

## Review

# Sperm migration, selection, survival, and fertilizing ability in the mammalian oviduct<sup>†</sup>

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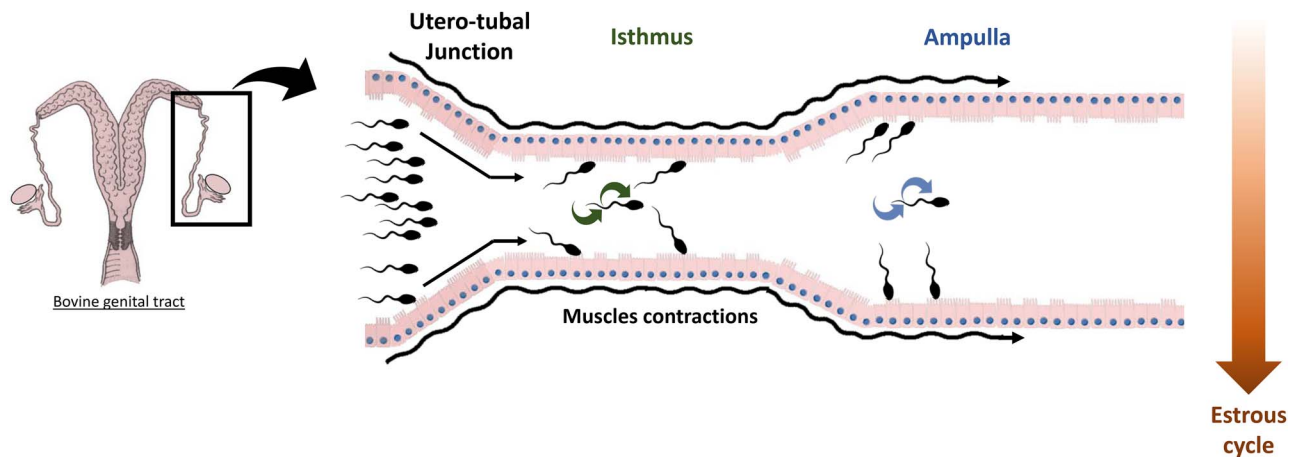
## Abstract

In vitro fertilization (IVF) gives rise to embryos in a number of mammalian species and is currently widely used for assisted reproduction in humans and for genetic purposes in cattle. However, the rate of polyspermy is generally higher in vitro than in vivo and IVF remains ineffective in some domestic species like pigs and horses, highlighting the importance of the female reproductive tract for gamete quality and fertilization. In this review, the way the female environment modulates sperm selective migration, survival, and acquisition of fertilizing ability in the oviduct is being considered under six aspects: (1) the utero-tubal junction that selects a sperm sub-population entering the oviduct; (2) the presence of sperm binding sites on luminal epithelial cells in the oviduct, which prolong sperm viability and plays a role in limiting polyspermic fertilization; (3) the contractions of the oviduct, which promote sperm migration toward the site of fertilization in the ampulla; (4) the regions of the oviduct, which play different roles in regulating sperm physiology and interactions with oviduct epithelial cells; (5) the time of ovulation, and (6) the steroid hormonal environment which regulates sperm release from the luminal epithelial cells and facilitates capacitation in a finely orchestrated manner.

## Summary sentence

Summary Sentence After mating or insemination, the region-specific and hormonally regulated morphology and secretions of the utero-tubal junction and oviduct lead to the selection of a limited sub-population of top quality spermatozoa at the site of fertilization.

