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Sperm membrane physiology and relevance for fertilization☆

B.M. Gadella *

Utrecht University, Faculty of Veterinary Medicine, Departments of Biochemistry and Cell Biology and of Farm Animal Health, Yalelaan 2, 3584 CM Utrecht, The Netherlands

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Abstract

This paper aims to overview recent insights in sperm surface remodelling pertinent to fertilization. A basic understanding of this remodelling is required to interpret the high amount of data appearing from high-throughput identification techniques for proteins presently applied in reproductive biology. From the extensive lists of protein candidates identified by proteomics, only a few are recognized to be directly involved in fertilization. Others are indirectly involved, but many are not yet considered to be involved in fertilization. Some of these newly identified and unexpected proteins may shed new light in the current molecular models for fertilization. However, the gathered lists of sperm proteins possibly involved in fertilization do only tell a part of the story regarding how fertilization is accomplished. When considering the identification of proteins involved in fertilization, one also needs to take into account the fundamental mechanisms involved in the redistribution of sperm surface proteins in membrane protein complexes and the involvement of cell signalling events that regulate their post-translational modification status. Both processes are likely requisite for protein configuration and grouping into functional membrane protein complexes necessary to elicit their delicate roles in fertilization. This paper emphasizes biochemical models for membrane surface modelling and their potential involvement for remodelling the sperm surface in the above described processes.

Keywords: Sperm capacitation; Sperm–zona binding; Acrosome reaction; Oolemma binding; Fertilization; Membrane protein complexes

1. Introduction

It is still not clear how sperm fertilize the oocyte, although it is clear that only functionally intact sperm can fertilize the egg and that this is somehow accomplished at the surface of the

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* Tel.: +31 30 2535386; fax: +31 30 2535492.
E-mail address: b.m.gadella@uu.nl.

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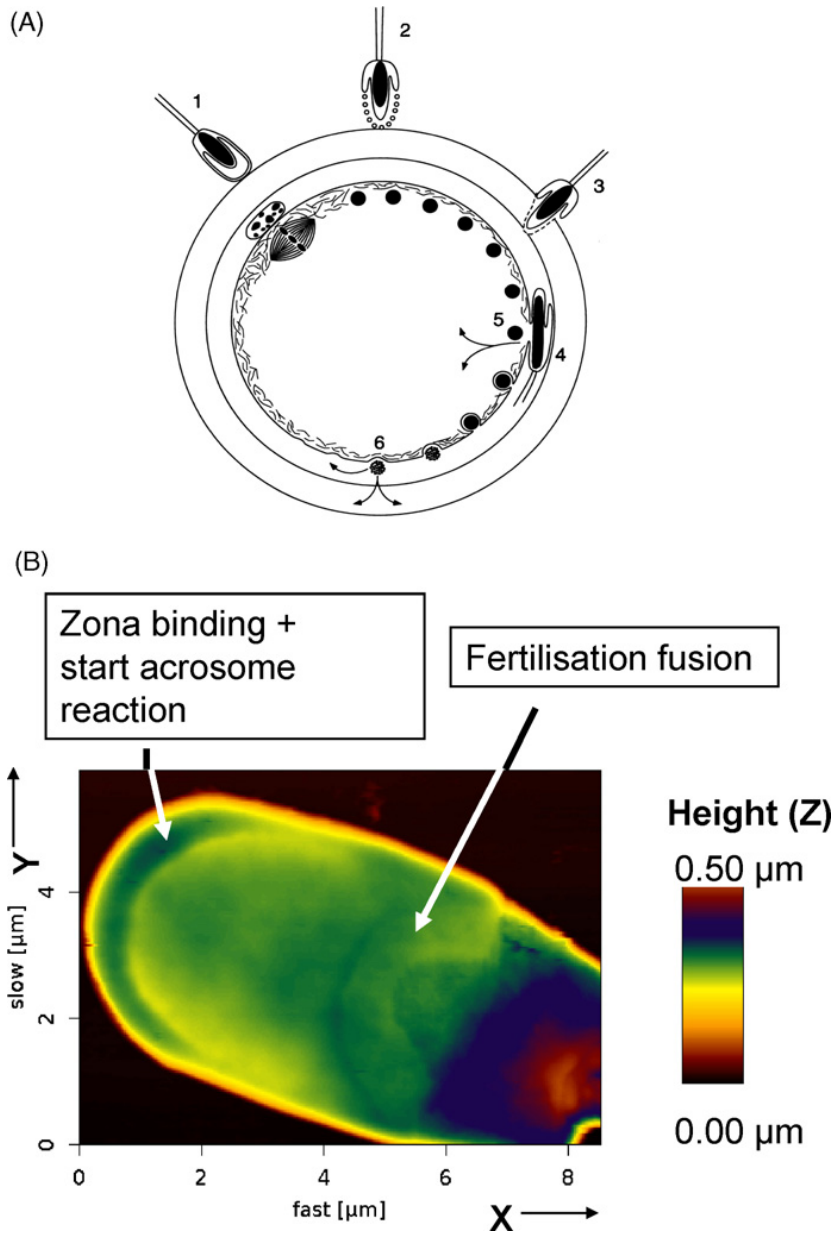

