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Roles of the oviduct in mammalian fertilization

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Abstract

The oviduct or Fallopian tube is the anatomical region where every new life begins in mammalian species. After a long journey, the spermatozoa meet the oocyte in the specific site of the oviduct named ampulla, and fertilization takes place. The successful fertilization depends on several biological processes which occur in the oviduct some hours before this rendezvous and affect both gametes. Estrogen and progesterone, released from the ovary, orchestrate a series of changes by genomic- and non-genomic pathways in the oviductal epithelium affecting gene expression, proteome and secretion of its cells into the fluid bathing the oviductal lumen. In addition, new regulatory molecules are being discovered playing important roles in oviductal physiology and fertilization. The present review tries to describe these processes, building a comprehensive map of the physiology of the oviduct, to better understand the importance of this organ in reproduction. With this purpose, gamete transport, sperm and oocyte changes in the oviductal environment and other interactions between gametes and oviduct are discussed in light of recent publications in the field.

Keywords

fertilization; oviduct; gamete transport; capacitation; hyperactivation; oocyte oviductal maturation

1. The spermatozoon in the oviduct

1.1. Arrival, binding to and releasing from epithelial cells

Depending on the species, the sperm are deposited in different sections of the female tract. In a large number of mammals, the semen is ejaculated into the anterior vagina during coitus (e.g. cows, sheep, rabbits, primates, dogs and cats). In others, sperm are placed in the cervix (e.g. sows) or directly spurted into the uterus (horses and many rodents). Regardless of where the sperm is initially dropped, to encounter the oocyte, the sperm is required to go through the utero-tubal junction and enter the oviduct.

However, not all the ejaculated spermatozoa reach this initial portion of the oviduct. Once ejaculated, most sperm are eliminated from the female tract by different mechanisms

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(Yanagimachi 1994). A very low percentage of the sperm population is able to reach the ampulla or the ampullar-isthmic junction, and a recent work using genetically modified mice models has shown that a critical step in sperm transport is their migration through the uterotubal junction (Tokuhiro *et al.* 2012). Although the molecular basis of this transport is not well understood, it has been observed that knock out models with deficiencies in this transport are infertile. Presently, analysis of sperm from eight different knockout mice has shown problems in uterotubal junction transport. The null mice models presenting this phenotype include those for: Ace (Hagaman *et al.* 1998), Adam1a (Nishimura *et al.* 2004), Adam2 (Cho *et al.* 1998), Adam3 (Shamsadin *et al.* 1999), Calr3 (Ikawa *et al.* 2011), Clgn (Ikawa *et al.* 1997), Tpst2 (Marcello *et al.* 2011) and PDILT (Tokuhiro *et al.* 2012). It is interesting that all these models appear to converge in the lack of ADAM3; therefore, it has been hypothesized that this molecule is central to uterotubal transport and the other knockout models presenting this phenotype are involved in the process and regulation of ADAM3.

Once in the isthmus, the spermatozoa are bound to the ciliated epithelial cells. This process seems to be mediated by carbohydrate residues present in the oviductal epithelial cells and lectin-like proteins on the sperm head (Suarez 2002). The molecules involved in this process vary among species (Talevi & Gualtieri 2010). In hamsters, sperm binding to oviductal epithelium is mediated by sialic acid (DeMott *et al.* 1995) and by galactose in horses (Dobrinski *et al.* 1996). In pigs, galactosyl and mannosyl residues seem to be involved in sperm-oviduct binding (Ekhlasi-Hundrieser *et al.* 2005). In cattle, strong evidence supports the involvement of fucose residues that are recognized by spermadhesin BSP1 (also called PDC-109) (Ignotz *et al.* 2001, Gwathmey *et al.* 2003, Sostaric *et al.* 2008), and in llamas (camelid), N-acetylgalactosamine and galactose have been observed that inhibit the sperm binding to the oviductal cells (Apichela *et al.* 2010).

From the sperm side, several proteins have been shown to have carbohydrate binding affinities and could then interact with the epithelial cells. Thus, it was previously reported that the spermadhesins AQN1 and AWN bind to the sequences Galβ1,3GalNAc and Galβ1,4GlcNAc (Dostàlovà *et al.* 1995, Calvete *et al.* 1996). AQN1 also bind to mannose residues (Ekhlasi-Hundrieser *et al.* 2005) and, in the bovine species, spermadhesin BSP1 is able to recognize fucose residues (Gwathmey *et al.* 2003).

Independently on the specific carbohydrate residues or the lectin-like proteins participating in the adhesion, the role of the oviduct in such sperm-epithelial cell interaction seems to be the formation of a sperm reservoir. The more plausible explanation for the formation of a sperm reservoir in different species of mammals is the sequential releasing of sperm to allow only a small quantity of them reaching the oocyte at any given time and therefore reducing the possibility of polyspermy (Hunter & Léglise 1971, Hunter 1973). Interestingly enough, the sperm release is modulated by the female estrous cycle with increased activity observed during the periovulatory period (Suarez 2008b) and it is probably related with the existence of unknown signaling between the recently attached *cumulus*-oocyte complex and the oviductal cells (Kölle *et al.* 2009) and with the progesterone levels (Bureau *et al.* 2002).

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Although the mechanisms responsible for sperm release are not well understood, it seems that the number of carbohydrate binding sites present in the oviductal epithelium surface is not greatly affected (Suarez *et al.* 1991, Lefebvre *et al.* 1995, Baillie *et al.* 1997). However, it has been proposed that this release is correlated with capacitation events (Smith & Yanagimachi 1991, Lefebvre & Suarez 1996). On the one hand, sperm release could be due to a loss of proteins involved in binding the sperm to the oviduct. As an alternative possibility, as part of the capacitation process, hyperactivation of the sperm motility might play an important role allowing these cells to escape the attachment by shear force (Demott & Suarez 1992, Pacey *et al.* 1995). Even though, it cannot be discarded that both mechanisms are coordinated to free the sperm from the epithelium, but additional hypothesis are also emerging:

Due to the complex protein composition found in the oviductal fluid (Avilés *et al.* 2010, Mondéjar *et al.* 2012a), two additional mechanisms could contribute to the regulation of the sperm oviductal interaction. First, activities for different glycosidases have been detected in the oviductal fluid showing variations along the estrous cycle (Carrasco *et al.* 2008a, Carrasco *et al.* 2008b). These enzymes could act on the specific carbohydrates residues present in the epithelial cells necessary for the sperm binding and contribute to the release of the sperm from the reservoir. Supporting this model, it is important to point out that the best characterized sperm-oviductal interaction was described in the bovine model (Figure 1). In this species, it was reported that sperm protein BSP1 recognizes specifically the fucose residues contained in the annexin present at the oviductal epithelium (Hung & Suarez 2010). Thus, fucosidase activity, present in the oviductal fluid (Carrasco *et al.* 2008b) could contribute to the regulation of the binding. Additionally, the presence of annexin in the oviductal fluid (Mondéjar *et al.* 2012a) could also participate in such regulation, since the atypical secretion of this protein has been reported previously (Christmas *et al.* 1991).