## Graphical Abstract



**Key words:** oviduct, spermatozoa, capacitation, sperm migration, fallopian tube, fertilization.

## Introduction

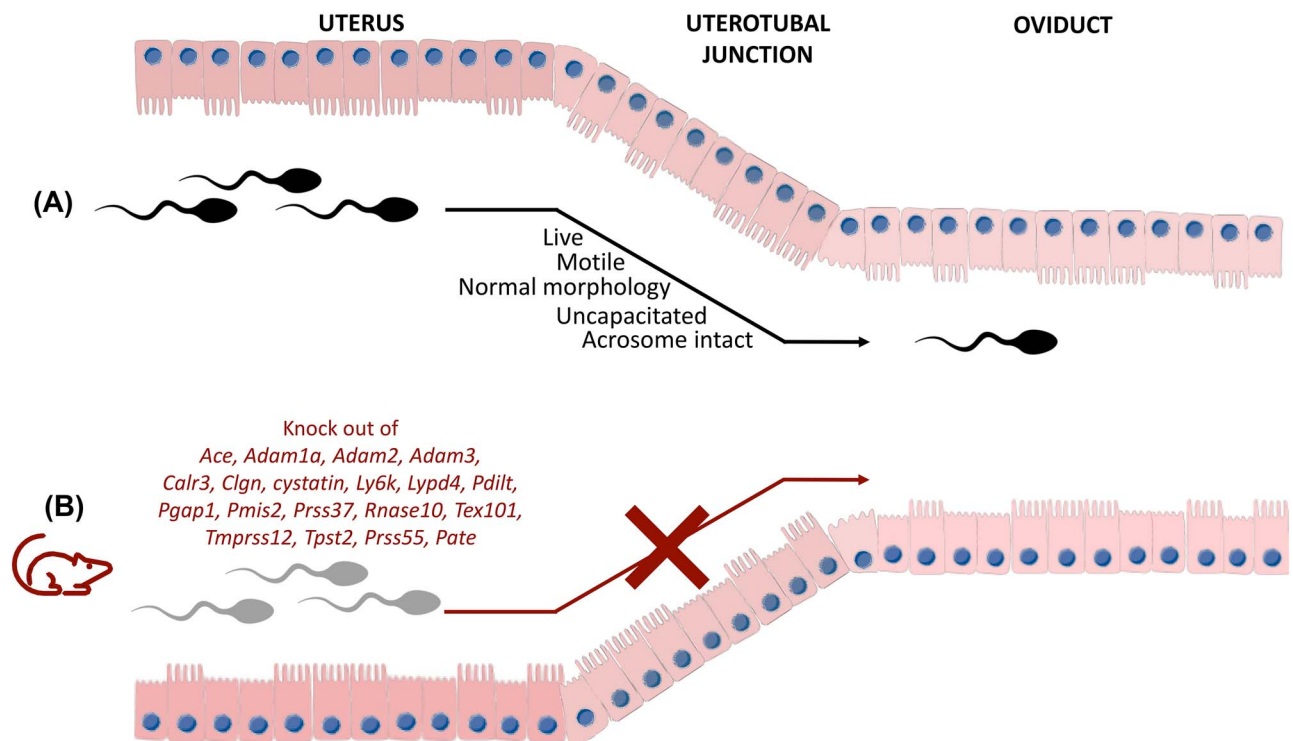
Although sperm capacitation and fertilization can occur *in vitro*, comparisons between data from *in vitro* and *in vivo* demonstrated the important role played by the maternal environment in successful monospermic fertilization. *In vivo*, only 13% of porcine fertilized oocytes are polyspermic [1–3], whereas polyspermic rates after *in vitro* fertilization (IVF) reach up to 75% in pigs [4, 5], 45% in cattle, and 30% in humans [6]. Moreover, more than 30% of *in vitro* produced embryos may present chromosomal abnormalities compared with an average of 15% *in vivo* in cattle [7, 8]. In horses and dogs, IVF remains unsuccessful despite many attempts made to improve both oocyte quality and sperm ability to fertilize [9, 10]. The comprehension of how the oviduct microenvironment affects gamete quality and that of underlying mechanisms are thus useful to improve assisted reproduction technologies and fertility. In mammals, the ability of the female reproductive tract to support sperm survival and storage relies on the binding of spermatozoa to the epithelium of the oviduct [11]. The understanding of the mechanisms supporting sperm storage *in vivo* may help to prolong sperm lifespan and to improve sperm quality outside the female body. This could also help to develop methods to improve sperm storage *in vivo*, in particular after insemination in farm animals. This might reduce the use of hormones to synchronize estrus (and induce ovulation just after insemination) or the time dedicated to detect natural estrus before insemination.

Although oocyte quality and fertilization have been investigated in numerous studies, only few studies focused on the effect of the maternal environment on sperm quality. The use of chemically defined efficient media for sperm capacitation in the IVF process and the success of intra-cytoplasmic sperm injection probably led us to underestimate the role of the female tract in selecting high-quality sperm and promoting sperm viability and fertilizing ability. Indeed, sperm migration in the female genital tract is a highly selective process. From millions of spermatozoa deposited in the vagina or uterus, only hundreds cross the utero-tubal junction (UTJ) and reach the oviduct [12, 13]. For this subpopulation of spermatozoa, major steps remain to be accomplished in the oviduct: migrate toward the oocyte and acquire fertilizing ability in a complex process of

membrane destabilization and changes in motility, named capacitation [14], and to survive for sometimes hours to days up to the time of ovulation. During their journey through the oviduct, spermatozoa undergo many interactions with luminal epithelial cells and secreted components of oviductal cells such as proteins and extracellular vesicles take place. Those interactions represent crucial steps not only to enable fertilization but also to reduce the number of spermatozoa reaching the site of fertilization. In this review, six female modulators of sperm migration, survival and/or capacitation in the oviduct were individually considered: (1) the UTJ, (2) sperm binding sites on epithelial cells, (3) the presence of region-specific (ampulla vs. isthmus) promoters of capacitation, (4) the contractions of the oviduct, (5) the time of ovulation and (6) steroid hormonal environment. For each of those items, sperm migration and interactions with female fluids components and epithelial cells as well as the effects of these interactions on sperm physiology are reviewed. A particular attention is given to farm animals, in view of the large amount of data acquired in those species and the economic significance of reproductive biotechnologies and fertility in the livestock industry.