Fig. 1. (Panel A) Schematic representation of the sequence of interactions between the male and female gamete leading to fertilization. 1, Sperm binding to the zona pellucida (restricted to the apical ridge subdomain area); 2, the acrosome reaction (fusions restricted to the apical ridge and pre-equatorial subdomain areas); 3, the penetration of the zona pellucida note that the equatorial membranes remain intact and that the mixed vesicles resulting from the acrosome reaction are shed of the penetrating sperm; 4, binding and fusion with the oolemma (restricted to the equatorial subdomain area); 5, activation of the fertilized oocyte by a soluble sperm factors; 6, polyspermy block by the cortical reaction. (Panel B) The surface height of a living porcine sperm cell measured with atomic force microscopy. The apical ridge area is specifically involved in zona binding and is the site were the acrosome reaction is initiated, the equatorial segment is the specific area of the sperm head involved in oolemma binding and in the fertilization fusion.

sperm head (Yanagimachi, 1994). The sperm head surface is heterogeneous and diverse surface regions can be observed microscopically (Fig. 1). From previous studies we know that sperm surface molecules exhibit lateral diffusion properties but remain entrapped into specific surface regions (Phelps et al., 1988; Gadella et al., 1995). A reordering of this mosaic sperm surface takes place when sperm reside in the proximity of the egg at the same time when sperm becomes competent to fertilize.

In mammals, complex changes at the surface of both gametes usually take place internally in the female genital tract (Tienthai et al., 2004; Rodriguez-Martinez et al., 2005) but can efficiently be mimicked under in vitro-fertilization conditions (Flesch and Gadella, 2000; Harrison and Gadella, 2005). A delicate reorientation and modification of sperm surface molecules takes place when sperm are activated by capacitation factors. These surface changes are probably required to enable the sperm to bind to the extracellular matrix of the oocyte (the zona pellucida, ZP; Gadella and Van Gestel, 2004). In fact, part of the sperm surface coating proteins; namely those that normally prevent this adhesion, are lost during the transit of sperm in the uterus and are recoated in the oviduct (for review see Suarez, 2007). The surface of the sperm cell is possibly also modified by the oviduct epithelium that adsorbs proteins from the sperm surface and also secretes glycoproteins with an unknown function in sperm–ZP binding. At the moment that the sperm meets the oocyte in the oviduct [or during in vitro fertilization (IVF), when mixed with the oocyte], the apical sperm head surface contains functional membrane protein complexes that recognize and bind to the ZP (van Gestel et al., 2007). This binding also induces the acrosome reaction (Yanagimachi, 1994). The enzymatic machinery (Mayorga et al., 2007; Tomes, 2007) required for the exocytosis and the initiation of ZP penetration is only functionally operative at the surface region of the sperm head in the proximity of ZP interaction (Tsai et al., 2007). After full ZP penetration, the sperm cell comes in direct contact with the oolemma (the plasma membrane of the egg). At this moment, another specific set of sperm surface proteins at another site of the sperm head surface (the equatorial region) is involved in adhesion and subsequent fusion of the two gametes at fertilization (Vjugina and Evans, 2008). All described steps are characterised by a specific spatial organization of sperm membrane molecules.

