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An update on post-ejaculatory remodeling of the sperm surface before mammalian fertilization

B.M. Gadella [a,b,*], A. Boerke [a,b]

[a] Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, The Netherlands
[b] Department of Biochemistry & Cell Biology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

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ABSTRACT

The fusion of a sperm with an oocyte to form new life is a highly regulated event. The activation—also termed capacitation—of the sperm cell is one of the key preparative steps required for this process. Ejaculated sperm has to make a journey through the female uterus and oviduct before it can approach the oocyte. The oocyte at that moment also has become prepared to facilitate monospermic fertilization and block immediately thereafter the chance for polyspermic fertilization. Interestingly, ejaculated sperm is not properly capacitated and consequently is not yet able to fertilize the oocyte. During the capacitation process, the formation of competent lipid-protein domains on the sperm head enables sperm-cumulus and zona pellucida interactions. This sperm binding allows the onset for a cascade reaction ultimately resulting in oocyte-sperm fusion. Many different lipids and proteins from the sperm surface are involved in this process. Sperm surface processing already starts when sperm are liberated from the seminiferous tubules and is followed by epididymal maturation where the sperm cell surface is modified and loaded with proteins to ensure it is prepared for its fertilization task. Although cauda epididymal sperm can fertilize the oocyte IVF, they are coated with so-called decapacitation factors during ejaculation. The seminal plasma–induced stabilization of the sperm surface permits the sperm transit through the cervix and uterus but prevents sperm capacitation and thus inhibits fertilization. For IVF purposes, sperm are washed out of seminal plasma and activated to get rid of decapacitation factors. Only after capacitation, the sperm can fertilize the oocyte. In recent years, IVF has become a widely used tool to achieve successful fertilization in both the veterinary field and human medicine. Although IVF procedures are very successful, scientific knowledge is still far from complete when identifying all the molecular players and processes during the first stages the fusion of two gametes into a new life. A concise overview in the current understanding of the process of capacitation and the sperm surface changes is provided. The gaps in knowledge of these prefertilization processes are critically discussed.

1. Introduction

When mammalian sperm cells are ejaculated, they are initially unable to fertilize the oocyte. *In vivo*, sperm are activated in the female genital tract and acquire fertilizing potential once reaching the isthmus of the oviduct [1,2]. Sperm activation is known as sperm capacitation and can be mimicked by incubating sperm in IVF media in which the composition of ions and metabolites resemble that of oviduct (synthetic oviductal fluid). Before putting ejaculated sperm into IVF media, semen is first precisely washed for almost total removal of seminal plasma components

* Corresponding author. Tel.: +31-30-2535386; fax: +31-30-2535492.
E-mail address: B.M.Gadella@uu.nl (B.M. Gadella).

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(decapacitation factors [3]). The IVF media facilitate capacitation medium by containing high concentrations of bicarbonate [4] allowing stimulation of protein phosphorylation and cells signaling leading to hyperactivated sperm motility and surface reorganizations [5]. *In vitro* fertilization media also typically contain millimolar extracellular Ca^{2+} ions that are required for stimulating the acrosome reaction, the fertilization fusion, and the activation of the oocyte [6,7]. Note that the induction of the acrosome reaction is prevented in liquid storage media used for porcine artificial insemination. For instance, Beltsville Thawing Solution that contains high concentrations of EDTA to scavenge all extracellular Ca^{2+} [8]. Finally, IVF media contain fatty acid–free albumin, which is required to extract free sterols from the surface of capacitating sperm (note that lipoprotein particles that are residing in the oviduct fluid are supposed to act similarly) [9–11]. The cholesterol removal can also be achieved by cyclodextrins [12], although success IVF rates are low, and only at a narrow cyclodextrin concentration range, high cyclodextrin levels have deteriorative effects on the sperm cell and the oocyte [12–14]. For two reviews on the concerted action of these three capacitation factors in IVF media on sperm surface changes during sperm capacitation, see [15,16]. The current review provides an update of our knowledge about the organization of the porcine sperm plasma membrane with special emphasis on the role of lipid microdomain dynamics in sperm physiology. It will deal with how lipid microdomain organization (also referred to as membrane rafts) is influenced by *in vitro* capacitation factors and how this relates to sperm cell signaling networks, sperm surface organization, and the consequences for sperm physiology in terms of (1) hyperactivated sperm motility, (2) zona binding, and (3) the acrosome reaction. These three different sperm transitions are prerequisites for sperm being able to penetrate the zona pellucida (ZP) and fertilize the oocyte, and all rely on a capacitation-induced aggregation of membrane rafts in the apical surface area of the sperm head.

2. Capacitation factors and the generation of hyperactivated sperm motility

Bicarbonate enters the cell facilitated by bicarbonate sodium co-transport [17]. Intracellular bicarbonate binds and activates soluble adenylyl cyclase (sAC) in a stoichiometry of 1:1 [18]. The production of cAMP in turn activates protein kinase A (PKA) and results in the phosphorylation of a certain proteins [19]. The bicarbonate-responsive cells require both albumin and Ca^{2+} for the removal of surface cholesterol [10,11,13]. The removal of cholesterol will be discussed in the next paragraphs, and this removal causes additional and activation of PKA [20]. The PKA-mediated phosphorylation of A-kinase anchoring proteins (AKAP-3) is of specific interest, as it is a protein that is present in the outer dense fibers of the sperm flagellum [21] and therefore links the PKA signaling pathway with the capacitation-specific induction of tyrosine kinase, which in turn causes extensive tyrosine phosphorylation of proteins in the sperm tail [22]. The altered protein phosphorylation status of tail proteins is responsible for the typical altered and hyperactivated sperm motility in which also additional glycolytic metabolism is stimulated [23]. In fact, specific glycolytic enzymes are present in the principal piece and are substrates for the activated protein kinases discussed previously [24].

Although the consensus is that the cAMP/PKA pathway plays a large role in the increase in tyrosine phosphorylation by Ca^{2+}-efflux and HCO_3^-, other protein kinases have also been found to be present in sperm. An example is protein kinase C, and treatment of sperm with phorbol esters (protein kinase C stimulators) gives rise to changes in sperm motility, the acrosome reaction, and cAMP levels [25]. Also, serine/threonine protein kinases have been identified to be activated during sperm capacitation such as the extracellular-signal-regulated kinases 1 or 2; glycogen synthase kinase 3; calmodulin-dependent protein kinase IV; casein kinase II, and members of the testis-specific serine kinase family [25]. One other interesting finding is that cholesterol efflux regulated a proline-directed phosphorylation [25]. These results together indicate that there are multiple pathways active during sperm capacitation [5].

3. Capacitation factors induce increased lipid fluidity in the sperm plasma membrane

As mentioned previously, activation of the sperm cell by bicarbonate/Ca^{2+}/albumin also causes sperm surface alterations [26]. The changes in the sperm head plasma membrane of the bicarbonate-responsive cells can be monitored by labeling sperm with specific surface stains such as chlortetracycline [27], merocyanine [28], or filipin [29]. These membrane dyes detect fluidity changes in capacitating sperm [30]. The alterations at the sperm surface coincide with the typical increased amount of tyrosine phosphorylation in the sperm tail and thus to cells with hyperactivated motility [29]. The subpopulation with surface responses to bicarbonate/albumin/Ca^{2+} all showed extensive tyrosine phosphorylation in the sperm tails. The increased membrane fluidity also coincides with a bicarbonate-dependent cholesterol uptake from the surface to lipid-free albumin [9,31]. Remarkably, the only type of lipids that is extracted by albumin from the surface of responsive sperm is free sterols (desmosterol and cholesterol [31]). These relatively rather hydrophobic lipids most likely do not passively diffuse from the sperm surface into the hydrophobic pocket of albumin. Therefore, a model was proposed in which cholesterol transfer from the sperm surface to a hydrophobic pocket of albumin is mediated by an active protein-mediated transport of sterol monomers [31]. Recently, the possible mechanism of reverse cholesterol transport has been reviewed [11] as well as the possibility that reverse cholesterol transport is regulated by a lateral reorganization of the membrane lipids (see Section 4). Another recent finding is that a small portion of cholesterol is converted to oxysterols during capacitation, and that this conversion is linked to the activation of reverse cholesterol transport [13]: Failure of oxysterol formation and reverse cholesterol (for instance, by administering the antioxidant vitamin A to the IVF medium) resulted in a complete inhibition of IVF (see Section 8).

4. Functional heterogeneity of the sperm surface

The sperm cell contains a typical polarized arrangement of organelles in the sperm head, the midpiece, and the tail: (1) the sperm head contains a large secretory vesicle called the acrosome and a nucleus containing the super-condensed protaminated DNA of male haploid genome; (2) the midpiece contains a proximal and immobile part of the flagellum and coiled at the periphery, thereof a couple of 100 mitochondria can generate ATP by aerobic metabolism; and (3) the tail (principal piece and end piece) with the distal and motile part of the flagellum and additional dense fibers and elastic fibrous sheath containing cytoskeletal structures surrounding the microtubular structures of the flagellum. These specific properties of these additional structures and the flagellum itself as well as the phosphorylation status of proteins within these structures determine the motility and hypermotility patterns of sperm [32].