## The UTJ selects a sperm sub-population to enter the oviduct

After uterine migration, spermatozoa are massively retained in the UTJ, which can be considered as the second major selective barrier beyond the cervix. In cows, the UTJ is characterized by a drastic restriction of the uterine diameter and by the presence of mucosal folds filling the lumen and forming impasses rather than channels to the isthmus, the lower part of the oviduct [15]. In sheep, 4 hours after insemination, increasing numbers of spermatozoa were observed from the base of the uterine horns to the UTJ but a sharp decline was seen in the oviducts [16]. More precisely, 42% of inseminated ram spermatozoa were found at the top of the uterine horn but only 0.5% in the isthmus [17, 18]. In cows, 18 h after mating, up to 20 000 spermatozoa were counted in the UTJ but only 2000 in the isthmus [12]. A large majority of spermatozoa reaching the UTJ are normal. In pigs, 98% of spermatozoa recovered from the UTJ lumen 24 h



**Figure 1.** Sperm selection by the utero-tubal junction (UTJ) in mammals. (A) The UTJ reduces the number of spermatozoa reaching the oviduct [16–18] and selects a sub-population of live boar spermatozoa [20], motile rat spermatozoa [21], acrosome-intact mouse spermatozoa [22], uncapsulated hamster spermatozoa, and morphologically normal mouse spermatozoa [24]. (B) Thanks to KO models, a list of genes required for sperm passage through the UTJ was identified in mice [25–30].

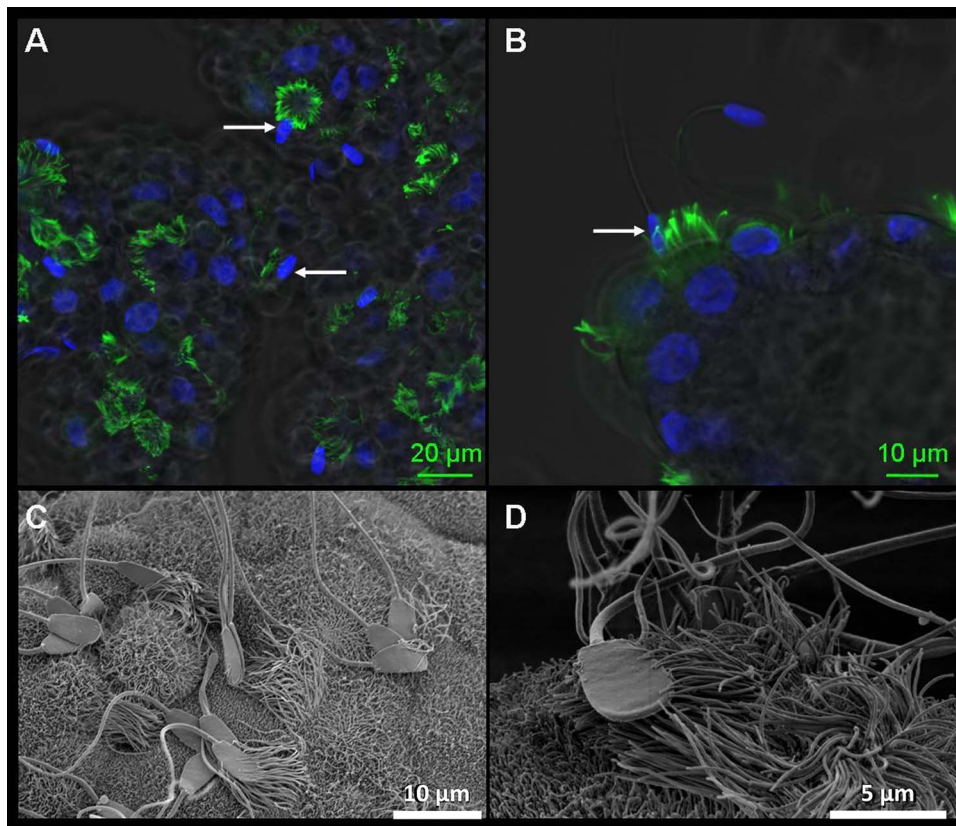
after insemination were found to be morphologically normal compared with 70% in the uterine backflow [19]. The UTJ drastically reduces the number of sperm entering the oviduct and also operates as a selective barrier for sperm. In pigs, rats, mice, and hamsters, only live [20], motile [21], acrosome intact [22], uncapsulated [23], and morphologically normal [24] spermatozoa were found in the lower part of the oviduct. In farm animals, the mechanisms underlying the selective role of the UTJ are still largely unknown. However, the use of knock out (KO) mouse models allowed the identification of more than 15 genes necessary for sperm passage through the UTJ, including one coding for A Disintegrin and Metalloproteinase (*Adam* 3), a glycosylated membrane protein normally present on the surface of spermatozoa, as well as several genes coding for proteins interacting with ADAM3 or required for the maturation of ADAM3 on sperm membrane [25–27] (Figure 1). However, whether ADAM3 interacts with specific proteins on luminal cells in the UTJ and how these interactions may allow sperm passage into the oviduct are still unknown. Furthermore, other genes required for sperm migration into the oviduct in mice, including *Ly6k*, *Pgap1*, and *Lypd4*, do not interfere with ADAM3 maturation on sperm, indicating that there are still unknown mechanisms that operate independently of *Adam3* [28–30].

Proteins on luminal cells in the UTJ and how these interactions may allow sperm passage into the oviduct are still unknown. Furthermore, other genes required for sperm migration into the oviduct in mice, including *Ly6k*, *Pgap1*, and *Lypd4*, do not interfere with ADAM3 maturation on sperm, indicating that there are still unknown mechanisms that operate independently of *Adam3* [28–30].