This communication serves as a short overview of the molecular events occurring on the sperm surface, without providing exhaustive lists of protein candidates for each process. This should aid the reader in comprehending the surface ergonomics of the sperm en route to fertilization.

2. Sperm capacitation

Over the last two decades, different research groups have reported that the sperm surface undergoes changes during sperm capacitation (for reviews see Flesch and Gadella, 2000; Harrison and Gadella, 2005). Basically, ejaculated sperm are transported through the uterus and are prepared to enter the oviduct. At that stage, a large portion of the extracellular coating of the sperm is removed (including the decapacitation factors from seminal plasma). Sperm also meet an environment with a high bicarbonate level. Although the process of capacitation that will take place in vivo in the oviduct is far from understood one can mimic this environment with IVF media under laboratory conditions. To this end, freshly ejaculated sperm are washed through a density gradient to remove seminal plasma but also to partially strip the extracellular coatings of the sperm. The resulting washed cells are mixed with a synthetic oviductal fluid (SOF) in which salt, carbohydrate, and metabolite composition resembles that of oviduct fluid. Furthermore, the IVF medium contains capacitation factors like bicarbonate, albumin and calcium and, in some cases, glycosaminoglycans, that may be required for further surface remodelling to enhance sperm capacitation (Chamberland et al., 2001). Capacitation is defined as the process that results in sperm becoming capable of fertilization. Major responses observed are hyperactivation of sperm motility (required for zona penetration) and a number of sperm surface rearrangements. Together these processes enable sperm to bind to the zona pellucida (see Section 3) and this subsequently induces the acrosome reaction (Section 4) and the penetration of the ZP to reach the oolemma (Section 5).

3. The zona binding

The zona contains a three-dimensional network of only limited types (three to four) of zona proteins which are heavily and differentially glycosylated (Jovine et al., 2005). This ZP protein network provides the landing substrate for the sperm. Biochemical procedures used to isolate the apical sperm plasma membrane, as well as to purify the zona pellucida have been employed to determine the primary sperm zona-binding proteins (Flesch et al., 1998, 2001). Using this approach, our group identified a number of proteins from porcine sperm (van Gestel et al., 2007). Some are of testicular origin (fertilin-β, Kim et al., 2006) while others (like the spermadhesin AQN-3, Töpfer-Petersen et al., 1998) are secreted by the seminal vesicles and remain tightly attached to the sperm during capacitation. It is known that the epididymis remodels the surface of the sperm (Sostaric et al., in press) and that lipid-modified proteins (for instance GPI-anchored proteins; Sharma et al., 2004) are added by an unknown mechanism to the sperm surface when sperm travel through the epidydimis (Sullivan et al., 2007). The most likely pathway is that such proteins are apocrine secreted from the epididymal epithelium in the form of exosomes (called epididymosomes) and are exchanged with the sperm surface by lipoprotein particles present in the epididymal fluid (Neumann et al., 2007); however, this concept has not been tested on epididymal epithelia, fluids and sperm. It is relevant that the zona-binding proteins accumulated from a more dispersed sperm surface localization onto the apical ridge area of the sperm head during sperm capacitation (van Gestel et al., 2005). During this accumulation, a multiple zona-binding protein complex is formed, which most likely also contains components required for induction of the acrosome reaction (see Section 4). The involvement of post-translational modifications, induced by capacitation, to establish this zona-binding complex, is probably important but not yet defined or established. IVF procedures probably mimic events that are occurring in the oviduct because IVF conditions lead to fertilization. However, one should keep in mind that the in vivo-fertilization process might be different and more efficient by further removal, addition and redistribution of sperm molecules, although this is much more difficult to study at the biochemical level. The sperm–zona interaction is highly species-specific and proteins identified for one species may not necessarily have orthologues in other species. Beyond this, rapid evolution of more common orthologues has been reported between closely related species and breeds (Torgerson et al., 2002; Swanson and Vacquier, 2002; Aagaard et al., 2006; Dean et al., 2008). Redundant mechanisms for zona binding are probably operative in sperm and this complicates genotypic knock-out (KO) animal model studies to unravel the function of one of the zona-binding proteins. Nevertheless, KO mice have been generated in which sperm–zona binding was inhibited, and the number of A Disintegrin and A Metalloproteases (ADAMs; including the fertilin β), calmegin- and angiotensin-converting enzyme (ACE)-knock-out genotypic mice have been shown to be infertile due to a deficiency in sperm–zona binding (Okabe and Cummins, 2007). Regarding other putative zona-binding candidates, such as galactose transferase (Lu and Shur, 1997) and SED1 (SED denotes a *S*ecreted protein containing a cleavable signal sequence, N-terminal Notch-like type II *E*GF repeats and C-terminal *D*iscoidin/F5/8 Complement domains; Ensslin and Shur, 2003) ZP-binding function was not fully supported by the knock-out genotypic mice because of fertility retention (Okabe and Cummins, 2007). For putative ZP binding proteins (like sp56, Buffone et al., 2008) knock-out genotypic mice have not yet been generated.