Similarly, the surface of the three sperm regions is also differentiated as two transmembrane protein ring structures, which separate the midpiece from the head and tail and are called the posterior and annular rings, respectively. These structures are lateral diffusion barriers allowing different lipid compositions in the sperm head, midpiece, and tail, respectively and functionally be compared with ring-shaped tight-junction structures [26]. The sperm head surface also has further lateral surface heterogeneity [26]. The boar sperm head surface can be subdivided into the apical ridge area, the pre-equatorial area, the equatorial area (together the entire acrosome region), and the post-acrosomal area. In part, these areas mirror the polar organization of underlying organelles; furthermore, the ergonomics of the fertilization cascade rely on specific features of these surface areas (see also Fig. 1): (1) sperm binding to the cumulus or ZP is restricted to the apical ridge area; (2) the acrosome reaction in which multiple fusions of plasma membrane and outer acrosomal membrane take place at the anterior area of the sperm surface but not at the equatorial membrane area of the sperm head [33]; and (3) this remaining intact equatorial surface area is the specific site of the sperm cell that, after zona penetration, becomes involved in the fusion with the oocyte membrane fertilization fusion [34]. Of specific interest is the finding on observing freeze-fracture replicas of the boar sperm head surface that no ultrastructural diffusion barriers can be observed [35]. In other words, the lipid microdomains are formed and reorganized by other forces than structures preventing lateral diffusion.

5. Lipid ordering and sperm membrane heterogeneity

The Singer–Nicolson model of the fluid-mosaic membrane was proposed as a prediction as to how cellular membranes are constructed [36]. Cholesterol, like phospholipids, behaves as an amphipathic molecule in the lipid bilayer, and the free hydroxyl group in cholesterol can be considered the hydrophilic part and the entire steroid backbone as hydrophobic stabilizing the lipid bilayer [37]. At physiological temperature, a large proportion of membrane lipids and membrane proteins have lateral mobility

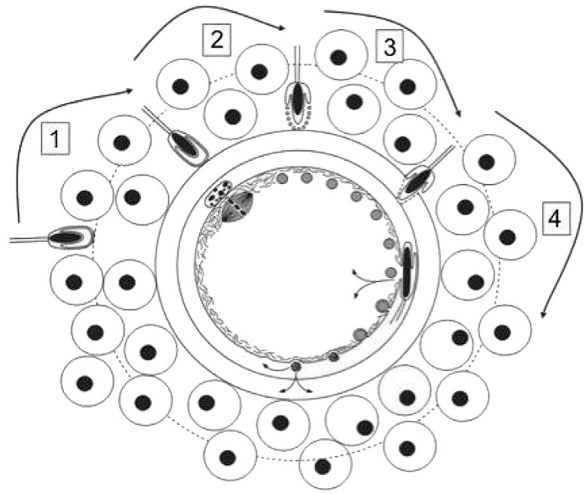

Fig. 1. The classical proposed sequence of events around fertilization related to sperm-zona interactions. Spermatozoa that have entered the oviduct will shed decapacitation factors that were adhered peripherally to the sperm surface. During this process, the acrosome docks to the sperm plasma membrane at the docking area, resulting in the formation of high-affinity zona binding complexes [7,33,52]. (1) It is not clear whether an association between the spermatozoa and the extracellular matrix of the expanded cumulus mass (here represented as a two-cell layer but, in reality, composed of more cell layers) surrounding the unfertilized oocyte results in the induction of some early steps of acrosomal fusion (as proposed by Jin et al. [107]) or whether acrosome-intact spermatozoa penetrate through the cumulus due to their hyperactive motility in combination with surface proteins [108]. Oviductal-secreted proteins have also been reported as important in determining the properties of the cumulus and zona pellucida (ZP) [109]. (2) Recognition of the ZP (primary zona binding to the ZP proteins ZP3/ZP4; [110]) and subsequent initiation of the acrosome reaction (or of the acute secretory phase of it; see [1] stage) that is induced by the ZP. (3) The acrosome reaction causes local modifications of zona proteins, and the hyperactivated spermatozoa can penetrate this structure due to secondary zona binding (to ZP2). The surface of the penetrating spermatozoa will be further remodeled, and this probably enables fertilization fusion [30]. (4) Immediately after fertilization fusion, the cortical reaction results in overall coating of the oolemma and the hardening of ZP, by chemically altering the zona proteins; in particular, cleavage of ZP2 appears to be instrumental for the release of spermatozoa from the ZP and for efficient block of polyspermy. Modified from [30].

[38], and the degree of lateral fluidity depends on the degree of acyl chain length saturation and the amount of cholesterol, as well as on the temperature [39,40]. Below the phase transition temperature, fluid membrane lipids freeze into a crystalline phase causing their lateral phase segregation from membrane lipids with still fluid properties [41]. Other causes for lipid or membrane protein immobilization can be their attachment either to the cytoskeleton or the glycocalyx (for models, see [20,42]). Not only sperm membrane proteins [43] but also glycolipids [44,45] and cholesterol [10] did not randomly diffuse over the sperm head but were localized in specific surface areas; and to our surprise, sperm capacitation treatments caused redistribution of membrane proteins [46] and lipids [26] into other surface areas of the sperm head: Seminolipid moved from the apical ridge toward the equatorial domain of the sperm head [44,45], and it was proposed that this negatively charged glycolipid is stabilizing the sperm surface for intracellular fusion events (plasma membrane with

the outer acrosomal membrane). Thus, the original apical distribution of seminolipid could be explained as to prevent the premature acrosome reaction after the ejaculation, whereas after redistribution to the equatorial surface area, it prevents the equatorial plasma membrane to fuse with the outer acrosomal. Interestingly, this surface area needs to remain intact as it is the specific area of the sperm head that is able to bind and fuse with the oolemma [34]. Note that a retrograde movement and concentration of cholesterol [11,13,26] is simultaneously occurring in the apical surface area of the sperm head, which is further detailed in Section 6.

6. Membrane microdomains and sperm surface heterogeneity

In the mid-1990s of the previous century, a refinement of the Singer–Nicolson model has been proposed by Simons and Toomre: The so-called lipid raft model predicts that small lipid-ordered microdomains contain larger proportions of cholesterol, sphingomyelin, gangliosides, and phospholipids with saturated long-chain acyl chains, as well as post-transcriptionally lipid-modified proteins such as GPI-anchored proteins and sterol or acyl-modified proteins (for a review, see [47]). A questionable [48] but widespread method to isolate these lipid-ordered membrane domains was to subject cells or cell homogenates to a low-ionic detergent at 4 °C. The non-raft fraction is dissolved by detergent (often 0.1% by volume of Triton X-100), whereas the fraction supposed to resemble the membrane raft remains non-dissolved. This so-called detergent resistant membrane (DRM) fraction can be isolated by discontinuous sucrose gradients.

Detergent resistant membranes are enriched with membrane-associated proteins that are considered to be raft marker proteins, and two of them (caveolin-1 and flotillin-1 [49,50]) were also shown to be present in sperm [31]. Indeed, the sperm DRM fraction is enriched in cholesterol and can be dissolved by cholesterol-disrupting agents, indicating that the DRM of sperm is comparable to that of Madin-Darby canine kidney cells or other cell lines [12]. Interestingly, caveolin and flotillin predominantly localized in the area of the sperm cell that is involved in sperm-zona binding and the zona-induced acrosome reaction [31]. Moreover, the capacitation-specific lateral redistribution and concentration at this apical sperm head area is followed by an albumin-mediated depletion of cholesterol [10]. Thus, studies were conducted to unravel the organization of lipid microdomains in sperm under *in vitro* capacitation/fertilization conditions. Both flotillin and caveolin migrated from the entire acrosome overlying plasma membrane toward the apical ridge area [51,52]. This process also involved oligomerization of caveolin, which indicates that sperm rafts aggregate in this membrane area. Albumin under the changed lateral organization of lipid microdomains extracts cholesterol from the non-DRM membrane area only [53]. The altered lipid composition of the fluid or lipid-disordered phase (Ld) membrane in capacitation-responsive cells may allow lipid microdomain aggregation (lipid-ordered phase of Lo) [11]. Although albumin is the protein of choice as a cholesterol acceptor during IVF treatment, likely it is not the acceptor of cholesterol in the oviduct. High density lipoprotein complexes are better suited for this as albumin in the oviduct will not be in fatty acid–free form, which is required for cholesterol extraction from boar sperm [10]. The reverse cholesterol transport also relies on other specialized proteins involved, and one group of sterol transporters that has been identified in mouse sperm is the ATP-binding cassette transporters ABCA-1, 7, 17 and ABCG-1 [11,54–56]. Interestingly, knockout mice for the ATP-binding cassette transporters have been shown to be infertile [57–59], showing their importance in fertilization processes. Another protein that is involved in reverse cholesterol transport is SRB-1 [60], but its presence on sperm has not been established yet. Interestingly, knockout mice for SRB-1 are also infertile [61], at least suggesting an important role for these proteins in mammalian fertilization (for a recent review on reverse cholesterol transport from the sperm surface, see [11]). Replacement of albumin by means of expressing sterol-depleting proteins, and use of purified batches to study their effect on sterol efflux on sperm should be considered in the future: Molecular biological tools can be used to modify proteins of interest to manipulate the efficiency of sterol depletion. One advantage of replacing albumin with *in vitro*–produced proteins is that more standardized protein material can be used for IVF with minimal risk contamination with biohazardous materials. Each new batch of albumin has intrinsic variations in efficiency on sperm activation and IVF and thus is a cause of increased experimental variation. Beyond this, as component extracted from blood, albumin is not chemically defined and could bear microbes such as viruses.