## Spermatozoa bind to oviduct epithelial cells in the sperm reservoir

### Sperm binding sites in the oviduct

The oviduct epithelium consists of ciliated and non-ciliated cells [31] and it is well established that spermatozoa bind preferentially to ciliated cells [32–34] (Figure 2). Sperm binding to oviduct epithelial cells (OECs) is reportedly mediated by proteins identified by protein affinity using purified sperm proteins and identification by mass spectrometry or immunohistochemistry [35, 36] or competition binding assays [37]. Sperm receptors identified on OECs include annexins A1, A2, A4, and A5 in cattle [35] and annexin A2 in pigs [36]; heat shock protein 5 (HSPA5) (also known as GRP78) in humans [38] and cattle [38], HSPA8 in cattle [39, 40] and pigs [40], HSP60 in cattle [41] and humans [42], and E-cadherin [43] and fibronectin [37] in cattle (Figure 3). Fibronectin was demonstrated to bind specifically to the integrin  $\alpha 5 \beta 1$  on bull sperm membrane [37]. Other proteins that coat sperm surface during maturation in the epididymis or at ejaculation were shown to be involved in the formation of the sperm reservoir: the binder of sperm proteins (BSPs) 1, 3, and 5 in cattle [44, 45], beta-defensin 126 (DEFB126) in macaque [46], and spermadhesin AQN1 [47] and lactadherin [48] in pigs. The use of a glycan array in pigs and cattle revealed that carbohydrates are primary species-specific components allowing sperm binding to the oviduct epithelium: boar sperm bind to the (6-Sialylated) N-acetyl-lactosamine and the Lewis X motifs, whereas bull sperm preferentially bind to the Lewis A motif [49]. Sialylated lactosamine was also abundantly observed on the apical side of OECs in pigs



**Figure 2.** Confocal and scanning electron microscopy images of bull sperm interacting with bovine oviduct epithelial cells. Bovine oviduct epithelial spheroids were co-incubated with frozen–thawed bull sperm *in vitro*. (A) Nuclei of oviduct epithelial cells and spermatozoa (white arrows) are stained with Hoechst (blue, DNA) and cilia are immunolocalized using an anti-acetylated tubulin antibody (green, cilia). (B) Higher magnification showing sperm head (white arrow) interacting with cilia at the cell surface. (C, D) Scanning electron microscopy pictures showing tight interactions between bull sperm heads and epithelial ciliated cells.

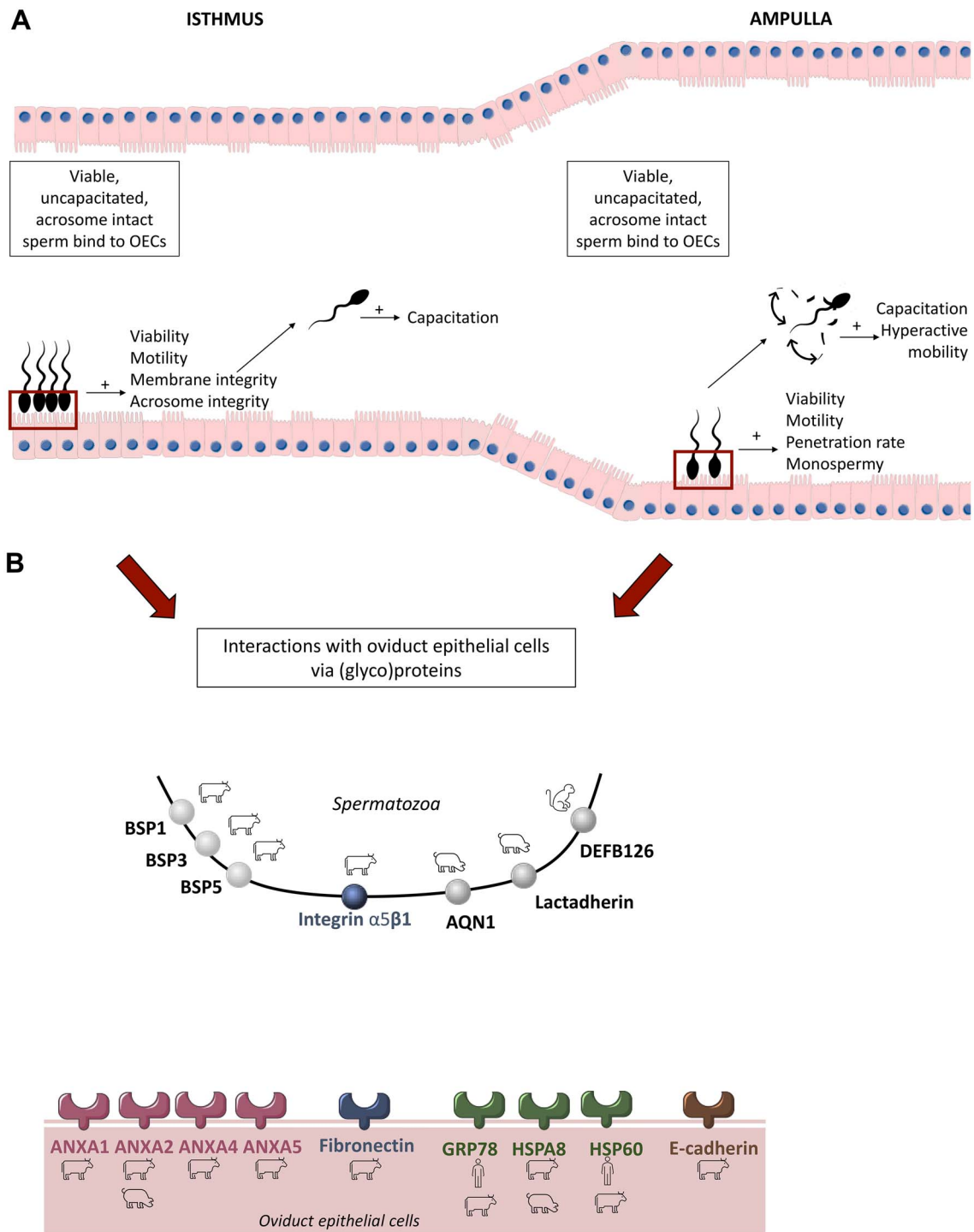
[50]. Those sperm-binding glycans are probably linked to membrane glycoproteins present on OEC cilia; however, their exact link with sperm protein receptors reported above remains to be clarified.