Second, as mentioned above, AWN has the ability to bind to carbohydrate residues. Unexpectedly, it was reported that this protein is secreted by the epithelial cells in the swine oviduct (Song *et al.* 2010) and, consequently, could compete with the sperm for oviductal carbohydrates suggesting its participation in the sperm releasing process. Additional experiments are necessary to confirm these different hypotheses. The development of improved experimental conditions as the use of labelled sperm, video microscopy and the *in vitro* system culture for oviductal epithelium will bring more light about the molecular mechanisms involved in the sperm-oviduct interaction (Miessen *et al.* 2011).

1.2. Capacitation

As explained above, it has been hypothesized that release of the sperm from the oviductal epithelium is dependent on their capacitated state (Smith & Yanagimachi 1991, Lefebvre & Suarez 1996). Discovered independently by Chang (Chang 1951) and Austin (Austin 1951) in 1951, capacitation has been defined as those physiological events that render the sperm able to fertilize. Discovery of capacitation was fundamental to allow development of *in vitro* fertilization. First demonstrated in rabbits in 1959 (Chang 1959), this technology led to the first test tube baby in 1978 when Mary Louise Brown was born (Steptoe & Edwards 1978). This success was recognized in 2010 when Dr. Roberts was awarded the Nobel Prize in

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Medicine. Although the initial experiments by Chang and Austin were conducted using artificial insemination in live female rabbits, most of what it is known about this process is derived from *in vitro* experimentation. *In vitro* capacitation in most mammalian species is achieved by incubation of the sperm in a simple media that mimics the oviductal milieu. In particular, capacitation-supporting media requires bicarbonate, calcium, energy sources and serum albumin as a cholesterol-binding compound.

One critical change in the sperm surrounding milieu after ejaculation is the change in HCO_3^- concentration (Figure 2). This anion plays a role in the regulation of the cAMP pathway (Visconti *et al.* 2011) through the stimulation of a unique type of adenylyl cyclase present in sperm, known as soluble adenylyl cyclase (SACY) (Buck & Levin 2011). SACY knock out mice are sterile (Hess *et al.* 2005, Xie *et al.* 2006) and their sterility phenotype is mapped to a lack of capacitation; in particular, sperm from the SACY null mice are not able to move actively and cannot hyperactivate (Hess *et al.* 2005). Consistent with the role of cAMP in capacitation, a similar phenotype is observed when the testis-specific PKA catalytic subunit splicing variant is eliminated by homologous recombination. Using sperm of these mice *in vitro*, the authors clearly showed PKA is required for the activation of flagellar beat and for the flagellar waveform asymmetry associated with hyperactivation (Nolan *et al.* 2004). In addition to genetic approaches, the role of cAMP in the regulation of sperm is also supported by biochemical and pharmacological approaches. Inhibitors of protein kinase A (PKA) such as H89 and rpScAMP and peptides that disrupt PKA binding to anchoring proteins block sperm motility and *in vitro* fertilization (Visconti *et al.* 1995, Vijayaraghavan *et al.* 1997). Downstream of the activation of a cAMP/PKA pathway, capacitation *in vitro* is also associated with an increase in protein tyrosine phosphorylation (for review see Visconti *et al.* 2011). Despite the fact that many groups have shown similar regulatory pathways in sperm from other species, there is still a limited knowledge on the identity and the role of proteins phosphorylated during capacitation.

Although it is believed that the regulation of signaling pathways *in vitro* mimic those happening *in vivo*, this possibility has not yet been demonstrated. Activation of PKA occurs immediately upon ejaculation, once the HCO_3^- concentration surrounding the sperm milieu increases from low mM levels in the cauda epididymis to ~25 mM concentration in the semen and female tract fluids. However, tyrosine phosphorylation as well as hyperactivation are not believed to occur until the sperm reach the oviduct.

As mentioned above, most of what it is known about the signaling events controlling sperm capacitation was obtained from *in vitro* experiments. While these observations are important, they do not address how the female tract controls the speed of capacitation and delivers freshly capacitated sperm to the ovulated eggs. The assumption that capacitation is regulated by the same signaling pathways *in vivo* as *in vitro*, although logical, has not yet been tested. Studies of *in vivo* capacitation are more complex because of the more difficult access to the sperm and also because of the smaller quantity of cells that can be obtained. Because of these limitations, novel approaches should be developed. Among them, the analysis of phosphorylation pathways could be performed by immunofluorescence analysis using anti phospho antibodies such as anti phospho PKA substrate or anti phosphotyrosine antibodies (Krapf *et al.* 2010). Some of the challenges presented by these experiments are:

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1) that these experiments should be conducted in static fixed sections what would not allow following the fate of the signaling changes in live sperm; and 2) that sections could cut the sperm in planes not compatible with anti phospho staining making it difficult to quantify the level of phosphorylation. Despite these perceived problems, this approach has the advantage that it does not need a lot of material as in the case of Western blots used in most *in vitro* experiments. In addition, sections of oviducts have been used successfully to visualize fluorescent mouse sperm in the oviduct (Tokuhiro *et al.* 2012) and confocal microscopy or even multiphoton microscopy can be used to optically assess thick sections of oviduct to provide more complete and clear images of different sperm compartments. Alternatively, it is predicted that the use of genetically modified mice in which fluorescent markers of capacitation are inserted through transgenic technology would allow investigating how the sperm behaves *in vivo*. This technology has been used to observe the acrosome reaction using sperm in which the green fluorescent protein is targeted to the acrosomal compartment (Nakanishi *et al.* 1999).

1.3. Sperm hyperactivation

The oviduct takes care of and modifies the sperm in such a way that it is able to fertilize. One of these modifications is the sperm pattern motility.