Attempts have been made to induce cholesterol depletion from sperm cells by artificial cholesterol acceptors. As mentioned in Section 4, high levels of cyclodextrin (a well-known cholesterol-depleting agent), was spermicidal to porcine sperm [12] and caused cholesterol depletion and disruption from the DRM and the Triton X-100 soluble sperm lipid fraction, consistent with observations from the group of Jones [62]. Nevertheless, cyclodextrins have been shown also to induce some capacitation responses in sperm [13]. Both cyclodextrins at a very narrow concentration range in absence of albumin result in IVF rates [13] under conditions in which the cyclodextrins already induce oocyte deterioration [13,14,63]. Recently, it has been shown that cholesterol incorporation into mammalian sperm by cholesterol-complexed cyclodextrins improves cryopreservation of sperm and subsequent IVF results of thawed treated sperm [64,65].

7. Aggregation of lipid microdomains and fertilization

As mentioned previously, the presence of the capacitation factors albumin, bicarbonate, and Ca^{2+} evokes reversible and irreversible cell signaling responses to a subpopulation of sperm (the responding cells [28]). The idea around the heterogeneity of individual sperm cells to respond either quickly or slowly to capacitation factors is that this allows *in vivo* an extended time frame, after ejaculation, for sperm cells to reach the oviduct and respond

appropriately to accomplish fertilization [19]. Capacitating sperm have signs of hyperactivated motility and surface remodeling. Advances have been made in the understanding of lipid microdomains in a variety of cell types [48,50]. In our laboratory, we found that one of the surface changes taking place in the capacitating sperm subpopulation is the aggregation of lipid microdomains in the apical ridge area of the sperm head, and that those cells also acquired hyperactivated motility [31,66]. The aggregated lipid microdomains are highly enriched in proteins that are involved in the primary binding of the ZP [31,52]. How the aggregation of lipid rafts is induced is to a large extend unknown although a lipocalin-2 (a phosphatidylethanolamine-binding lipid) has been shown to be involved in a PKA-dependent movement of rafts in mouse sperm [67]. Interestingly, in our laboratory, the relationship between the capacitation-induced apical raft aggregation and the acrosome reaction was investigated [66]. A first observation was that the specific proteins involved in membrane docking and fusion between the plasma membrane and the outer acrosomal membrane were indeed concentrated in the DRM fraction [66]. More of interest was that of these so-called SNARE proteins not only aggregation was noted at the sperm surface but also at the outer acrosomal membrane. Laborious experiments using dedicated cell disruption techniques showed that capacitation indeed caused docking of the two membranes [7] by the formation of trans-trimeric SNARE complex exactly at the area where raft aggregation was monitored. In fact, the trimeric complex was heat stable and with a boiling dithiothreitol treatment could be dissociated into the three SNARE monomers. Interestingly, the trans-SNARE complexes did not convert into cis-SNARE complexes (which would execute the fusion of the two docked membranes and thus initiate the acrosome reaction). This premature acrosome reaction is prevented by locking the SNARE complex to complexin [33]. Of further note is the observation that caveolin-1 (a marker protein for rafts) and CDC42 are involved in the regulation of the acrosome reaction [68]. In summary, the raft aggregation did cause SNARE aggregation and docking of the acrosome rendering this structure ready to carry out multiple membrane fusions at a whole apical surface area in response to the acrosome reaction inducing agent [7]. It is at the moment uncertain for the pig whether or not the ZP is involved in inducing the acrosome reaction or the intercellular matrix of the cumulus layers (as discussed in an earlier review [69]). At any rate, the priming agent will allow Ca^{2+} entry in the space between the plasma membrane and the docked acrosomal membrane, and this immediately executes the acrosome fusions by forcing a Ca^{2+}-dependent trans to cis conversion of the trimeric SNARE complexes and the unlocking of complexin [33]. It is also noteworthy to mention that cholesterol removal at the sperm surface is activating phospholipase B and by doing so stimulates acrosome exocytosis [70,71]. This shows that lateral redistribution of membrane components, the reverse cholesterol transport, the acrosome docking, and the acrosome reaction are regulated in a complex and partly synergistic mode. Recently, the surface organization of the oolemma and the interaction of sperm with these microdomains have been reported to be of potential interest for fertilization of the oocyte [72]; they observed CD9 to be present in rafts at the oocyte's surface, and CD9 is involved in the sperm-oolemma contact/fertilization fusion [34,73].

GPI-anchored proteins are enriched in DRMs and are covalently linked the lipid bilayer *via* a conserved glycolipid anchor (Fig. 2). As a consequence, a GPI-anchored protein is covalently attached to the sperm surface at the outer lipid monolayer of the plasma membrane. This class of intrinsic membrane proteins is described to have a preference for residing in cholesterol-rich domains [74]. Cholesterol-rich domains are also termed membrane rafts and possess low lateral fluidity of lipids when compared to non-raft membrane fractions [47]. It is proposed that the membrane fluidity changes in the sperm membrane and that cholesterol extraction is necessary for proper microdomain formation [11]. The formed microdomain in turn is hypothesized to lead to a functional ZP-binding complex [52,75]. However, the exact role of GPI-anchored proteins in those processes is still elusive despite the fact that these proteins are highly enriched in the ZP-binding area of the capacitated sperm. It is known that GPI-anchored proteins are involved in the protection of sperm in the genital tract [76–80]. However, recent reports also showed that sperm GPI-anchored proteins play a role in the recognition of the ZP [31,52]. For example, PH-20 is known to have hyaluronidase activity, which enables it to penetrate the ZP [81,82]. Interestingly, there are reports in literature that a testis-specific angiotensin-converting enzyme is capable of cleaving of GPI-anchored proteins from sperm. Kondoh et al. [82] reported that knockout mice lacking the expression of this protein were infertile. This suggests that shedding of GPI-anchored proteins is an important step for sperm to gain fertilizing competence [82]. We have shown that there is also spontaneous release of GPI-anchored proteins CD55 and CD52 under the influence of bicarbonate [83]. Both are members of a family of proteins involved in protecting cells from being attacked by the immune system [78,84]. These results might imply that *in vivo,* sperm migrating through the female genital tract, with increasing concentrations of bicarbonate higher up the tract, release these proteins progressively. Beyond the release of such proteins, other peripheral surface proteins such as the 15 to 25 kDa wheat germ agglutinin-binding protein are released [85]. The release of this sperm plasma membrane marker protein [86] may have a functional role in the process of sperm oocyte interaction. At any rate, after the removal of GPI-anchored proteins and peripheral proteins, the membrane is rearranged in such a way that the surface of sperm has a fully competent apical ridge that is in place for ZP recognition and penetration [52,87]. An alternative way to release GPI-anchored proteins from the sperm surface is the treatment with phosphatidylinositol-specific phospholipase C (PI-PLC; [83]). PI-PLC treatment caused and altered the aggregation of flotillin-1 in the apical ridge area of the sperm head in absence of bicarbonate. This suggests that PI-PLC mediated loss of GPI-anchored proteins induces a similar lateral aggregation of lipid rafts to the apical ridge area as was induced by bicarbonate and that both treatments elicit this by GPI-anchored protein removal. Full capacitation in presence of PI-PLC probably leads to excessive removal of

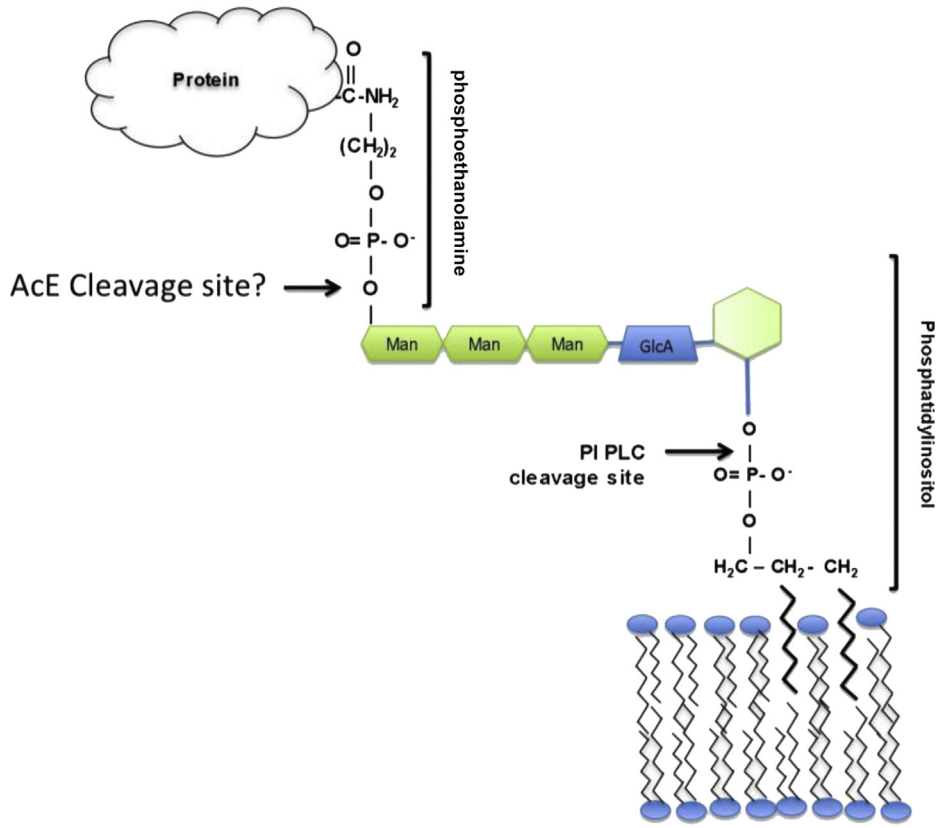

Fig. 2. Schematic overview of a GPI-anchored protein and the cleavage site of phosphatidylinositol-specific phospholipase C. The cleavage site of the angiotensin-converting enzyme is still not fully known, but it is expected to cleave somewhere close to the mannose residues near the GPI anchor [82].