Except for some bat species capable of storing sperm for months during hibernation, sperm storage in the tract of most mammalian females lasts generally no more than 2 days [11]. There is evidence from the observation of oviducts after mating or insemination that the main sites for sperm binding are located in the caudal part of the isthmus, as reported in cattle [12], rabbits [51], pigs [17, 18], and hamsters [52]. However, the surgical infusion of sperm through the ampulla ostium (or infundibulum) of heifers showed that spermatozoa became attached to epithelial cells in both the ampulla and isthmus, suggesting that the sperm reservoir is located in the isthmus because it is the first region encountered by sperm beyond the UTJ [32]. Some *in vitro* data demonstrated a higher number of spermatozoa bound to OECs recovered from the isthmus than from the ampulla [53–55]. However, most studies reported equivalent densities of sperm binding sites on oviductal explants and monolayers from both the ampulla and isthmus in cats [56], cattle [32], pigs [34, 57], and sheep [58]. *In vivo*, frequent detachments and reattachments of spermatozoa were observed in the isthmus but also in the ampulla of inseminated mice during contractions of the oviduct [59]. Therefore, sperm binding to OECs is not restricted to the caudal isthmus and may support important physiological functions in the remaining segments of the oviduct. Whether these successive binding and release from luminal epithelial

cells occur throughout the oviduct in domestic mammals is currently not known.

#### Prerequisite to bind to OECs and impact on sperm physiology

After crossing the UTJ, only a sub-population of spermatozoa of high quality seem able to bind to oviduct luminal epithelial cells. *In vitro* studies showed that only viable [60], uncapacitated [61–63], and acrosome-intact [64, 65] spermatozoa bound to OECs in humans, pigs, horses, and cattle (Figure 3). It has been proposed that this selected subpopulation forms a “functional sperm reservoir” able to provide motile and capacitated spermatozoa at the time of ovulation [66]. Accordingly, binding to OECs *in vitro* maintained sperm viability and motility up to 24 h in pigs [34, 67], cattle [41, 64, 68], and humans [69, 70]. Furthermore, sperm attached to OECs had lower levels of membrane and acrosome disruption and also less chromatin abnormalities compared with unattached sperm in humans [60] cattle [64], pigs [57], and horse [61]. Thus, binding to OECs may be considered as a selective process for sperm. However, little is known about the local female factors regulating sperm binding to OECs. In particular, the proteins involved in sperm binding to the apical side of OECs are also present in the oviductal fluid (OF) and expected to compete with sperm binding sites on the cells. This binding competition may also play a role in sperm release from OECs at the time of ovulation (see section, The time of ovulation regulates sperm interactions with oviductal cells and capacitation).



**Figure 3.** Mechanisms involved in sperm interactions with oviductal epithelial cells (OECs) and functional consequences on sperm physiology. After crossing the utero-tubal junction, a sub-population of sperm [60–65] is able to bind to OECs of the isthmus, forming a sperm reservoir [12, 17, 18, 41, 67], and also of the ampulla close to the fertilization site [32, 34, 56–58]. Binding to OECs maintains sperm viability [34, 41, 64, 67–70], membrane, and acrosome integrity [57, 60, 61, 64] and increases the rates of oocyte penetration and monospermy in vitro [68, 73–75] especially in the ampulla [112]. Subsequent release of spermatozoa from OECs induced sperm capacitation and hyperactive motility [74, 104–106, 109]. The insert below indicates the proteins on sperm head (up dark line) [37, 44–47] and on the apical side of OECs (down pink rectangle) [35–43] identified as playing a role in sperm binding to OECs.