Sperm within the epididymis are unmotile or poorly motile (inactivated). When they are released from the epididymis and mixed with the seminal plasma, they become activated. The term "activated motility", described by Yanagimachi (Yanagimachi 1994), means that the sperm start to swim straight and vigorously with symmetrical flagellar beats. Once the activated sperm are in the female tract after mating, only a small population (hundreds to thousands) will be able to reach the oviduct and become established in the sperm reservoir (described above). Spermatozoa in this storage place are attached and stabilized during the pre-ovulatory interval with suppressed motility and intact surface membranes (for review see Hunter 2012), but later sperm will detach acquiring another motility pattern named "hyperactivated motility (Figure 3a). This condition was defined for the first time by Yanagimachi (Yanagimachi 1970) who observed that hamster sperm in the oviduct had a very vigorous motility pattern with high amplitude and asymmetrical flagellar beating. In the years following that discovery, it was demonstrated that the "hyperactive motility" is essential for the sperm to fertilize. So, this term was re-defined as the swimming pattern shown by most sperm retrieved from the oviductal ampulla at the time of fertilization (Suarez & Ho 2003).

But, when do mammalian spermatozoa become hyperactivated? One of the known triggers is the increase of intracellular Ca^{2+} ($[Ca^{2+}]_i$) (Publicover *et al.* 2008). This is probably prompted by ovulation and is a consequence of progesterone secretion influencing the oviduct epithelium (for review see Hunter 2012) and helping sperm transport to the fertilization place (Chang & Suarez 2012). And, how is Ca^{2+} mobilized into the cytoplasm to induce hyperactivation? Spermatozoa require Ca^{2+}-channels in the plasma membrane which have been identified and named as CatSper proteins (CatSper 1 to 4), located in the principal piece of the flagellum (Kirichok *et al.* 2006, Qi *et al.* 2007). In fact, several reports have shown that male mice null mutant for CatSper genes are infertile (Ren *et al.* 2001,

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Quill *et al.* 2003, Jin *et al.* 2007, Qi *et al.* 2007), suggesting that the reason is the lack of hyperactivation in the spermatozoa of these animals (Ren *et al.* 2001, Quill *et al.* 2003). Ho et al. (Ho *et al.* 2009) supported this hypothesis because spermatozoa from CatSper-null mutants did not detach from the epithelium or show deep asymmetrical flagellar bending. As indicated above, progesterone could be involved in sperm detachment (Bureau *et al.* 2002) and hyperactivation. It is known that progesterone rises $[Ca^{2+}]_i$ levels in human sperm (Publicover *et al.* 2007) but it is not known by which mechanism. Recently, Strunker et al. and Lishko et al. (Lishko *et al.* 2011, Strünker *et al.* 2011) explained the action of this hormone on human spermatozoa where the progesterone, in combination with an elevation of intracellular pH, activates the CatSper channels involved in Ca^{2+} human sperm intake. The extracellular pH increase in oviductal fluid during oestrus could possibly be the primary factor for inducing hyperactivation in the oviduct, activating CatSper and raising intracellular pH (Suarez 2008a). HCO_3^- levels could play an important role for this purpose.

Summarizing the oviductal influence on spermatozoa physiology, an increasing body of research suggests that peri-ovulatory changes in pH, temperature, $[Ca^{2+}]$ and HCO_3^- levels in the oviductal fluid modulate different aspects of sperm function, including releasing from the epithelial cells, membrane modifications leading to capacitation, and the hyperactive motility that addresses them to the oocyte vicinity (Hunter & Nichol 1986, Rodriguez-Martinez *et al.* 2001, Rodriguez-Martinez 2007, Coy *et al.* 2010, Zumoffen *et al.* 2010, Kumaresan *et al.* 2012).

2. The oocyte in the oviduct

2.1. Arrival to the fertilization site

The role of the oviduct on the oocyte transport has been classically attributed to the cilia beating and smooth muscle activity, speeded up by estrogens and slowed down by progesterone, when administrated in adequate dosages and times (Chang 1966). This principle is still valid, and modern video microscopy and biochemical techniques have contributed to describe the process in detail.

From studies in hamsters it was shown the importance of the *cumulus* cells and the extracellular matrix of the *cumulus*-oocyte complex (COC) on the picking up and initial adhesion to the infundibulum cilia. Slight changes in the level of COCs expansion affected the initial adhesion and made their further transportation difficult (Talbot *et al.* 2003). Cilia that cover the exterior surface of the infundibulum beat in the direction of the ostium, induce a current of oviductal fluid and move the COC into the oviduct. It was suggested in mice and humans that the progesterone receptor (PR), localized in the lower half of the motile cilia of oviduct ciliated epithelial cells, may regulate directly ciliary beat frequency (Teilmann *et al.* 2006), thus confirming the initial discoveries by Chang (Chang 1966).

Although it is a key factor, ciliary beating alone cannot provide the propulsive force behind oocyte movement along the oviduct. Spontaneous contractions of the oviduct are also necessary, and interstitial cells of Cajal associated with the smooth muscle cells along the entire length of the oviduct are responsible for electrical slow-wave events that couple in a one-to-one relationship with phasic contractions of the myosalpinx (Dixon *et al.* 2009).

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Dixon et al. demonstrated that these slow waves are not initiated by neural inputs, but they are driven by pacemaker activity provided by the oviductal cells of Cajal.

Another interesting body of research about oviductal motility and oocyte transport is that concerning the role of Nitric Oxide Synthases (NOS). Lapointe et al. (Lapointe *et al.* 2006) showed in the bovine oviduct that expression of inducible NOS is selectively up-regulated by estradiol during the temporal window of oocyte transport. Since Nitric Oxide (NO) plays a role as a relaxing agent in mammalian oviduct and its inhibition accelerates oocyte transport (Perez Martinez *et al.* 2000), regulation of NOS by estradiol suggests a fine tuned equilibrium of the oviductal motility. Such equilibrium seems to be reached by non genomic pathways of estradiol action including estradiol bound to its receptors, activation of cAMP and partial participation of PKA (Orihuela *et al.* 2003) that again confirm Changś discoveries.

