisms accounting for the presence of distinct lipid domains

The intramembranous protein particles seem to play an important role in the formation of membrane microdomains. Nevertheless, in sperm plasma membrane, lipid-lipid interactions could be responsible for the immobilization of lipids and appear to contribute to the microdomain formation (83). This was demonstrated by FRP in liposomes formed exclusively with lipids extracted from the anterior region of head plasma membrane (10). The very unusual lipid composition of plasma membrane of sperm is very similar in its major components (one saturated fatty acid chain and one polyunsaturated 22:6 chain in the same phospholipid molecule) to the outer membrane of rods of the retina. Biophysical studies have provided evidence of lateral phase separation

(formation of lipid domains) in the retinal membranes (84), as was found for sperm membranes. This lateral phase separation results from differences in lipid chain saturation, chain length, head group and charge and the concentration of cholesterol within the membrane (62). Wolf *et al.* (30) reported, at physiological temperature, two major phase transitions in the plasma membrane of ram sperm by using DSC, suggesting the co-existence of liquid-crystalline and gel phase lipids of different domains. Large amounts of polyunsaturated plasmalogens would segregate from other lipids and form fluid domains, whereas more saturated lipids would form more rigid domains at physiological temperature (30).

Cholesterol preferentially interacts with sphingomyelin or saturated phospholipids whereas it is unlikely to associate with highly unsaturated phospholipids, like 16:0-20:4 and 16:0-22:6 phosphatidylcholine. In systems with a highly unsaturated fatty acid content, there could be lateral separation into cholesterol-rich and cholesterol-depleted microdomains. Consequently, this would create highly saturated and unsaturated lipid domains (85, 86).

Lipid diffusion barriers might also be mantained by lipid structures such as glycolipids (sulfo-galactosylglycerolipids or seminolipids) (65). Seminolipids have been localized in the equatorial segment of the acrosome reacted sperm (67). Lipid regionalization may also lead to protein regionalization by virtue of the preferential solubility of the proteins in different sites.

6. ROLE OF FUSOGENIC LIPIDS IN CAPACITATION AND ACROSOME REACTION

A required condition for membrane fusion between two membrane bilayers is that they have to be close to each other (87). However even though two membranes are proximal, fusion will not occur unless the bilayer structure is transiently disrupted. Such disruption may result from internal components of the membrane or from the external environment. For instance, calcium can induce membrane fusion in some bilayers rich in negatively charged lipids (88).

Phospholipids can form a variety of nonlamellar lipid assemblies. Most of these nonlamellar structures have been proposed as being involved as intermediates in membrane fusion. A qualitative concept based on "molecular shape" has successfully predicted some phase preference behaviors (89, 90). Cylindrical-shaped phospholipids, like phosphatidylcholine, prefers bilayer phases. Cone shaped phospholipids, such as unsaturated ethanolamine plasmalogens, have a small polar head and a large hydrophobic region; they tend to bend the

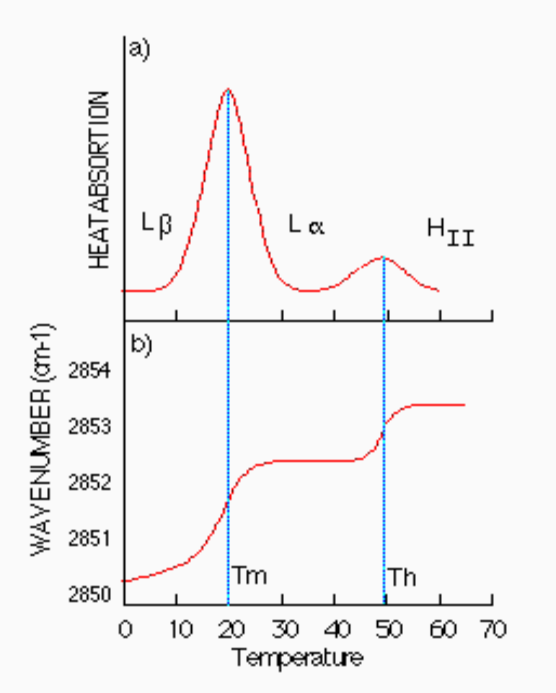

Figure 3 - Phase behavior of egg yolk phosphatidyl-ethanolamine as a function of temperature, studied by two techniques: a) Differential scanning calorimetry (DSC) and b) Fourier transform infrared spectroscopy (FTIR) (see the text for details). Adapted from Mantsch *et al.*. (80).

bilayer and to facilitate the formation of non-bilayer phases, like hexagonal II phase (Figure 4). Inverted cone-shaped phospholipids, such as lysophosphatidyl-choline, have only one acyl chain and also tend to form curved bilayers and non-bilayer phases.