**Impact of sperm binding to OECs on fertilization**

The surgical removal of the isthmus promoted polyspermy in pigs and rabbits [1, 2], leading to the hypothesis that the binding of

spermatozoa to the isthmus followed by their progressive release around the time of ovulation provide a small population of spermatozoa around the oocyte(s) in a way that precludes polyspermy

[71, 72]. Accordingly, the pre-incubation of spermatozoa with OECs reduced polyspermy and increased IVF rates in pigs compared with no OECs [73]. Similarly in cattle, the pre-incubation of bull sperm with OECs maintained sperm fertilizing capacity for 30 h [68] and when co-incubated with *in vitro* matured oocytes, increased cleavage and blastocysts rates compared with the control group with no pre-incubation with OECs [74]. Recently, the use of an oviduct-on-a-chip system was also shown to improve monospermic fertilization in cattle [75] (Figure 3).

### The contractions of the oviduct promote sperm progression toward the ampulla

Spermatozoa in the oviduct are submitted to contractions assumed to help their progression toward the ampulla. The injection of ink directly in the isthmus of mice highlighted an upward fluid flow created by isthmic contractions toward the ampulla [76]. After insemination, the chemical inhibition of oviductal contractions reduced the number of spermatozoa able to migrate through the isthmus in mice [77] and rabbits [78] and decreased the fertilization rate by 27% compared with untreated controls in mice [76, 77]. The number of spermatozoa in the ampulla was slightly reduced by the inhibition of contractions but to a lesser extent than in the isthmus [77], supporting the idea that oviductal contractions are required for sperm to migrate through the isthmus but that other factors like sperm motility may be more important for migration through the ampulla. In addition, live-cell imaging of mouse oviducts *ex vivo* revealed that sperm detach from both the isthmus and ampulla during strong contractions of the oviduct, suggesting that contractions of the oviduct may help sperm to successively bind and then be released from luminal epithelial cells in their progression toward the fertilization site [59].

Few studies examined the endocrine and local regulation of oviductal contractions. Oxytocin and prostaglandins are hormones with well-described roles in uterine contractions at the time of parturition [79, 80]. Their roles in sperm migration in the female genital tract have been little explored. The administration of oxytocin to women during the follicular phase of cycle was reported to amplify the uterine contractions and increase the transport of labeled particles toward the ipsilateral fallopian tube [81–83]. Nanomolar concentrations of prostaglandins E2 and F2 $\alpha$  were detected in the oviduct of cows around the time of ovulation [84, 85] but further investigations are needed to understand their role in oviductal contractions and sperm transport.

### Regions of the oviduct play different roles in regulating sperm physiology and interactions with OECs

#### Region-specific promoters of capacitation in the OF

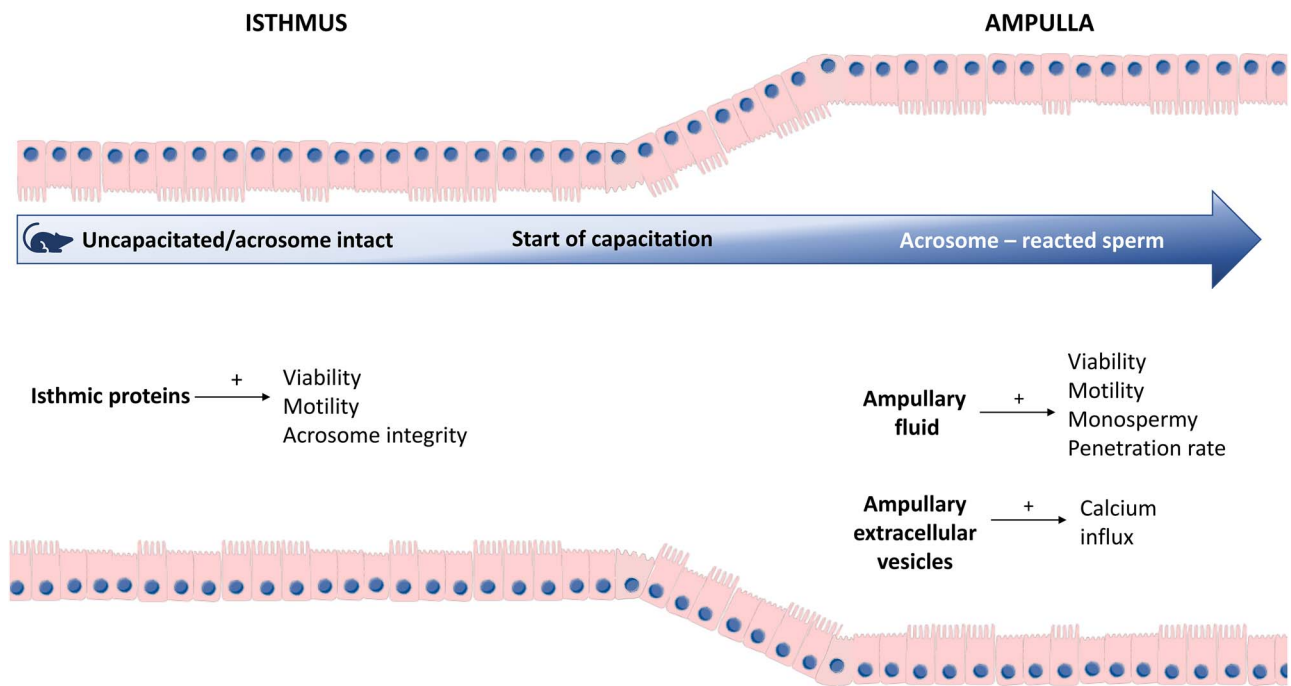
Capacitation involves several mechanisms including membrane the efflux of membrane cholesterol, changes in intracellular concentrations of Ca<sup>2+</sup> and Zn<sup>2+</sup>, an increase in intracellular pH, and protein tyrosine phosphorylation. These changes prepare spermatozoa to undergo the acrosome reaction (AR) and fertilize an egg [14]. Another characteristic of capacitation is the change in the flagellar movements toward higher amplitude and asymmetrical beating, called hyperactivated motility [14]. There is evidence that sperm encounter region-specific changes in their capacitation status when progressing through the oviduct but with species-specific patterns (Figure 4). As observed by *in vivo* imaging in mice, most