GPI-anchored proteins, which turned out to be destructive for sperm and cannot be recommended as a sperm treatment before IVF or artificial insemination [83]. However, in absence of either albumin, bicarbonate, or both, PI-PLC induced several other capacitation-like responses in sperm such as increased tyrosine phosphorylation and hyperactivated motility. This shows that regulated removal of GPI-anchored proteins may be required to prepare sperm for proper interactions with the cumulus oocyte complexes at the site of fertilization. Beyond the aforementioned endogenous enzyme angiotensin-converting enzyme , which has been reported to be capable of cleaving of GPI-anchored proteins on sperm cells, it is possible that *in vivo* sperm cells migrating through the uterus and oviduct will interact with other surface modifying extracellular compounds such as the metalloproteinases [88–90]. Therefore, it is of great importance for future studies to investigate whether other extracellular modifying enzymes are present on the sperm surface and/or are present in the female genital tract and how these enzymes might play a role in sperm activation and the subsequent binding of sperm to the ZP. For instance, the glioma parthenogenesis-related 1-like protein 1 is a sperm raft associated protein that becomes covalently GPI-linked

Table 1
Oxysterol-forming enzymes.

Enzymes	Cellular distribution	Subcellular localization	Oxysterol
24-hydroxylase (CYP46A1)	Neurons, neural retina, hepatocytes	Endoplasmatic reticulum and golgi apparatus	24S-hydroxycholesterol
25-hydroxylase	Hepatocytes	Endoplasmatic reticulum and golgi apparatus	25-hydroxycholesterol
27-hydroxylase (CYP27)	Hepatocytes macrophages, endothelial cells	Mitochondria	27-hydroxycholesterol
7α-hydroxylase (CYP7A1)	Hepatocytes ovary cells	Endoplasmatic reticulum	7α-hydroxycholesterol
CYP3A4	Hepatocytes	Microsomes	4β-hydroxycholesterol
11β-hydroxysteroid dehydrogenase type-1	Hepatocytes	Microsomes	7-ketocholesterol, 7β-hydroxycholesterol

The distribution and subcellular localization for different enzymes capable of oxidizing cholesterol (CYP-cytochrome P450). Adapted from [96].

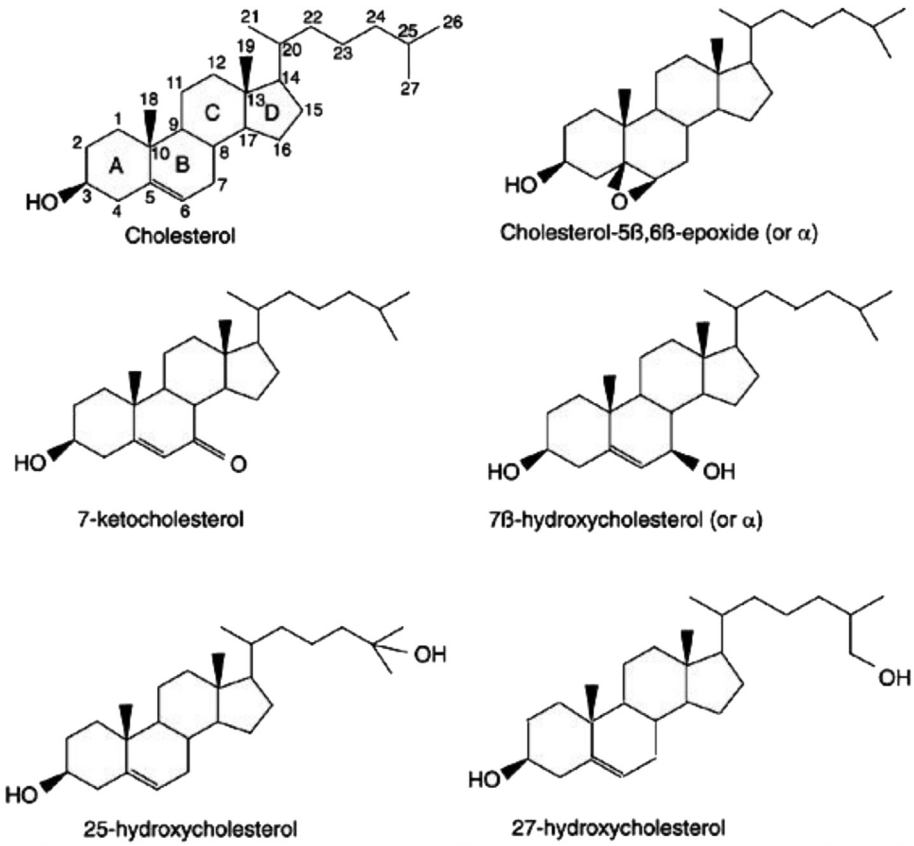

Fig. 3. Structure of cholesterol and the major oxysterols resulting from autooxidation (7-ketocholesterol; 7α-hydroxycholesterol, and its enantiomer 7β-hydroxycholesterol, cholesterol-5α, 6α-epoxide and its enantiomer cholesterol-5β,6β-epoxide) and enzymatic oxidation of the 25-hydroxycholesterol and 27-hydrocholesterol are also enzymatic products from respectively cholesterol 25-hydroxylase and 27-hydroxylase (CYP27). Adapted from [96].

during bovine sperm transit through the epididymis and is after capacitation still present and functionally involved in sperm-zona binding [91].

8. Sperm capacitation reactive oxygen species and cholesterol removal

One of the interesting observations in recent years is the fact that oxidative stress on sperm not only has detrimental effects and should be avoided but that oxidative stress also can impose stimulatory effects on sperm [92,93]. High levels of reactive oxygen species (ROS), and especially H_2O_2, have harmful effects on important sperm functions such as membrane integrity, cell viability, and motility [93]. On the other hand, it has become clear that mild ROS production has a positive effect on the capacitation of sperm and the subsequent acrosome reaction [94]. Reactive oxygen species has been described as one of the regulators that influence protein tyrosine phosphorylation [92], which, as described previously, is an indicator of sperm capacitation. Reactive oxygen species has also been hypothesized to activate PKA and the extracellular-signal-regulated kinases pathways acting independently on the modulation of tyrosine phosphorylation such as bicarbonate [5].

Although capacitation leads to the formation of ROS in sperm, it is still not exactly known how raft dynamics and membrane fluidity are regulated during sperm capacitation and which role ROS plays in this process. The reported increase in membrane fluidity and exact mechanism behind the efflux of cholesterol from the membrane is still unknown. Interestingly, cholesterol is a substrate for enzymatic oxidation with oxysterols as a final product and can be regarded as a ROS [95]. The cholesterol molecule can be divided into three regions: (1) a lateral chain composed of hydrocarbons, (2) a four hydrocarbon ring structure (termed A, B, C, and D), and (3) a hydroxyl group (Fig. 3). The B hydrocarbon ring and its double bond is one of the suitable targets for free radical attack and is therefore the most sensitive for autooxidation. The autooxidation of the B hydrocarbon ring leads to different species of oxysterols, 7α- and 7β-hydroxycholesterol, 7-ketocholesterol, 5α,6α-epoxycholesterol, 5α,6β-epoxycholesterol, and cholestane-3β, 5α,6β–triol [96]. The formed oxysterols are more polar compounds when compared to cholesterol. Oxysterols are known to cause lateral membrane expansion, reduce the size of the membrane bilayer, and thus cause higher membrane permeability. Due to this, the thickness differences between cellular membranes are thought to aid in sorting membrane proteins to their intended place in the membrane [97–100]. Autooxidation processes do not only