4. The acrosome reaction

Prior to the fertilization fusion, sperm need to secrete their acrosomal enzymes in order to penetrate the ZP. This so-called acrosome reaction is triggered by the ZP and is a multi-point

fusion event between two closely positioned membranes (Mayorga et al., 2007; Tomes, 2007). In somatic cells, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor proteins (SNAREs) are involved in the membrane-fusion machinery responsible for secretion (Sørensen, 2005). SNAREs required for exocytosis have been identified in mammalian sperm (De Blas et al., 2005). We identified in rat and porcine sperm (also reported in human and mouse sperm) all SNAREs that are required to form the SNARE complex involved in membrane fusion (Tsai et al., 2007). In porcine sperm, these syntaxins (plasma membrane SNAREs) showed similar aggregation towards the apical ridge area of the sperm surface as was observed for the zona-binding proteins when sperm were capacitated. In addition, the vesicle-associated membrane proteins (VAMPs; the vesicle, in our case the acrosomal SNAREs) aggregated at the apical tip of the outer acrosome membrane at the same time (Tsai et al., 2007). We also demonstrated that SNAREs of the plasma membrane are not interacting with SNAREs in the acrosome in ejaculated sperm (Tsai et al., 2007) and that sperm activation is required for this interaction (preliminary results). The regulation of the formation of SNARE protein fusion complexes (and additional adhering proteins participating in the acrosome reaction) is currently under study and, to a large extent, remains unknown. The acrosome reaction is relatively easy to detect on live sperm (Mayorga et al., 2007) due to the extraordinary size of the surface area where membrane docking and the multiple fusions take place during this secretion event. This makes it easier to study whether or not post-translational modifications of the relevant proteins are involved in regulating the sperm acrosome reaction. Another interesting hypothesis worthy of investigation is the possibility that the zona-binding protein complex (see Section 3) is orchestrating the assembly of the acrosome–fusion protein complex. In this respect, a putative K^+ channel has been identified in the zona-binding complex (van Gestel et al., 2007; see Section 3) which may be indirectly involved in the influx of calcium into the sperm head which is, in turn, required for SNARE zippering and conformational changes of the SNARE fusion complex to establish acrosomal fusions.