2.2. Oocytés changes in the oviduct

Once in the ampulla, transported by the cilia beating and smooth muscle contractions, the COC remains attached to the epithelium for a variable period of time. While previous *in vivo* studies in the pig model indicated that unfertilized oocytes reached the ampullar-isthmic region within 30-45 min from the beginning of ovulation (Hunter 1974) and that spermatozoa met them in this site, digital video microscopical studies in the cow oviduct have suggested that as soon as the mature COC enter the ampulla, they are immediately firmly attached to the oviductal epithelium (Kölle *et al.* 2009). Whether inter-specific differences between the pig and cow or methodological differences between the experiments (*in vivo* in the pig and *ex vivo* in the cow) explain this controversy remains elusive.

One important change for the oocyte at this time is related with the removal of its investments: in some species, such as cows and sheep, *cumulus* cells are rarely detected around recently ovulated oocytes, whereas in primates and in pigs *cumulus* cells and oocytes are immersed in a dense plug (Hunter 1989). Even in this case, the plug is dissolved in the porcine species a few hours later and oocytes collected from the oviduct become naked within 1 hour (Coy *et al.* 1993). However, a general consensus exists, based on wide experimental evidence, about the improving of the fertilization rates in most mammalian species in the presence of *cumulus* cells (Campos *et al.* 2001, Zhuo *et al.* 2001, Van Soom *et al.* 2002), underlying the importance of a rapid response of the oviduct, releasing the sperm from the reservoir, as soon as the oocyte arrives surrounded by the *cumulus*.

During the process of *cumulus* expansion and disaggregation, zona pellucida (ZP) becomes more accessible to the oviductal fluid permitting its modification by different molecules. Differences in ZP among oviductal and follicular oocytes, or zona maturation, have been referred by a number of authors, most of them related to changes at the ultrastructural level (Funahashi *et al.* 2001), and a few of them identifying specific molecules in the oviductal ZP that are not present in the ovarian ZP. Among them, oviduct-specific glycoprotein (OVGP1), osteopontin and lipocalin-type prostaglandin D synthase (L-PGDS) were demonstrated to associate with the bovine ZP (Goncalves *et al.* 2008). Moreover, OVGP1 and heparin-like glycosaminoglycans (GAGs) from the oviductal fluid have been demonstrated in the pig and cow to participate in the functional modification of the ZP that, before fertilization, make it

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more resistant to enzymatic digestion and to sperm penetration, contributing to the control of polyspermy (Coy *et al.* 2008). This mechanism is represented in Figure 4. Finally, there is also a significant change in the sugar moieties of glycoproteins in the ZP following ovulation (Aviles *et al.* 1996, Aviles *et al.* 1997, El-Mestrah & Kan 2001) although the specific role of these changes needs to be investigated. Studies completing the list of proteins and sugars binding to the ZP in the oviduct could contribute to the comprehension of the molecular events affecting the sperm-oocyte interaction and to the definitive description of a model assigning to each molecule its specific role in this complex mechanism.

3. Oocyte-sperm-oviduct interactions

3.1. Oviductal influence on the initial sperm approach to the oocyte

Once the sperm are hyperactivated and released into the oviduct, how do the sperm know which direction to travel in order to reach the oocyte? The sperm in the reservoir (Fig. 3a) are like “the boats docking in a port”...they are attached, but when they are released (“sailing on the sea”) they need a guide (“navigator”) to reach the objective that in the sperḿs case is the fertilization place. Thermo- and chemo-taxis have been defined, in terms of fertilization, as the process by which sperm are guided by a temperature gradient (cooler to warmer; for review see Eisenbach & Giojalas 2006) or by a chemical gradient (Chang & Suarez 2010) to reach the oocyte. There are only a few studies about the motility on mammalian sperm responses to these gradients. Some substances have been identified as potential chemo-attractants; for example the progesterone that is released during the ovulation (present in follicular fluid) and is produced by the *cumulus* cells that surround the oocytes (Chang & Suarez 2010). It has been postulated that $[Ca^{2+}]_i$ increases during sperm chemotaxis (i.e. progesterone) induce turning swimming with asymmetric flagellar bending (for review see Yoshida & Yoshida 2011). Other components in oviductal fluid have been identified as chemo-attractant; that is the case for natriuretic peptide precursor, that modifies the sperm pattern motility and enhance $[Ca^{2+}]_i$ levels, whose receptor has been recently shown in mouse spermatozoa (Bian *et al.* 2012). Temperature also seems to play a role in the levels of $[Ca^{2+}]_i$. Temperature stimulation activates the release of the internal sperm Ca^{2+} store affecting flagellar bending (Bahat & Eisenbach 2010). In addition to chemo- and thermo-taxis, other factors as the movement of oviductal fluid, oviductal contractions, oviductal ephitelium, and the internal structure of the oviduct (Burkitt *et al.* 2012) could also influence the sperm transport and guidance, although the evidence of these aspects has not yet been demonstrated *in vivo.*

After the sperm are hyperactivated and guided to the oocyte, they have to propel themselves through the viscous glycoprotein secretion in the oviduct toward the ampullar-isthmic junction (Figure 3b). During the pre-ovulatory stage the mucus within the oviduct is extremely viscous before ovulation and may contribute to the suppression of sperm motility (Hunter *et al.* 2011); after ovulation, it become less viscous which would facilitate an adequate flagellar beat and progression of spermatozoa towards the ampulla (Suarez & Dai 1992, Hunter *et al.* 2011). Suarez and Dai (Suarez & Dai 1992) showed an increase in flagellum propulsion when mouse sperm was confronted with an increase viscosity gradient

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medium. So, it seems that spermatozoa escape from the viscous fluid thanks to the hyperactivation. The flagellar beating during hyperactivation has been widely reported *in vitro*, but only a few experiments show the flagellar behavior simulating real conditions. Recently, Chang and Suarez (Chang & Suarez 2012) have recorded mice sperm in the oviduct in conditions very close to *in vivo*. These authors showed a different motility pattern called anti-hook instead of pro-hook beating (amplitude of the bend in the same orientation as the hook of the head) described before in *in vitro* situations. The cause of these differences could be found (among other factors) in the composition (mucoid type) and viscosity of the oviductal fluid that are absent in most of the *in vitro* media used, making the sperm to propels themselves (better than to swim) in such a semi solid environment.

What seems to be obvious it is that sperm are exposed to a different microenvironment (viscosity, chemical agents, temperature, etc) in their travel through the oviduct and they are continuously re-adapting their pattern motility to these conditions. As suggested by Brenker et al. (Brenker *et al.* 2012) CatSper could function as a polymodal translator for the chemical and physical code of each microenvironment into Ca^{2+} patterns to reach the site of fertilization.