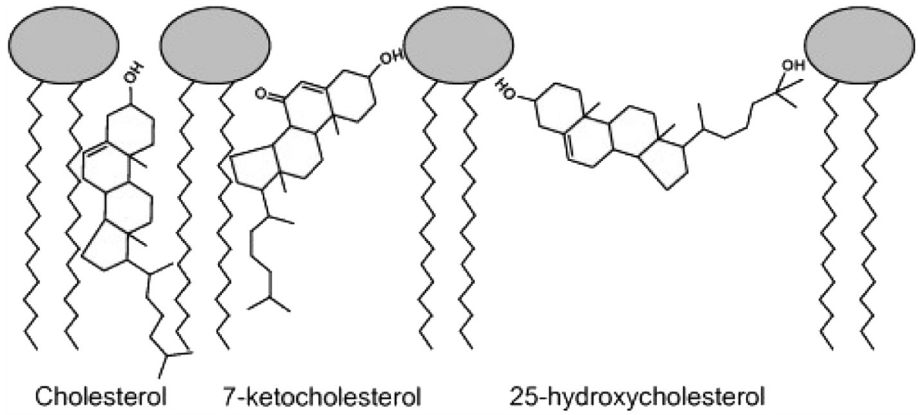

Fig. 4. Cholesterol and oxysterol behavior in model membranes. The additionally added hydroxyl groups cause another topology of the oxysterols in the lipid bilayer (here only drawn for the outer monolayer). Oxysterols are increasingly reoriented more to the polar headgroup side as compared with their native cholesterol molecules. Adapted from [97].

form oxysterols but there are also enzymes capable of changing cholesterol into oxysterols. Species of oxysterol generated by enzymes are 24S-hydroxycholesterol, 25-hydroxycholesterol, 27-hydroxycholesterol, and 7α-hydroxycholesterol (see Table 1 for matching enzymes) [96]. Oxysterols have been shown to be important in processes related to cholesterol turnover, atherosclerosis, apoptosis, and among others [99]. Current consensus is that the oxysterols are physiological mediators involved in cholesterol-induced metabolic effects.

The mechanism of how bicarbonate-activated sperm cells oxidize sterols and how this relates to the reverse transport of cholesterol to lipoproteins or *in vitro* to albumin is not understood. In recent literature [96,97,101], it has become evident that cholesterol is a substrate for autooxidation by intrinsic formed ROS or alternatively oxysterols can be formed by specific enzymes. Therefore, the emergence of oxysterols and the partition of sterols as well as oxysterols were measured between sperm cell pellets and supernatants of centrifuged sperm samples under various capacitation incubation conditions [11,13,102]. Brouwers et al. [102] showed that when bovine sperm were incubated in several ways to investigate whether these incubations led to the formation of oxysterols. Addition of active prooxidants was tested for detection of autooxidation of cholesterol in sperm [102]. We have found that HPLC and mass spectrometry–based techniques can detect in the fmole-mmole detection range of sterol species quantification and identification of the various oxidation products formed out of cholesterol and desmosterol [102]. The differences in molecular composition of oxysterols formed after prooxidant treatments either of reconstituted sperm membrane vesicles or intact sperm when compared to *in vitro* capacitation conditions are also of interest. More detailed analysis of these differences may elucidate autooxidation and enzymatic steps involved in oxysterol formation [61,103,104] and those prooxidation as well as antioxidation pathways that are switched on or are inhibited during the process of sperm activation. This knowledge may be useful to minimize adverse effects of oxysterol formation in sperm processed before cryopreservation.

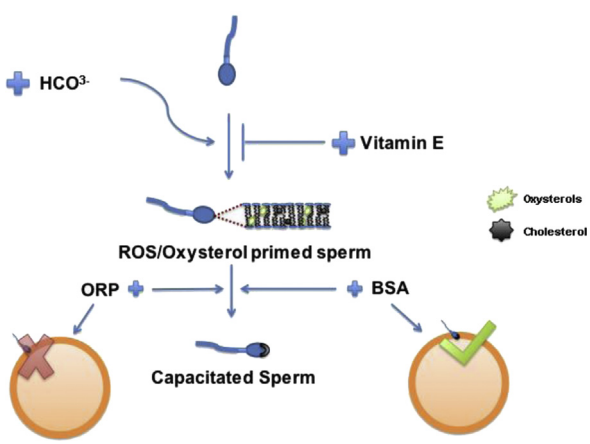

Fig. 5. Model depicting the influence of oxysterols on sterol efflux during sperm capacitation and fertilization of the oocyte. Bicarbonate induces reactive oxygen species/oxysterol primed sperm. Vitamin E inhibits this process. Incubation with oxysterol-binding protein leads to capacitation signs but does not result in sperm capable of fertilizing the oocyte. Using albumin as a sterol acceptor in IVF procedures does lead to sperm competent in fertilizing the oocyte (based on results of Boerke et al. [13]).

Oxysterol formation also takes place under *in vitro* capacitation conditions in porcine and mouse sperm [13]. Indeed, in both mammalian species, sperm capacitation led to the formation of oxysterols and the depletion of free sterols [13]. The formation of oxysterols was bicarbonate-dependent and could also be induced by prooxidants in absence of bicarbonate or blocked by hydrophobic antioxidants in presence of bicarbonate. The capacitation conditions that resulted in oxysterols formation also allowed the depletion of cholesterol and desmosterol in both species. However, addition of prooxidants did not have such effects showing that the relationship between oxysterol formation and sterol depletion is more complex. Moreover, it is of note that oxysterol formation in cryopreserved sperm was not succeeded by sterol removal from the sperm surface indicating that the different topologies (Fig. 4) of oxysterols

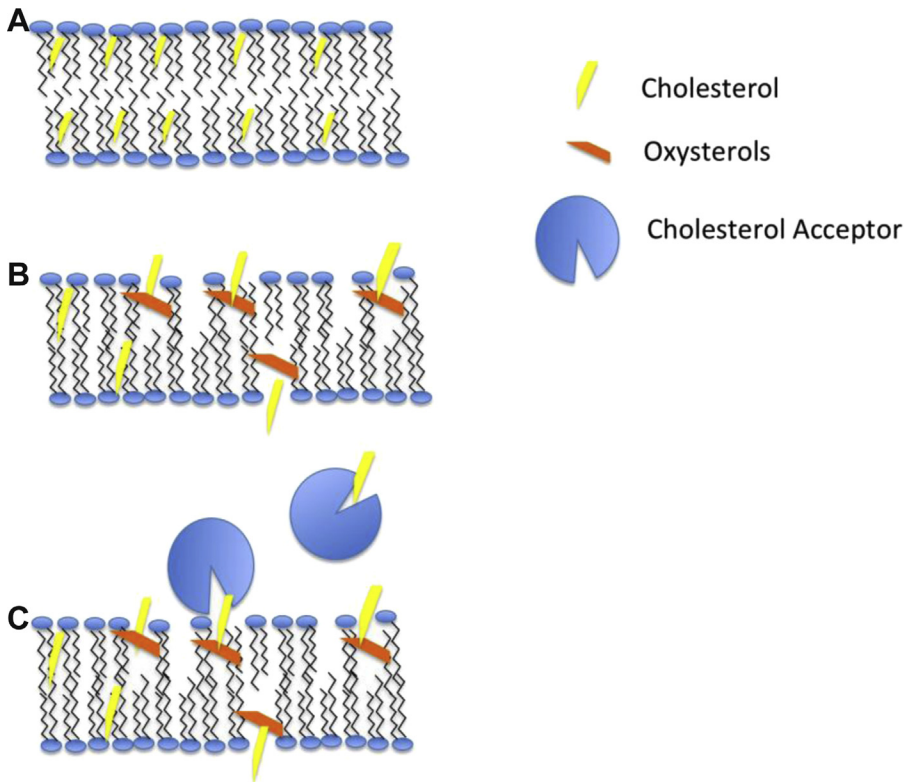

Fig. 6. Proposed model of cholesterol efflux mediated by the oxysterol induced changes in the membrane. (A) Depicts a rigid membrane with cholesterol. (B) When oxysterols are formed, they may push the cholesterol partially out of the outer lipid monolayer of the plasma membrane. This mechanism is explained by the parallel position of oxysterols relative to the polar headgroups of phospholipids in the lipid monolayer. (C) In the presence of oxysterols, cholesterol is now accessible to a cholesterol acceptor (for IVF, this would be albumin). Active cholesterol may also be preferentially transported by sterol transporter proteins such as the ATP-binding cassette transporters ABCA-1, 7, 17 and ABCG-1 [11].

and free sterols in biomembranes are not *per se* allowing cholesterol depletion. Capacitation-dependent sterol depletion is one of the steps required for downstream sperm activation processes such as increased protein tyrosine phosphorylation in the sperm tail and a related induction of hyperactivated motility (see Sections 2 and 7). Cholesterol loss also induces lateral rearrangements of membrane components in the sperm head and increases membrane fluidity (Section 3). Such preparative steps are required for optimal binding of capacitated sperm to the ZP and to allow rapid induction of the acrosome reaction in sperm after the initial recognition of the ZP (Section 6). Failure of proper capacitation also blocks fertilization. We observed that indeed capacitation was required for not only oxysterol formation and sterol depletion but also the generation of tyrosine phosphorylation, hyperactivated motility, and rearrangements of molecules at the sperm head surface [13]. Under IVF conditions, these changes were required for the induction of the acrosome reaction after zona binding and were required for fertilization. When IVF was carried out under conditions in which hydrophobic antioxidants were added to the medium, this not only blocked oxysterol formation but also fertilization of the oocyte (Fig. 5). The amount of sperm bound to the ZP was not significantly affected. Moreover, the polyspermic fertilization rates of fertilized oocytes in control versus antioxidant conditions also were identical. Both observations indicate that the antioxidant inhibition effect was specific for sperm and that the oocyte's developmental competence was unaffected.