5. The sperm–oocyte binding and fusion (fertilization)

The zona-induced acrosome reaction enables the hyperactive motile sperm to penetrate through the zona pellucida. Once one sperm reaches the perivitelline space, its destiny is to bind to and fuse with the oolemma. In contrast to the zona-binding event (Section 3), this event is far less species-specific (Ponce et al., 1993) and probably has some homology with virus membrane fusions and the fusions involved in the formation of multinucleated cell syncitia (for instance muscle fibers from myoblasts). The hamster oocyte test reveals the non-species specificity of the fertilization fusion. In this test, human sperm are brought into a fluid with zona free hamster oocytes and the incidence of male pronucleus formation provides an IVF clinic with a diagnostic parameter for male-factor fertility (Zainul Rashid et al., 1998). The sperm–oolemma fusion takes place at the equatorial sperm head surface. This is the area of the sperm where the plasma membrane still covers the acrosome and where acrosome fusions (see Section 4) did not occur. Interestingly, the transfer of oocyte membrane protein components to the sperm surface has been observed in the perivitelline space prior to fertilization (Barraud-Lange et al., 2007). This may imply that, even within the perivitelline space, the sperm surface undergoes final alterations that prepare it for fertilization. The first-entering sperm could thus be facilitated (feed forward principle) to fertilize. After the cortical reaction, redundant sperm are eliminated to prevent polyspermic fertilization. Although a number of candidate molecules on the sperm plasma membrane and on the oolemma have been described, the exact molecular mechanism of fertilization is still not resolved. From the

egg side, the potential proteins involved in fertilization fusion are grouped into the tetraspanins (such as CD9), and GPI-anchored proteins and integrins are mentioned. From the sperm side, IZUMO, ADAMs and cysteine-rich secretory proteins (CRISPs) are described in literature (for a recent review, see Vjugina and Evans, 2008). The CRISP family of proteins is secreted at different sites in the epididymis and seminal vesicles (Nolan et al., 2006) and specific types of CRISP remain firmly attached to the sperm surface, even after IVF incubations (Da Ros et al., 2007; Cohen et al., 2007). Other interesting sperm proteins that may have a role in fertilization are sperm lysosomal like protein 1 (SLLP-1; Herrero et al., 2005) and a multifunctional thiol-disulfide oxidoreductase that can efficiently catalyze disulfide reduction, disulfide isomerization and dithiol oxidation in substrate proteins that is called ERp57 (Ellerman et al., 2006). In general, the big problem in studying the fertilization fusion is that a proper biochemical assay is difficult to develop. The ultimate fusion assay needs to be done with a large proportion of purified plasma membrane preparations of the two merging gametes. Another thing which complicates the story is the question of whether the sperm proteins involved in fertilization of the oocyte are of sperm plasma membrane origin, or from the acrosomal membrane and only are exposed after the acrosome reaction. It is most likely that the sperm plasma membrane at the equatorial segment has a fundamentally different membrane organization when compared to the apical sperm plasma membrane involved in the zona binding (Section 3) and the acrosome reaction (Section 4).

6. Conclusions

The molecular architecture of the sperm surface, and the interplay of sperm surface proteins needed to initiate contact with proteins of the zona pellucida, the acrosome and the oolemma, are far from elucidated. Modern proteomic technology enables the newest generation of scientists to identify sperm surface proteins involved in these important first steps before and during the start of new life. The technology not only provides us with the identification of proteins, but also allows us to detect a variety of post-translational modifications that may take place before and during fertilization. For a very nice recent experimental update on new emerging proteomic technologies and applications see O'Connor and Hames (2008). This technology probably will aid in resolving the enigmas in the field of reproductive biology. Gametes are almost inaccessible for approaches of molecular biology research because the male gamete has no translation and transcription properties and precursor cells need to be manipulated (Boerke et al., 2007). The oocyte, on the other hand, has an enormous reserve of mRNA which will not be translated until the first cleavage of the zygote. Therefore, introduction of interfering RNA probably has little effect on the processes prior to and at fertilization, but may influence post-fertilization events instead.

Hopefully this paper has helped introducing the reader to the current "conceptual" thinking of the mammalian conceptus. It is still not known how the sperm fertilizes the egg, but the emerging high-throughput lists of proteins candidates involved in the fertilization process (Stein et al., 2006) and the recent biochemical and cell biological models of how the two gametes could interact should bring us to much deeper insights. Applied-research scientists that want to optimize field storage of sperm should take into consideration that all processing steps may alter the membrane architecture of the sperm head and thus may influence the viability and the fertilization capability of sperm.

Conflict of interest

None of the authors (B.M. Gadella) has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the paper entitled "Sperm membrane physiology and relevance for fertilization.

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