sperm undergo capacitation and the AR during their passage from the isthmus to the ampulla [86, 87]. This observation led to reconsider the former widely accepted concept that the zona pellucida of the oocyte causes the AR *in vivo*. Although the thinness and transparency of the mouse oviduct allow live-cell imaging of spermatozoa (with fluorescent acrosomes) within the oviductal lumen, most studies in domestic mammals used *in vitro* incubation of spermatozoa with OF (collected by oviduct cannulation), oviductal flushings (oEVs) or epithelial cells collected post-mortem, thus far from *in vivo* conditions. Ejaculated bull sperm incubated with ampullary OF fertilized more oocytes [88] and promoted a higher rate of monospermic fertilization [89] than sperm treated with isthmic OF. In another study in cattle, more oocytes were fertilized when sperm were pre-incubated in isthmic OF and oocytes in ampullary OF than if both gametes were incubated in ampullary OF [90], supporting the idea that sperm passage through the isthmus may be beneficial for *in vivo* fertilization. However, in rabbits [91] and sheep [92], when sperm were directly injected into the ampulla via surgical or intra-peritoneal insemination, fertilization still occurred (up to 100% of oocytes were fertilized) questioning the exact role of the isthmus in selecting spermatozoa for fertilization.

The OF is a complex mixture of molecules [93] among which proteins appear to play important modulating roles on sperm functions. There is evidence that *in vitro* OF-extracted proteins promote the viability and delay the AR of bull sperm *in vitro* [94]. Moreover, some proteins highly abundant or specifically present in the OF including oviductin (OVGP1), annexin A1, HSPAS, and myosin 9 interact with the sperm surface, at least *in vitro* [38, 95–97], but the functional roles of these interactions need to be clarified. To date, few studies examined the region-specific effect of the OF proteins on sperm. In buffalos, proteins extracted from the isthmic flushings maintained higher post-thaw sperm viability, motility, and acrosomal integrity than proteins from the ampullary flushings, suggesting a protective effect of isthmic flushings on cryopreserved sperm [98]. Sperm interactions with specific proteins in the ampulla and isthmus might explain region-specific effects of the OF on sperm. Bull spermatozoa incubated with biotinylated OF and subsequent western blot of sperm membrane proteins revealed no difference between ampullary and isthmic protein signals that associate with bull spermatozoa [99]. It is worth noting that several sperm-interacting proteins identified in the OF have also been identified in extracellular vesicles isolated from oEVs, also called oviductosomes [97, 100]. These oEVs have been recently reported to modulate sperm physiology via interactions with sperm membrane in cats [101], cattle [102], and pigs [103]. Furthermore, oEVs isolated from isthmic and ampullary flushings were both able to interact with bull sperm membrane [102]. In particular, oEVs from the ampulla induced a higher Ca<sup>2+</sup> influx in sperm compared with oEVs from the isthmus [102], suggesting that oEVs may be involved in the regional effect of the oviduct on sperm capacitation. However, the concentrations of oEVs used in these experiments may not reflect the *in vivo* situation.

#### Region-specific modulation of sperm interactions with oviductal cells

There is evidence from *in vitro* studies in cattle that binding followed by progesterone- or heparin-induced release from OECs triggers rapid sperm membrane remodeling, hyperactivated motility, increase in intracellular Ca<sup>2+</sup> and tyrosine phosphorylation, all associated with capacitation [74, 104–106]. However, it is still not known whether the binding to isthmic and/or ampullary cells is a



**Figure 4.** Region-specific effects of the oviductal fluid, proteins, and extracellular vesicles on sperm capacitation and fertilizing ability. In vivo observation in mice closely analyzes the functions of the lower and upper isthmus on sperm capacitation [86, 87], whereas in cattle, the effect of proteins purified from the isthmis OF were shown to have beneficial effect on sperm viability [98]. The ampulla stimulates capacitation [102], monospermy [89], and fertilization rate [88].