Interestingly, sperm incubated with oxysterol-binding proteins (ORPs) also showed—in part—signs of sperm capacitation [13]. The ORPs induced sperm protein tyrosine phosphorylation, hyperactivated motility, lateral rearrangements of molecules at the sperm head, and affinity for the ZP. In fact, the ORP interaction with the sperm surface scavenged oxysterol formation by greater than 50% and induced premature acrosome reactions of incubated sperm in capacitation media. However, ORPs did not allow IVF or the efflux of free sterols from the sperm surface (Fig. 5).

An alternative way to deplete the sperm surface from (oxy)sterols is treatment with cyclodextrin (see also Section 6). Successful depletion of cholesterol and desmosterol was observed with methyl-beta-cyclodextrin (MBCD), which results in tyrosine phosphorylation, hyperactivated motility, and moderate IVF rates (about 30% instead of the usual 60%) at 2-mM MBCD levels [13]. Lower levels of MBCD did not result in these effects, and higher levels were detrimental to sperm [12] and oocytes. Oocyte deterioration was also manifested in 30% of the oocytes; thus, the permissive concentration range for IVF using MBCD is narrow, and adverse effects on oocytes are

forming a coinciding problem (a process also noted for mouse oocytes [13,14]).

It is of special interest to sperm physiology that oxysterol formation may enable altered lateral and bilayer behavior of cholesterol and/or desmosterol in the membrane: oxysterols can rigidify model membranes [97]. This could be detrimental, for instance for cryopreserved sperm; and thus, the presence of oxysterols can be used for diagnostics in assessing the cryopreservability of semen donors. Another possibility is that oxysterols can promote sterol depletion. Sterols are flat hydrocarbon steroid structures with one polar hydroxyl group in the polar headgroup region of phospholipid monolayers and the full steroid structure in parallel oriented with the fatty acids esterified to phospholipids. However, oxysterols by virtue of additional polar hydroxyl groups will orient to a more surface-exposed orientation (i.e., in parallel with the polar headgroups; see Fig. 6). This phenomenon may not only lead to changes in the fluidity of the membrane, but has also been predicted to tilt free sterols into a more accessible topology for sterol accepting proteins. If this is the case in sperm cells, the oxysterol formation could be a preparative step for sterol depletion.

9. Conclusions

In this review, we have overviewed the current understanding of the process of sperm capacitation with special emphasis on the effects of the *in vitro*–induced surface alterations and their role in sperm signaling, membrane fusion, and IVF. New information on the role of lipid microdomain dynamics, cholesterol efflux, cholesterol oxidation, and GPI-anchored protein release is included. Recently, it has been established that just preovulatory oviductal fluid improves IVF when added to fresh porcine sperm [105]. Likewise, oviduct interactions modulate sperm physiology [106]. The effects of the intraoviductal environment on the sperm surface remains a largely undisclosed area that needs to be further elucidated in the future. The picture is far from complete, but fundamental understanding of the ergonomics of the sperm surface rearrangements may help to improve IVF. Likewise, the methods to store sperm may have negative side effects on the capacitation-specific surface rearrangements, and better understanding of this may help in the design of better sperm preservation protocols (see also [11]).

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References

[1] Rodriguez-Martinez H, Saravia F, Wallgren M, Tienthai P, Johannisson A, Vazquez JM, et al. Boar spermatozoa in the oviduct. Theriogenology 2005;63:514–35.
[2] Tienthai P, Johannisson A, Rodriguez-Martinez H. Sperm capacitation in the porcine oviduct. Anim Reprod Sci 2004;80:131–46.
[3] Fraser LR, Adeoya-Osiguwa SA, Baxendale RW, Gibbons R. Regulation of mammalian sperm capacitation by endogenous molecules. Front Biosci 2006;11:1636–45.
[4] Rodriguez-Martinez H, Ekstedt E, Einarsson S. Acidification of epididymal fluid in the boar. Int J Androl 1990;13:238–43.
[5] Gadella BM, Luna C. Cell biology and functional dynamics of the mammalian sperm surface. Theriogenology 2014;81:74–84.
[6] Darszon A, Lopez-Martinez P, Acevedo JJ, Hernandez-Cruz A, Trevino CL. T-type Ca2+ channels in sperm function. Cell Calcium 2006;40:241–52.
[7] Tsai PS, van Haeften T, Gadella BM. Preparation of the cortical reaction: maturation-dependent migration of SNARE proteins, clathrin, and complexin to the porcine oocyte's surface blocks membrane traffic until fertilization. Biol Reprod 2011;84:327–35.
[8] Johnson LA, Aalbers JG, Willems CM, Rademaker JH, Rexroad Jr CE. Use of boar spermatozoa for artificial insemination. III. Fecundity of boar spermatozoa stored in Beltsville liquid and Kiev extenders for three days at 18 C. J Anim Sci 1982;54:132–6.
[9] Ehrenwald E, Foote RH, Parks JE. Bovine oviductal fluid components and their potential role in sperm cholesterol efflux. Mol Reprod Dev 1990;25:195–204.
[10] Flesch FM, Brouwers JF, Nievelstein PF, Verkleij AJ, van Golde LM, Colenbrander B, et al. Bicarbonate stimulated phospholipid scrambling induces cholesterol redistribution and enables cholesterol depletion in the sperm plasma membrane. J Cell Sci 2001;114:3543–55.
[11] Leahy T, Gadella BM. New insights into the regulation of cholesterol efflux from the sperm membrane. Asian J Androl 2015;17:561–7.
[12] van Gestel RA, Helms JB, Brouwers JF, Gadella BM. Effects of methyl-ß-cyclodextrin-mediated cholesterol depletion in porcine sperm compared to somatic cells. Mol Reprod Dev 2005;72:386–95.
[13] Boerke A, Brouwers JF, Olkkonen VM, van de Lest CH, Sostaric E, Schoevers EJ, et al. Involvement of bicarbonate-induced radical signaling in oxysterol formation and sterol depletion of capacitating mammalian sperm during in vitro fertilization. Biol Reprod 2013;88:21.
[14] Buschiazzo J, Ialy-Radio C, Auer J, Wolf JP, Serres C, Lefèvre B, et al. Cholesterol depletion disorganizes oocyte membrane rafts altering mouse fertilization. PLoS One 2013;8:e62919.
[15] Gadella BM, Tsai PS, Boerke A, Brewis IA. Sperm head membrane reorganisation during capacitation. Int J Dev Biol 2008;52:473–80.
[16] Boerke A, Tsai PS, Garcia-Gil N, Brewis IA, Gadella BM. Capacitation-dependent reorganization of microdomains in the apical sperm head plasma membrane: functional relationship with zona binding and the zona-induced acrosome reaction. Theriogenology 2008;70:1188–96.
[17] Demarco IA, Espinosa F, Edwards J, Sosnik J, De La Vega-Beltran JL, Hockensmith JW, et al. Involvement of a Na+/HCO-3 cotransporter in mouse sperm capacitation. J Biol Chem 2003;278:7001–9.
[18] Okamura N, Tajima Y, Soejima A, Masuda H, Sugita Y. Sodium bicarbonate in seminal plasma stimulates the motility of mammalian spermatozoa through direct activation of adenylate cyclase. J Biol Chem 1985;260:9699–705.
[19] Gadella BM, van Gestel RA. Bicarbonate and its role in mammalian sperm function. Anim Reprod Sci 2004;82-83:307–19.
[20] Gadella BM, Visconti PE. Regulation of capacitation. In: De Jonge C, Barrett C, editors. The sperm cell production maturation fertilization regeneration. Cambridge UK: Cambridge University Press; 2006. p. 134–69.
[21] Ficarro S, Chertihin O, Westbrook VA, White F, Jayes F, Kalab P, et al. Phosphoproteome analysis of capacitated human sperm. Evidence of tyrosine phosphorylation of a kinase-anchoring protein 3 and valosin-containing protein/p97 during capacitation. J Biol Chem 2003;278:11579–89.
[22] Leclerc P, de Lamirande E, Gagnon C. Cyclic adenosine 3',5'monophosphate-dependent regulation of protein tyrosine phosphorylation in relation to human sperm capacitation and motility. Biol Reprod 1996;55:684–92.
[23] Miki K. Energy metabolism and sperm function. Soc Reprod Fertil Suppl 2007;65:309–25.
[24] du Plessis SS, Agarwal A, Mohanty G, van der Linde M. Oxidative phosphorylation versus glycolysis: what fuel do spermatozoa use? Asian J Androl 2015;17:230–5.
[25] Visconti PE, Krapf D, de la Vega-Beltran JL, Acevedo JJ, Darszon A. Ion channels, phosphorylation and mammalian sperm capacitation. Asian J Androl 2011;13:395–405.
[26] Flesch FM, Gadella BM. Dynamics of the mammalian sperm plasma membrane in the process of fertilization. Biochim Biophys Acta 2000;1469:197–235.