The balanced co-existence of bilayer forming lipids with non-bilayer inducing lipids can result in "frustrated" structures that are in a transient and unstable situation. External changes, such as temperature increase, could alter the balance between structural factors and trigger a transition from bilayer $L\alpha$ phase to non-bilayer H_{II} phase (Figure 3). Alternatively, a change in the lipidic composition that would account for an increase in non-bilayer-forming lipids could also induce the phase transition. In biological membranes, proteins stabilize the bilayer phases. A decrease in the membrane protein concentration would favor the non-bilayer formation. These non-lamellar structures seem to be involved as intermediates in the membrane fusion (88).

In acrosomal membranes of spermatozoa, unsaturated ethanolamine plasmalogens are abundant. These lipids prefer hexagonal phases. On the other hand, cholesterol sulfate also present in these membranes, due to its large charged and hydrated

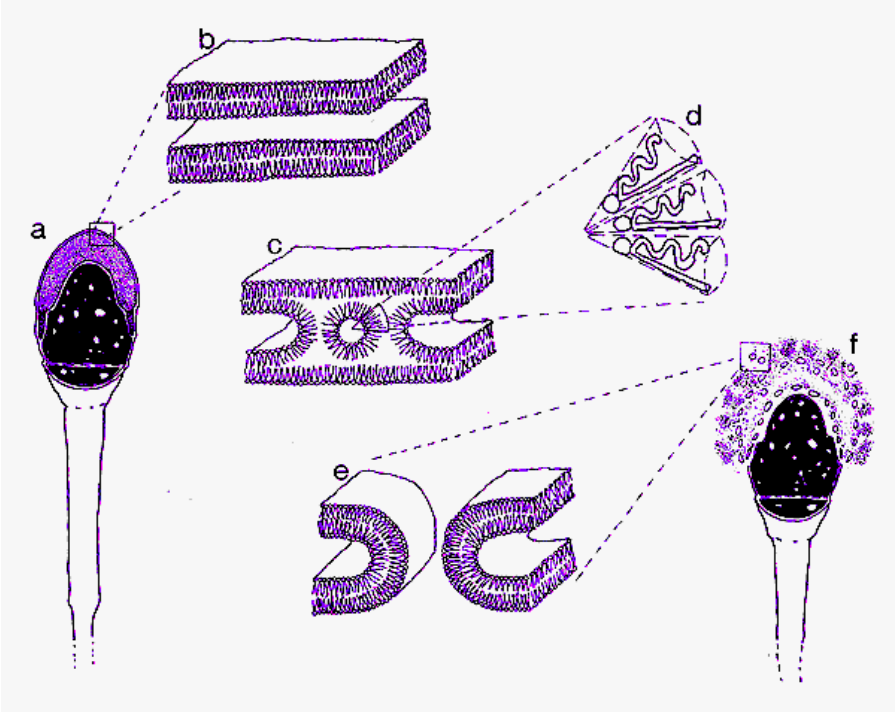

Figure 4 - Schematic presentation of the role of lipids in the proposed mechanism of membrane fusion, via the formation of nonlamellar inverted micellar intermediates. a) human non capacitated sperm; b) apposed plasma membrane (PM) and outer acrosomal membrane (OAM) from human sperm; c) inverted micellar intermediate; d) disposition of the cone-shaped lipids (*e.g.* polyunsaturated phosphatidylethanolamine plasmalogen) in the formation of a rod inverted mycelle; e) formation of lamellar vesicles; f) acrosome reacted sperm. Adapted from Yeagle (88) and Verkleij (91)

polar sulfate group, is a bilayer stabilizer (49). Drastic changes in the lipidic composition during capacitation would lead to fusogenicity (Figure 4). The migration of membrane proteins from the acrosomal head towards the equatorial region could also facilitate membrane fusion.