Once the sperm reach the oocytes, they have to cross the *cumulus* cells surrounding them (Figure 3c). Sperm GPI-anchored surface hyaluronidases and hyperactivated sperm motility are thought to be sufficient for the sperm to get through the *cumulus* (for review see Yin *et al.* 2009). Carrasco et al. (2008a, b) have described some hexosaminidases in oviductal fluid (as it has been indicated in section 1.1.) which could be responsible for *cumulus* cells disaggregation from ovulated oocytes helping the sperm to cross this barrier. In addition, it was previously reported that SPAM1 (with hyaluronidase activity) is secreted by the oviduct and consequently can participate in the *cumulus oophorus* dispersion (Griffiths *et al.* 2008). Preliminary data from our laboratory also described the presence of the SPAM1 in the porcine and bovine oviduct (Acuña *et al.* 2011).

Moreover, in the latest reports, acrosome reaction (AR) has been observed also during the sperm pass through the *cumulus* (Yin *et al.* 2009, Jin *et al.* 2011). Indeed, as Yanagimachi pointed out recently (Yanagimachi 2011), the place where AR begins in mammalian sperm before fertilization has been a controversial topic. On one side of the debate some researchers think that the AR takes place while the sperm advance through the *cumulus*, while on the other side, other scientists think that AR occurs on the surface of the ZP. Combining information from the latest reports, what seems to be clear is that the ZP may not be the only site of AR; therefore *cumulus* cells play an important role in sperm AR. Among the potential chemo-attractants, it is interesting to note that progesterone secreted by *cumulus* cells is known to induce or promote the AR of spermatozoa of various species, but the spermatozoa in the oviduct are acrosome-intact or occasionally reacted (for review see Sun *et al.* 2011). The reason that the sperm in the oviduct are not reacted by progesterone action is probably due to its concentration; the possibility is that sperm stimulation by low levels of progesterone (microM-mM) does t induce AR (Publicover *et al.* 2008) (Figure 5). Gahlay et al. (Gahlay *et al.* 2010) using transgenic mice ($ZP2^{Mut}$, $ZP3^{Mut}$) suggested that sperm binding at the surface of the ZP is not sufficient to induce sperm AR. In fact, Jin et al. (Jin *et al.* 2011) recorded that the spermatozoa begining the AR before reaching the zona

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were able to penetrate it. A new protein called NYD-SP8 has been recently identified in the interaction *cumulus*-sperm triggering Ca^{2+} mobilization and progesterone release from *cumulus* cells inducing the AR (Yin *et al.* 2009). It should be noted that no one has ever followed a single spermatozoon from the beginning of the AR until the end of fertilization, so the exact place where fertilizing spermatozoa begin their AR and what triggers the AR remain to be determinated (Yanagimachi 2011).

All the described steps related to the sperm modifications since they are ejaculated are probably addressed to enable them to bind and penetrate the extracellular matrix of the oocyte, the ZP (Gadella 2010). This is the last barrier before gamete fusion (Figure 3d). Although the true mechanism is still unclear, and it is not the objective of the present review to describe the different models proposed for the sperm-ZP binding in the different species, it has been hypothesized that the sperm penetration through the ZP is dependent either entirely or partly on the mechanical force that provided the hyperactivated sperm (Kim *et al.* 2008). In fact when hyperactivation was blocked in hamster sperm bound to the ZP they were unable to penetrate it (Stauss *et al.* 1995) and, in this sense, hyperactivation induced by the oviduct can be considered one more role of this organ in the fertilization process.

3.2. Does the oviductal fluid have any effect on fertilization itself?

Apart from its roles on the female and male gamete preparation for their meeting, discussed above, the question about the specific function of the oviduct or their secretions on the fertilization process itself, once the spermatozoon has bound to ZP, has not received significant attention in research literature. Until recently, no molecules present in the oviductal fluid, other than OVGP1, already mentioned as a molecule reducing the number of sperm bound to ZP (Coy *et al.* 2008), had been demonstrated to directly affect the fertilization but new information is coming out every day supporting this hypothesis.

First, quantification of activity for five glycosidases in the oviductal fluid, with changes along the estrous cycle, has brought about different proposals, such as the possible role of oviductal hexosaminidase in the sperm-ZP binding, hydrolyzing the β-N-acetyl-glucosamine moieties at ZP (Carrasco *et al.* 2008b). Oviductal β-D-galactosidase could also regulate the sperm binding sites present in the ZP since β-galactosyl residues in the ZP oligosaccharides have shown to be involved in porcine sperm-egg binding (Yonezawa *et al.* 2005). Further studies are necessary to describe the specific function of each glycosidase in the oviduct.

Second, plasminogen, a serum zymogen mainly produced by the liver, has also been quantified in the oviductal fluid and demonstrated to bind oocytes at ZP and oolemma level (Mondéjar *et al.* 2012b). Moreover, the different components of the plasminogen-plasmin system, including activators and inhibitors are present in the oviduct and a model has been proposed by which, upon sperm contact to the oolemma, plasminogen activators are released from the oocyte (Figure 6) and increase the conversion of the plasminogen into plasmin; such plasmin seems to remove spermatozoa attached to the ZP, thus contributing to the regulation of sperm penetration in the oocyte (Coy *et al.* 2012).

Finally, attention must be paid to recent studies about changes in the oviductal secretory proteome and transcriptome induced by the arrival of oocytes or spermatozoa to this organ

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(Fazeli *et al.*, 2004, Georgiou *et al.* 2005). It seems clear that gametes modulate their own microenvironment and it can be anticipated that in the immediate future new molecules of oviductal origin participating in the fertilization process will be identified. Data from the whole oviductal transcriptome in different animal models and at the different phases of the estrous cycle would be very useful to complete the puzzle of the molecular pathways playing a role in the beginning of a new life (Mondéjar *et al.* 2012a).

Concluding remarks

A number of molecules participating in the oviductal signaling affects different steps in the fertilization process, including sperm binding and releasing from the oviductal epithelium, sperm capacitation and hyperactivation, oocyte oviductal maturation and pre-fertilization ZP hardening, sperm-ZP binding and fertilization itself. Although descriptive genomic and proteomic studies have identified a high amount of candidate molecules participating in these processes (Fazeli *et al.*, 2004, Georgiou *et al.* 2005), functional data are now necessary to understand the specific role of each molecule in each pathway. Only with these studies could be reached a significant advance in the comprehension of the fertilization process and, consequently, novel tools to modulate it, could be developed.