[27] Mattioli M, Barboni B, Lucidi P, Seren E. Identification of capacitation in boar spermatozoa by chlortetracycline staining. Theriogenology 1996;45:373–81.
[28] Harrison RA, Ashworth PJ, Miller NG. Bicarbonate/CO2, an effector of capacitation, induces a rapid and reversible change in the lipid architecture of boar sperm plasma membranes. Mol Reprod Dev 1996;45:378–91.
[29] de Vries KJ, Wiedmer T, Sims PJ, Gadella BM. Caspase-independent exposure of aminophospholipids and tyrosine phosphorylation in bicarbonate responsive human sperm cells. Biol Reprod 2003;68:2122–34.
[30] Tsai PS, Gadella BM. Molecular kinetics of proteins at the surface of porcine sperm before and during fertilization. Soc Reprod Fertil Suppl 2009;66:23–36.
[31] van Gestel RA, Brewis IA, Ashton PR, Helms JB, Brouwers JF, Gadella BM. Capacitation-dependent concentration of lipid rafts in the apical ridge head area of porcine sperm cells. Mol Hum Reprod 2005;11:583–90.
[32] Gadella BM, Ferraz MA. A review of new technologies that may become useful for in vitro production of boar sperm. Reprod Domest Anim 2015;50:61–70.
[33] Tsai PS, Brewis IA, van Maaren J, Gadella BM. Involvement of complexin 2 in docking, locking and unlocking of different SNARE complexes during sperm capacitation and induced acrosomal exocytosis. PLoS One 2012;7:e32603.
[34] Gadella BM, Evans JP. Membrane fusions during mammalian fertilization. Adv Exp Med Biol 2011;713:65–80.
[35] De Leeuw FE, Chen HC, Colenbrander B, Verkleij AJ. Cold-induced ultrastructural changes in bull and boar sperm plasma membranes. Cryobiology 1990;27:171–83.
[36] Singer SJ, Nicolson GL. The fluid mosaic model of the structure of cell membranes. Science 1972;175:720–31.
[37] Chapman D. Fluidity and phase transitions of cell membranes. Biomembranes 1975;7:1–9.
[38] Scandella CJ, Devaux P, McConnell HM. Rapid lateral diffusion of phospholipids in rabbit sarcoplasmic reticulum. Proc Natl Acad Sci U S A 1972;69:2056–60.
[39] Blok MC, van der Neut-Kok EC, van Deenen LL, de Gier J. The effect of chain length and lipid phase transitions on the selective permeability properties of liposomes. Biochim Biophys Acta 1975;406:187–96.
[40] Cullis PR. Lateral diffusion rates of phosphatidylcholine in vesicle membranes: effects of cholesterol and hydrocarbon phase transitions. FEBS Lett 1976;70:223–8.
[41] Feigenson GW. Phase behavior of lipid mixtures. Nat Chem Biol 2006;2:560–3.
[42] Cherry RJ. Rotational and lateral diffusion of membrane proteins. Biochim Biophys Acta 1979;559:289–327.
[43] Myles DG, Primakoff P, Bellve AR. Surface domains of the guinea pig sperm defined with monoclonal antibodies. Cell 1981;23:433–9.
[44] Gadella BM, Lopes-Cardozo M, van Golde LM, Colenbrander B, Gadella Jr TW. Glycolipid migration from the apical to the equatorial subdomains of the sperm head plasma membrane precedes the acrosome reaction. Evidence for a primary capacitation event in boar spermatozoa. J Cell Sci 1995;108:935–46.
[45] Gadella BM, Gadella TW, Colenbrander B, van Golde LM, Lopes-Cardozo M. Visualization and quantification of glycolipid polarity dynamics in the plasma membrane of the mammalian spermatozoon. J Cell Sci 1994;107:2151–63.
[46] Myles DG, Primakoff P. Localized surface antigens of guinea pig sperm migrate to new regions prior to fertilization. J Cell Biol 1984;99:1634–41.
[47] Simons K, Toomre D. Lipid rafts and signal transduction. Nat Rev Mol Cell Biol 2000;1:31–9.
[48] Lingwood D, Simons K. Detergent resistance as a tool in membrane research. Nat Protoc 2007;2:2159–65.
[49] McGuinn KP, Mahoney MG. Lipid rafts and detergent-resistant membranes in epithelial keratinocytes. Methods Mol Biol 2014;1195:133–44.
[50] Smart EJ, Graf GA, McNiven MA, Sessa WC, Engelman JA, Scherer PE, et al. Caveolins, liquid-ordered domains, and signal transduction. Mol Cell Biol 1999;19:7289–304.
[51] Bickel PE, Scherer PE, Schnitzer JE, Oh P, Lisanti MP, Lodish HF. Flotillin and epidermal surface antigen define a new family of caveolae-associated integral membrane proteins. J Biol Chem 1997;272:13793–802.
[52] van Gestel RA, Brewis IA, Ashton PR, Brouwers JF, Gadella BM. Multiple proteins present in purified porcine sperm apical plasma membranes interact with the zona pellucida of the oocyte. Mol Hum Reprod 2007;13:445–54.
[53] van Gestel R.A. Membrane characteristics of sperm cells during capacitation. PhD Thesis Utrecht University ISBN 90-393-3964-3 Ridderprint Press, The Netherlands, Ridderkerk, pp 133.
[54] Morales CR, Marat AL, Ni X, Yu Y, Oko R, Smith BT, et al. ATP-binding cassette transporters ABCA1, ABCA7, and ABCG1 in mouse spermatozoa. Biochem Biophys Res Commun 2008;376:472–7.
[55] Ban N, Sasaki M, Sakai H, Ueda K, Inagaki N. Cloning of ABCA17, a novel rodent sperm-specific ABC (ATP-binding cassette) transporter that regulates intracellular lipid metabolism. Biochem J 2005;389:577–85.
[56] Byrne K, Leahy T, McCulloch R, Colgrave ML, Holland MK. Comprehensive mapping of the bull sperm surface proteome. Proteomics 2012;12:3559–79.
[57] Aiello RJ, Brees D, Francone OL. ABCA1-deficient mice: insights into the role of monocyte lipid efflux in HDL formation and inflammation. Arterioscler Thromb Vasc Biol 2003;23:972–80.
[58] Tarling EJ, Bojanic DD, Tangirala RK, Wang X, Lovgren-Sandblom A, Lusis AJ, et al. Impaired development of atherosclerosis in Abcg1-/-Apoe-/- mice: identification of specific oxysterols that both accumulate in Abcg1-/- Apoe-/- tissues and induce apoptosis. Arterioscler Thromb Vasc Biol 2010;30:1174–80.
[59] Tarling EJ, Edwards PA. Dancing with the sterols: critical roles for ABCG1, ABCA1, miRNAs, and nuclear and cell surface receptors in controlling cellular sterol homeostasis. Biochim Biophys Acta 2012;1821:386–95.
[60] Nieland TJ, Chroni A, Fitzgerald ML, Maliga Z, Zannis VI, Kirchhausen T, et al. Cross-inhibition of SR-BI- and ABCA1-mediated cholesterol transport by the small molecules BLT-4 and glyburide. J Lipid Res 2004;45:1256–65.
[61] Trigatti B, Rigotti A. Scavenger receptor class B type I (SR-BI) and high-density lipoprotein metabolism: recent lessons from genetically manipulated mice. Int J Tissue React 2000;22:29–37.
[62] Shadan S, James PS, Howes EA, Jones R. Cholesterol efflux alters lipid raft stability and distribution during capacitation of boar spermatozoa. Biol Reprod 2004;71:253–65.
[63] Nagao Y, Ohta Y, Murakami H, Kato Y. The effects of methyl-beta-cyclodextrin on in vitro fertilization and the subsequent development of bovine oocytes. Zygote 2010;18:323–30.
[64] Leahy T, Gadella BM. Sperm surface changes and physiological consequences induced by sperm handling and storage. Reproduction 2011;142:759–78.
[65] Belmonte SA, López CI, Roggero CM, De Blas GA, Tomes CN, Mayorga LS. Cholesterol content regulates acrosomal exocytosis by enhancing Rab3A plasma membrane association. Dev Biol 2005;285:393–408.
[66] Tsai PS, De Vries KJ, De Boer-Brouwer M, Garcia-Gil N, Van Gestel RA, Colenbrander B, et al. Syntaxin and VAMP association with lipid rafts depends on cholesterol depletion in capacitating sperm cells. Mol Membr Biol 2007;24:313–24.
[67] Watanabe H, Takeo T, Tojo H, Sakoh K, Berger T, Nakagata N, et al. Lipocalin 2 binds to membrane phosphatidylethanolamine to induce lipid raft movement in a PKA-dependent manner and modulates sperm maturation. Development 2014;141:2157–64.
[68] Baltiérrez-Hoyos R, Roa-Espitia AL, Hernández-González EO. The association between CDC42 and caveolin-1 is involved in the regulation of capacitation and acrosome reaction of guinea pig and mouse sperm. Reproduction 2012;144:123–34.
[69] Gadella BM. Dynamic regulation of sperm interactions with the zona pellucida prior to and after fertilisation. Reprod Fertil Dev 2012;25:26–37.
[70] Asano A, Nelson-Harrington JL, Travis AJ. Phospholipase B is activated in response to sterol removal and stimulates acrosome exocytosis in murine sperm. J Biol Chem 2013;288:28104–15.
[71] Asano A, Nelson-Harrington JL, Travis AJ. Membrane rafts regulate phospholipase B activation in murine sperm. Commun Integr Biol 2013;6:e27362.
[72] Van Blerkom J, Caltrider K. Sperm attachment and penetration competence in the human oocyte: a possible aetiology of fertilization failure involving the organization of oolemmal lipid raft microdomains influenced by the ΔΨm of subplasmalemmal mitochondria. Reprod Biomed Online 2013;27:690–701.
[73] Jégou A, Ziyyat A, Barraud-Lange V, Perez E, Wolf JP, Pincet F, et al. CD9 tetraspanin generates fusion competent sites on the egg membrane for mammalian fertilization. Proc Natl Acad Sci U S A 2011;108:10946–51.
[74] Garner AE, Smith DA, Hooper NM. Sphingomyelin chain length influences the distribution of GPI-anchored proteins in rafts in supported lipid bilayers. Mol Membr Biol 2007;24:233–42.