7. A MODEL FOR ORGANIZATION OF LIPIDS DURING CAPACITATION

7.1. Cholesterol efflux

A mechanism for free (un-esterified) cholesterol efflux from liposomes has been widely demonstrated, in which cholesterol molecules desorb from the donor lipid-water interface and diffuse throughout the aqueous phase until they collide with an acceptor particle (for reviews, see references 92, 93). Cholesterol may desorb very rapidly (half time of 30 minutes) from small unilamellar vesicles (SUV). The kinetics of cholesterol release from biological membranes has been described to be slower and cell-dependent (half time 1-50 hours).

The efflux of cholesterol from several mammalian biological membranes can be explained by the above mechanism.However, this process, due to the presence of an "unstirred" or immobile water layer around the cells, which is not present around vesicles, is more complex. This layer acts as a diffusion barrier between the cell surface and the "bulk" water; then, cholesterol concentrates in the surrounding layer until an acceptor enters this area and encounters it. Therefore, cholesterol efflux from membranes involves both the entrance of the cholesterol acceptor into the immobile water layer and collision with the desorbed cholesterol molecules (92-95). The high density of charged hydrophilic oligosacharide side-chains of glycoproteins of the glycocalyx region retains a layer of immobile water. The plasma membrane of sperm head exhibits a thick glycocalyx, consisting of glycoproteins anchored to the bilayer, extending 100-150 Å from the phospholipid-water interface. The morphological features of this glycocalyx are different in the acrosomal cap from those of the postacrosome (8).

Ejaculated non-capacitated sperm adsorb proteins in their surface, mainly of seminal plasma origin. The amount of one kind of coating protein that belongs to the family of spermadhesins is sufficient to

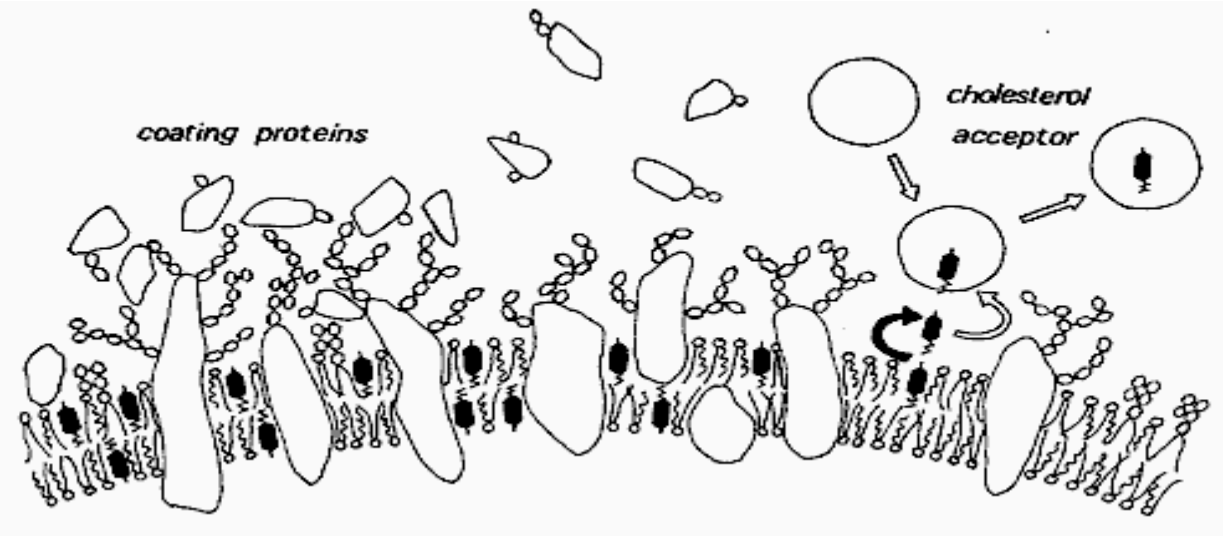

Figure 5 - Hypothetical model for the mechanism of cholesterol efflux in human sperm capacitation. This model shows the phospholipid-water interface to which cholesterol migrates and the glycocalyx zone consisting of glycoproteins anchored to the plasma membrane. Ejaculated non-capacitated sperm adsorb several coating proteins on the external charged surface. These proteins progressively dissociate by binding to the glycosaminoglycans in the female genital tract or by *in-vitro* incubation with heparin. Then, some cholesterol acceptors (93), such as albumin, may bind cholesterol and the lipidic bilayer becomes more fusogenic. Some receptors for hormones (progesterone) or for oligosaccharides (mannose-ligand) become accessible after capacitation, and acrosome reaction may be induced by progesterone or by zona pellucida.