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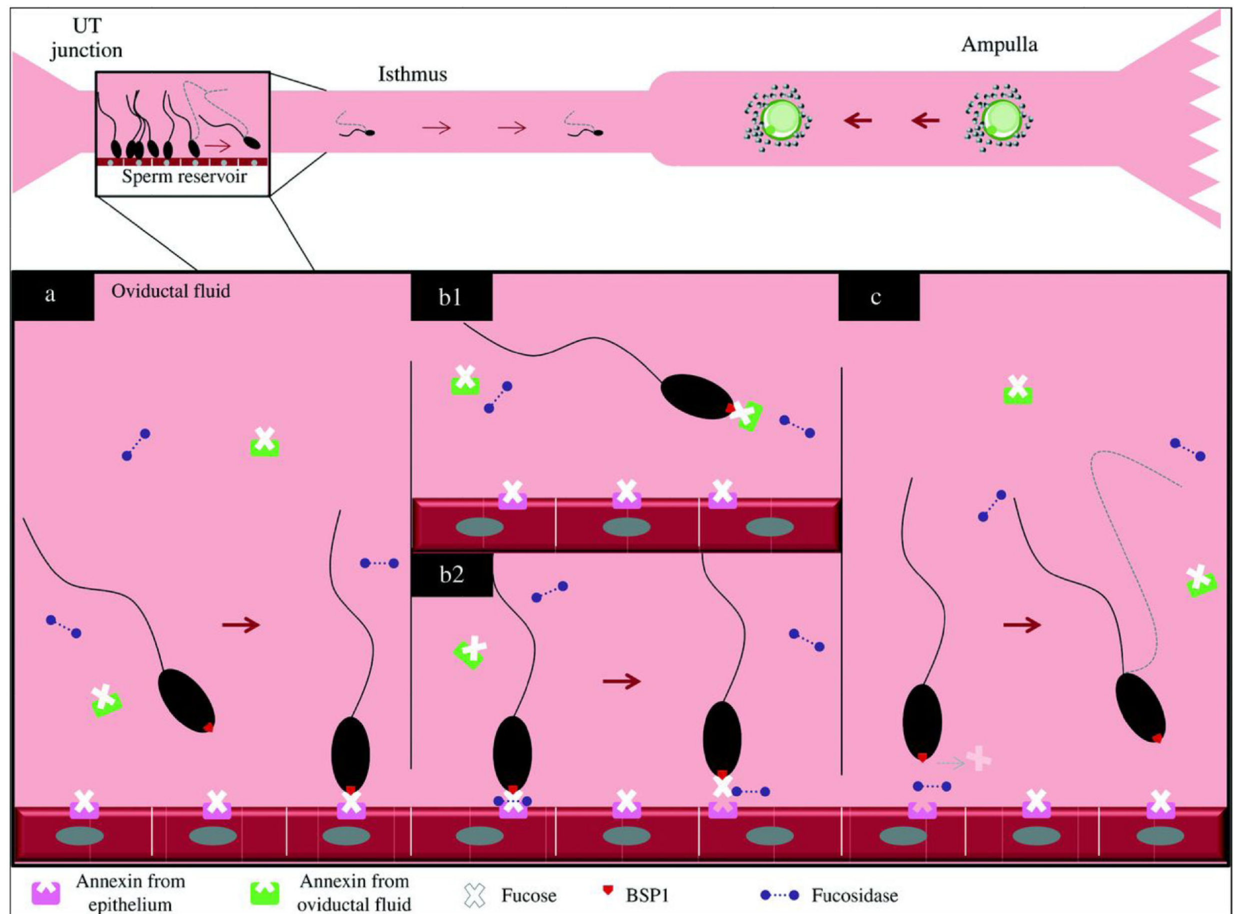

Figure 1.

Mechanism for the sperm binding and releasing from the oviduct in the bovine model. a) Sperm binding is mediated by lectin like protein as BSP1 present in the sperm plasma membrane that recognize fucose contained in the annexin molecule bound to the epithelial cell membrane. b) Sperm binding to the oviduct could be modulated by two different mechanisms that can act at the same time. b1) Annexin present in the oviductal fluid compete for the BSP1 binding site present on the sperm. b2) Fucosidase enzymes present in the oviductal fluid can remove fucose residues contained in the annexin present in the oviductal epithelium. c) These different mechanisms and the development of hyperactivative motility allow the sperm release from the oviductal reservoir.

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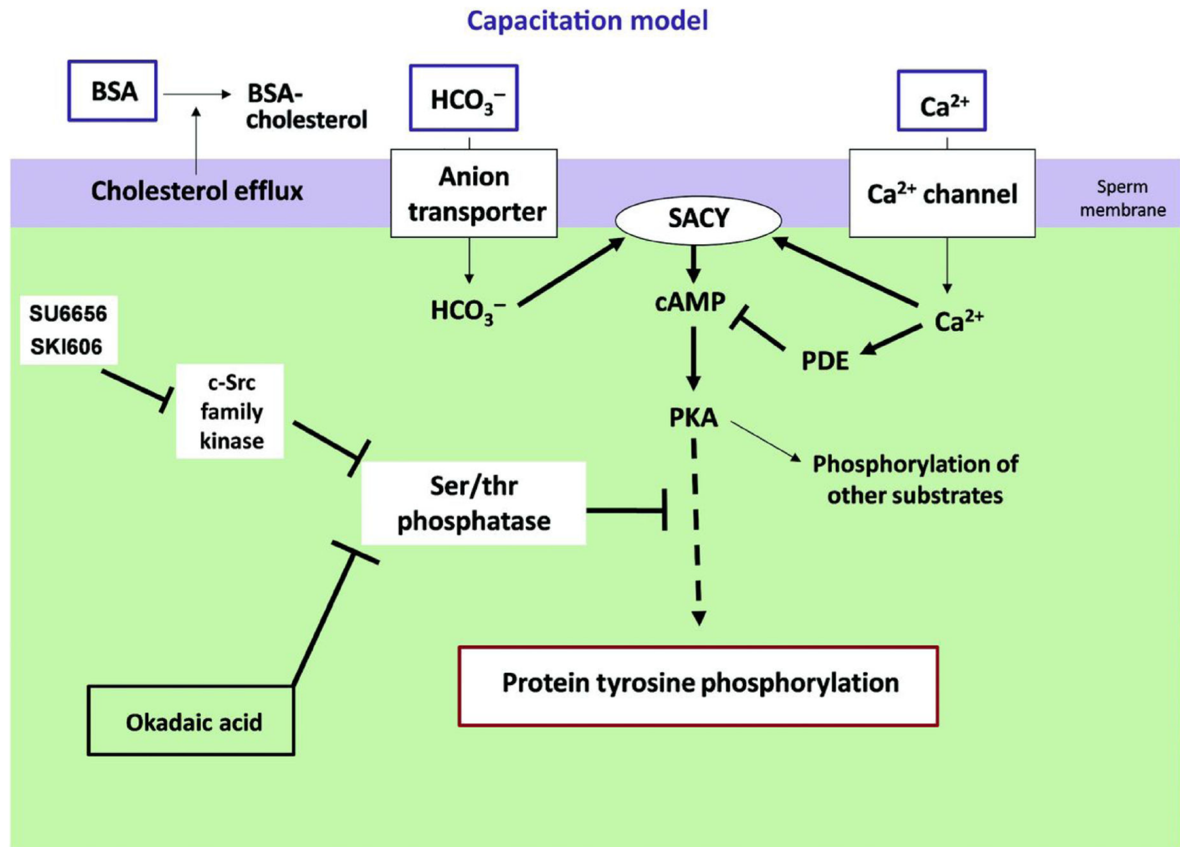