[75] Kongmanas K, Kruevaisayawan H, Saewu A, Sugeng C, Fernandes J, Souda P, et al. Proteomic characterization of pig sperm anterior head plasma membrane reveals roles of acrosomal proteins in ZP3 binding. J Cell Physiol 2014;230:449–63.
[76] Koyama K, Hasegawa A, Komori S. Functional aspects of CD52 in reproduction. J Reprod Immunol 2009;83:56–9.
[77] Flori F, Ermini L, La Sala GB, Nicoli A, Capone A, Focarelli R, et al. The GPI-anchored CD52 antigen of the sperm surface interacts with semenogelin and participates in clot formation and liquefaction of human semen. Mol Reprod Dev 2008;75:326–35.
[78] van Beek J, van Meurs M, 't Hart BA, Brok HP, Neal JW, Chatagner A, et al. Decay-accelerating factor (CD55) is expressed by neurons in response to chronic but not acute autoimmune central nervous system inflammation associated with complement activation. J Immunol 2005;174:2353–65.
[79] Donev RM, Sivasankar B, Mizuno M, Morgan BP. The mouse complement regulator CD59b is significantly expressed only in testis and plays roles in sperm acrosome activation and motility. Mol Immunol 2008;45:534–42.
[80] Perez de la Lastra JM, Harris CL, Hinchliffe SJ, Holt DS, Rushmere NK, Morgan BP. Pigs express multiple forms of decay-accelerating factor (CD55), all of which contain only three short consensus repeats. J Immunol 2000;165:2563–73.
[81] Cherr GN, Yudin AI, Overstreet JW. The dual functions of GPI-anchored PH-20: hyaluronidase and intracellular signaling. Matrix Biol 2001;20:515–25.
[82] Kondoh G, Tojo H, Nakatani Y, Komazawa N, Murata C, Yamagata K, et al. Angiotensin-converting enzyme is a GPI-anchored protein releasing factor crucial for fertilization. Nat Med 2005;11:160–6.
[83] Boerke A, van der Lit J, Lolicato F, Stout TA, Helms JB, Gadella BM. Removal of GPI-anchored membrane proteins causes clustering of lipid microdomains in the apical head area of porcine sperm. Theriogenology 2014;81:613–24.
[84] Mizuno M, Donev RM, Harris CL, Morgan BP. CD55 in rat male reproductive tissue: differential expression in testis and expression of a unique truncated isoform on spermatozoa. Mol Immunol 2007;44:1613–22.
[85] Kasekarn W, Kanazawa T, Hori K, Tsuchiyama T, Lian X, Garénaux E, et al. Pig sperm membrane microdomains contain a highly glycosylated 15-25-kDa wheat germ agglutinin-binding protein. Biochem Biophys Res Commun 2012;426:356–62.
[86] Flesch FM, Colenbrander B, van Golde LM, Gadella BM. Capacitation induces tyrosine phosphorylation of proteins in the boar sperm plasma membrane. Biochem Biophys Res Commun 1999;262:787–92.
[87] Reid AT, Redgrove K, Aitken RJ, Nixon B. Cellular mechanisms regulating sperm-zona pellucida interaction. Asian J Androl 2011;13:88–96.
[88] Ferraro GB, Morrison CJ, Overall CM, Strittmatter SM, Fournier AE. Membrane-type matrix metalloproteinase-3 regulates neuronal responsiveness to myelin through Nogo-66 receptor 1 cleavage. J Biol Chem 2011;286:31418–24.
[89] Saengsoi W, Shia WY, Shyu CL, Wu JT, Warinrak C, Lee WM, et al. Detection of matrix metalloproteinase (MMP)-2 and MMP-9 in canine seminal plasma. Anim Reprod Sci 2011;127:114–9.
[90] Tentes I, Asimakopoulos B, Mourvati E, Diedrich K, Al-Hasani S, Nikolettos N. Matrix metalloproteinase (MMP)-2 and MMP-9 in seminal plasma. J Assist Reprod Genet 2007;24:278–81.
[91] Caballero J, Frenette G, D'Amours O, Belleannée C, Lacroix-Pepin N, Robert C, et al. Bovine sperm raft membrane associated glioma pathogenesis-related 1-like protein 1 (GliPr1L1) is modified during the epididymal transit and is potentially involved in sperm binding to the zona pellucida. J Cell Physiol 2012;227:3876–86.
[92] O'Flaherty C, de Lamirande E, Gagnon C. Positive role of reactive oxygen species in mammalian sperm capacitation: triggering and modulation of phosphorylation events. Free Radic Biol Med 2006;41:528–40.
[93] Aitken RJ, Jones KT, Robertson SA. Reactive oxygen species and sperm function—in sickness and in health. J Androl 2012;33:1096–106.
[94] Griveau JF, Renard P, Le Lannou D. An in vitro promoting role for hydrogen peroxide in human sperm capacitation. Int J Androl 1994;17:300–7.
[95] Travis AJ, Kopf GS. The role of cholesterol efflux in regulating the fertilization potential of mammalian spermatozoa. J Clin Invest 2002;110:731–6.
[96] Vejux A, Malvitte L, Lizard G. Side effects of oxysterols: cytotoxicity, oxidation, inflammation, and phospholipidosis. Braz J Med Biol Res 2008;41:545–56.
[97] Olkkonen VM, Hynynen R. Interactions of oxysterols with membranes and proteins. Mol Aspects Med 2009;30:123–33.
[98] Bielska AA, Schlesinger P, Covey DF, Ory DS. Oxysterols as non-genomic regulators of cholesterol homeostasis. Trends Endocrinol Metab 2012;23:99–106.
[99] Bjorkhem I, Diczfalusy U. Oxysterols: friends, foes, or just fellow passengers? Arterioscler Thromb Vasc Biol 2002;22:734–42.
[100] Hynynen R, Suchanek M, Spandl J, Back N, Thiele C, Olkkonen VM. OSBP-related protein 2 is a sterol receptor on lipid droplets that regulates the metabolism of neutral lipids. J Lipid Res 2009;50:1305–15.
[101] Massey JB. Membrane and protein interactions of oxysterols. Curr Opin Lipidol 2006;17:296–301.
[102] Brouwers JF, Boerke A, Silva PF, Garcia-Gil N, van Gestel RA, Helms JB, et al. Mass spectrometric detection of cholesterol oxidation in bovine sperm. Biol Reprod 2011;85:128–36.
[103] Chen H, Cheung MP, Chow PH, Cheung AL, Liu W, O WS. Protection of sperm DNA against oxidative stress in vivo by accessory sex gland secretions in male hamsters. Reproduction 2002;124:491–9.
[104] Bucak MN, Tuncer PB, Sariozkan S, Baspinar N, Taspinar M, Coyan K, et al. Effects of antioxidants on post-thawed bovine sperm and oxidative stress parameters: antioxidants protect DNA integrity against cryodamage. Cryobiology 2010;61:248–53.
[105] Ballester L, Romero-Aguirregomezcorta J, Soriano-Úbeda C, Matás C, Romar R, Coy P. Timing of oviductal fluid collection, steroid concentrations, and sperm preservation method affect porcine in vitro fertilization efficiency. Fertil Steril 2014;102:1762–8.
[106] Talevi R, Gualtieri R. Molecules involved in sperm-oviduct adhesion and release. Theriogenology 2010;73:796–801.
[107] Jin M, Fujiwara E, Kakiuchi Y, Okabe M, Satouh Y, Baba SA, et al. Most fertilizing mouse spermatozoa begin their acrosome reaction before contact with the zona pellucida during in vitro fertilization. Proc Natl Acad Sci U S A 2011;108:4892–6.
[108] Florman HM, Jungnickel MK, Sutton KA. Regulating the acrosome reaction. Int J Dev Biol 2008;52:503–10.
[109] Avilés M, Gutiérrez-Adán A, Coy P. Oviductal secretions: will they be key factors for the future ARTs? Mol Hum Reprod 2010;16:896–906.
[110] Gupta SK, Bhandari B, Shrestha A, Biswal BK, Palaniappan C, Malhotra SS, et al. Mammalian zona pellucida glycoproteins: structure and function during fertilization. Cell Tissue Res 2012;349:665–78.