cover one-third of the entire sperm head surface. Spermadhesins are acidic proteins of low molecular weight (15-17 kDa); these heparin binding proteins are carbohydrate- and zona pellucida-binding proteins and are thought to be adsorbed to this glycocalyx. Alternatively, spermadhesins and other coating proteins may interact and bind to phospholipids (96). They are involved in at least two important aspects of fertilization: sperm capacitation and sperm-egg interaction (97, 98). Spermadhesins seem to be related to the major proteins from bull seminal plasma, designated BSP (bovine seminal plasma), since they also have an affinity to heparin (99, 100).

It has been established both *in vivo* and *in vitro* that heparin or glycosaminoglycans capacitates sperm (101, 102). First and Parrish have suggested that capacitation of bovine sperm by glycosaminoglycans includes removal of decapacitation proteins from seminal plasma (15, 103, 104).

Removal of these proteins appears to be a prerequisite for the acrosome reaction. Ehrenwald *et al*. established that the rate of cholesterol exchange between lamellar liposomes and epididymal sperm is three to four times greater than for the ejaculated sperm (53). Taking all these data into consideration, the early events of sperm capacitation may be explained by the following (Figure 5): the layer of coating proteins covers the glycocalix zone, prevents the entrance of cholesterol acceptors and interferes with the removal of the cholesterol that might have migrated from sperm plasm membrane to the underlying viscous glycocalix. Once the coating proteins are removed from the surface of sperm, cholesterol acceptors are free to interact with the cholesterol which has already been removed from the sperm membrane, thus facilitating cholesterol efflux.

7.2. Effects of cholesterol efflux

Cholesterol is not randomly distributed within biological membranes. Some areas of membrane are cholesterol-rich whereas other domains are cholesterol-poor (36). After cholesterol efflux in capacitation, changes in the physical state of the sperm plasma membrane are observed: the lipid diffusibility becomes regionalized. A lipid re-distribution after capacitation would not be surprising, as cholesterol is known to alter dramatically the lateral domain organization of lipidic bilayers. Such reorganizations also affect the function of membrane proteins.

The anterior acrosome region of the human sperm plasma membrane, due to its high concentration of anti-fusogenic sterols, seems to be resistant to immediate fusion. It is the formation of sterol-depleted patches in the anterior acrosomal region that renders it susceptible to membrane fusion (51). Lipid re-distribution during capacitation appear to provide the

fusogenic domains required for membrane fusion in the acrosome reaction (50).

One of the important consequences of cholesterol efflux is the massive influx of extracellularCa^{2+}, this is considered a prerequisite for the acrosome reaction to occur. The entrance of calcium may be a consequence of the changes in the fluidity of the membrane that renders the membrane more permeable to Ca^{2+}. Alternatively, there is an opening of voltage-dependent Ca^{2+} channels, probably by stimulation of phospholipase C (105).

Increased intracellular Ca^{2+} concentration can trigger different pathways involved in the acrosome reaction: generation of diacylglycerol (DAG) through phospho-inositide breakdown; DAG stimulation of Ca^{2+} dependent phospholipase A_2 and participation in the membranefusion itself. Phospholipase A_2 action on phospholipids gives rise to lysophospholipids and arachidonic acid or other fatty acids, which are known to be highly fusogenic. (106, 107).

Ca^{2+} could also act directly on negatively charged membrane lipids, by neutralizing anionic phospholipids or cholesterol sulfate. This may induce membrane destabilization and fusogenic intermediates that are thought to induce a massive acrosome reaction in the vicinity of oocyte envelopes (49).

8. ACKNOWLEDGMENTS

We are greatful to M. Campillo from the Laboratorio de Medicina Computacional, Facultad de Medicina (Universitat Autònoma de Barcelona), for drawing the diagram presented in Figure 2. We acknowledge Pilar Fuentes for her contribution in drawing Figures 4 and 5 and Antoni Iborra for his help in the final typing of the manuscript; both from the Unidad de Inmunologia (Universitat Autònoma de Barcelona).

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