Figure 2.

Model for the regulation of sperm capacitation. Removal of cholesterol by BSA modulates the influx of HCO^{-}_{3} and Ca^{2+}. These ions regulate the activity of the sperm soluble adenyl cyclase (SACY), increasing intracellular cAMP and activating PKA. The activation of cSrc family kinase sensitive to both SU6656 and SKI606 down-regulates a ser/thr phosphatase, which modifies the phosphorylated steady state of PKA substrates. As a consequence, the onset of PKA phosphorylation is followed by the promotion of tyrosine phosphorylation associated with sperm capacitation. Okadaic acid is a known ser/thr phosphatase inhibitor and can induce some of the capacitation-associated processes.

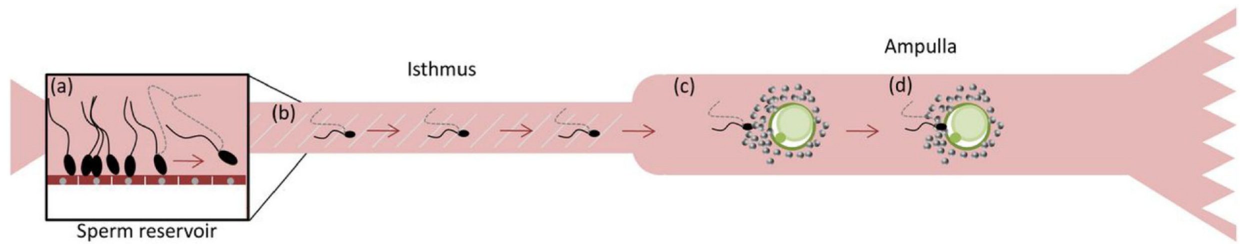

Figure 3.

Roles of the hyperactivation induced by the oviduct on the sperm during the approach to the oocyte: a) Detaching of the sperm from the epithelial cells; b) Transport to the fertilization site; c) Cross through *cumulus* cells and d) Penetration of zona pellucida.

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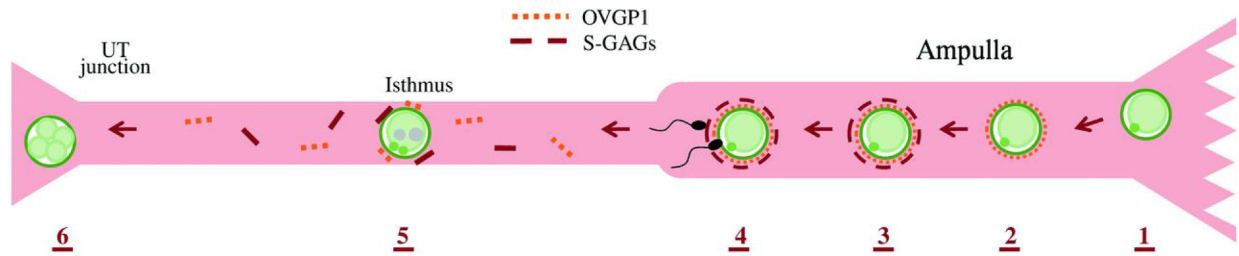

Figure 4.

Pre-fertilization ZP hardening (adapted from Coy & Avilés 2010). (1) When the oocyte is shedding in the ampulla soon after ovulation, oviduct-specific glycoprotein (OVGP1) surround it in a “shell” (2) that is responsible for the ZP resistance to proteolysis. (3) Heparin-like GAGs in the oviduct fluid stabilizes and reinforces the binding of OVGP1 with ZP, which determines the interaction of selected spermatozoa (4) with such a modified ZP. (5) In the transit towards the uterus, the system is destabilized and OVGP1 is partially unbound or internalized. (6) Thus, ZP in the embryo reaching the uterus returns to the low resistance to proteolysis showed by the ovarian oocyte in 1.

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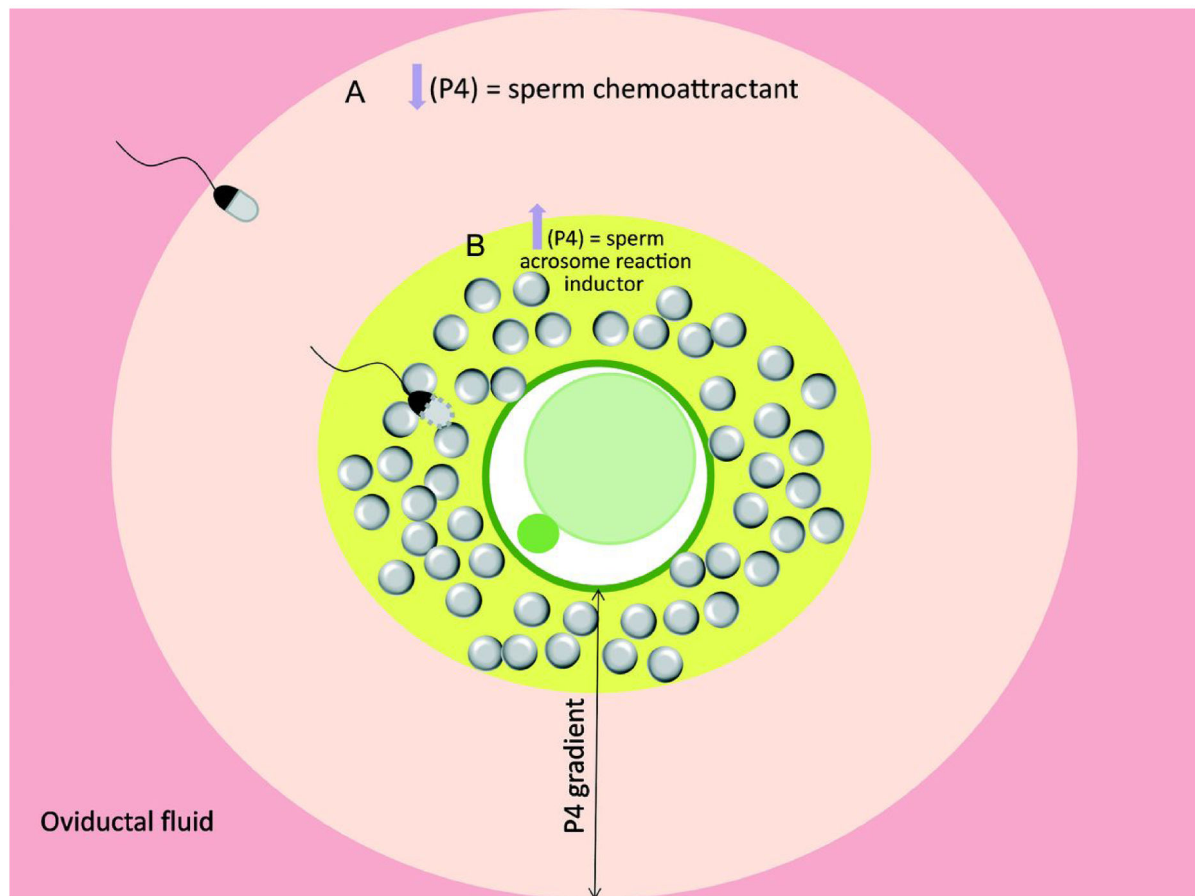

Figure 5.
Progesterone (P4) levels close to the fertilization location and its effect on sperm. A) Low progesterone levels acting like a chemo-attractant driving the sperm towards the oocyte. B) High progesterone levels secreted by *cumulus* cells induce acrosome reaction.

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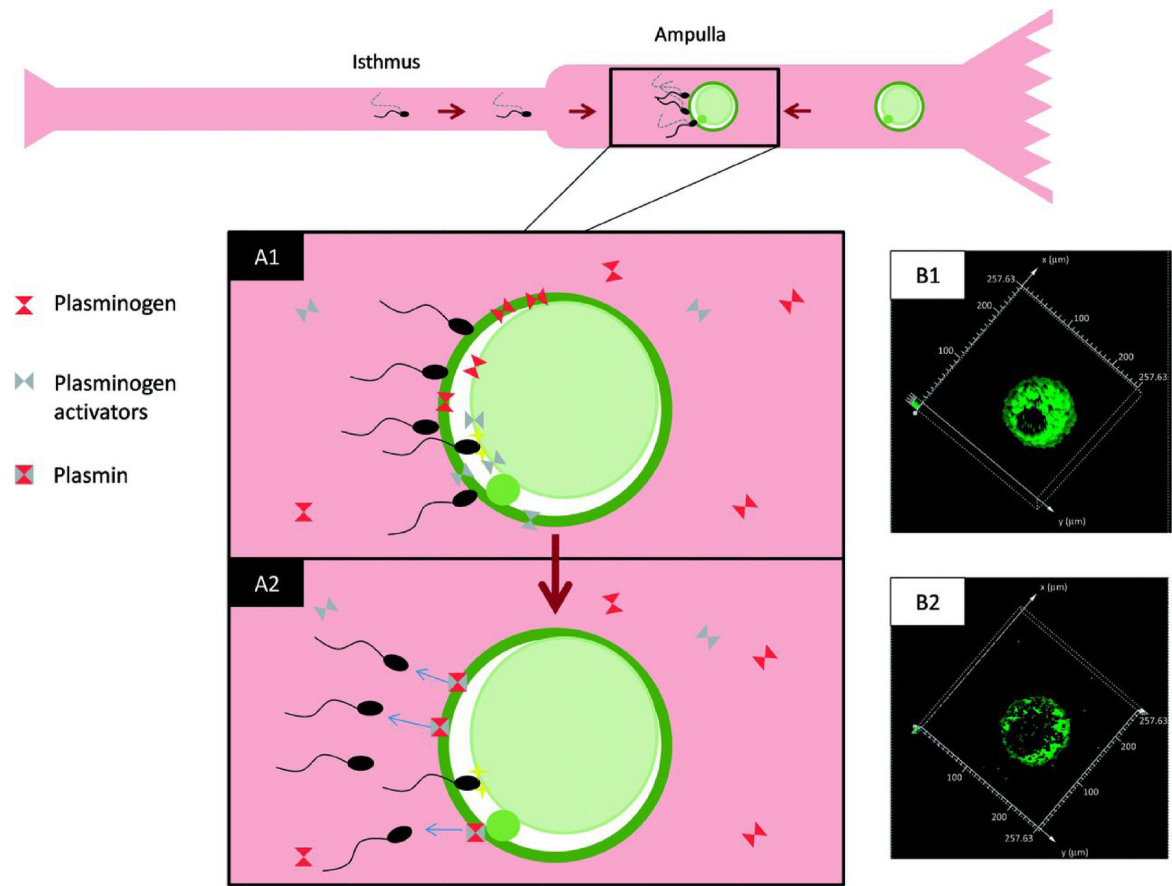

Figure 6.

Proposed model for the role of the plasminogen-plasmin system during fertilization. Plasminogen and plasminogen activators are present in the oolemma and ZP of the oocyte (A1). Oocyte immunostaining with antiboidies against plasminogen activators shows the oolemma strongly labeled (B1). When the spermatozoa bind the oolemma, plasminogen activators are released and increase the activation of the plasminogen into plasmin. Plasmin detaches additional spermatozoa bound to ZP (A2). The labeling in the oolema decreases a few minutes after sperm binding (B2).