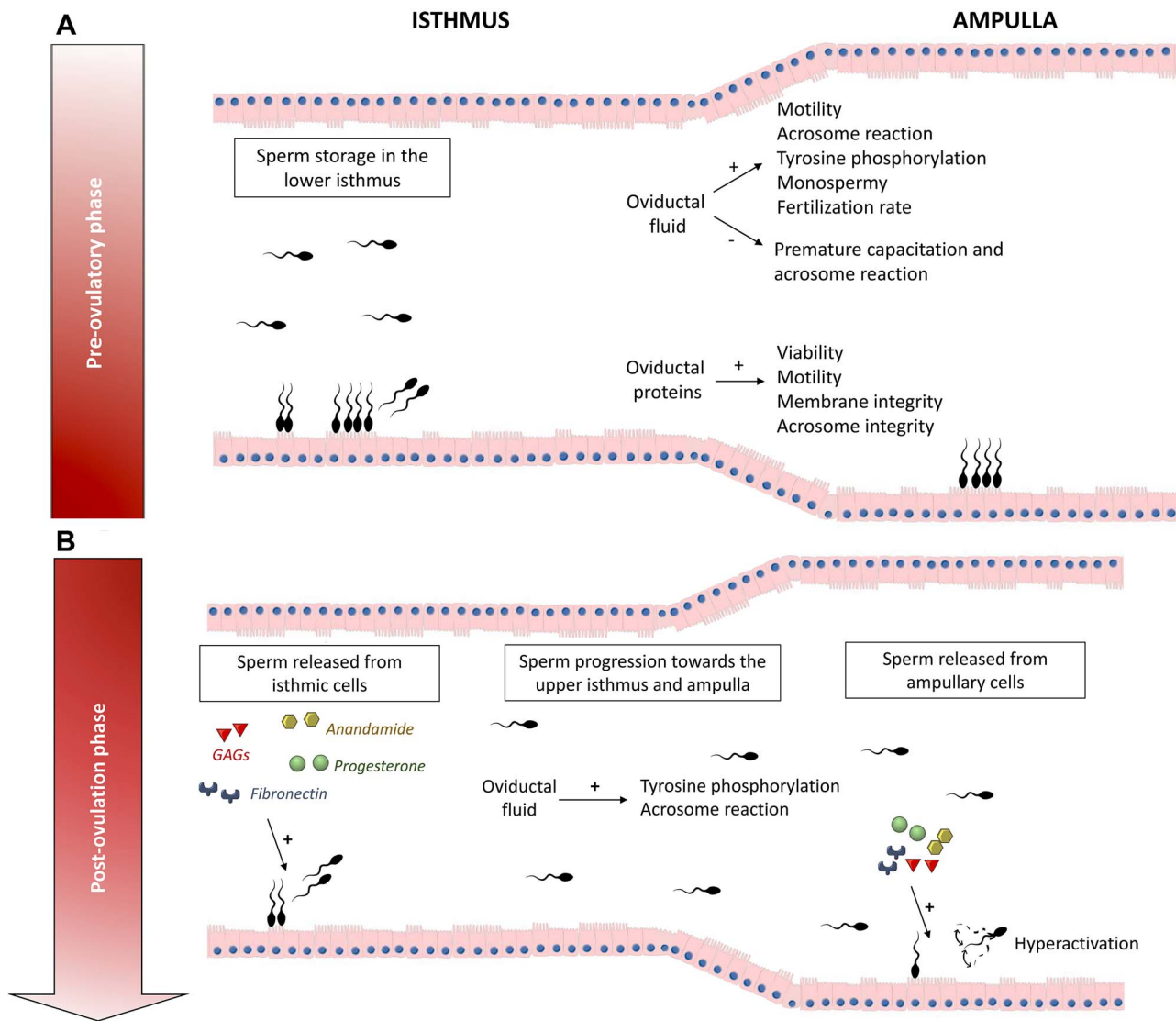
prerequisite for in vivo fertilization. Furthermore, the mechanisms of sperm detachment may differ depending on the oviductal region and proximity to the oocyte. In cattle, heparin-like GAGs are present in high abundance in the OF before ovulation [107] and heparin-binding sites are present on the BSP proteins covering the spermatozoa at ejaculation [108]. The addition of heparin to a bovine sperm-OECs co-culture was sufficient to release all sperm from isthmis but not ampullary cells; in the ampulla, an additional induction of sperm hyperactivated motility was necessary to release bull spermatozoa [109]. This suggests that sperm binding may be stronger in the ampulla than in the isthmus. In addition, the proportion of bull sperm exhibiting hyperactivated motility in vitro was higher in the presence of oviduct explants from the ampulla than from the isthmus [110]. Moreover, for the optimization of cryopreservation media in cattle, the addition of proteins from OECs apical membrane was shown to better maintain post-thaw sperm membrane integrity when using isthmis rather than ampullary cells [111]. In sheep, the penetration of oocytes and monospermic fertilization were promoted by the addition of ampullary but not isthmis OECs to the fertilization medium [112]. Taken together, these data indicate that at least in ruminants, the micro-environment of the ampulla may be more capacitating, whereas that of the isthmus may be more conducive to sperm survival.

### The time of ovulation regulates sperm interactions with oviductal cells and capacitation

#### Sperm interactions with oviductal epithelial cells related to ovulation time

The role of the time of ovulation on sperm binding to OECs has also been studied in vitro but with a lack of consistency among studies and species. In cattle, pigs, and horses, sperm binding to

oviduct explants was not affected by the time relative to ovulation (pre-ovulatory vs. post-ovulatory) at which the tissues were collected [32, 55, 57]. However, in one study in cattle, a higher number of sperm bound to pre-ovulatory compared with post-ovulatory oviduct explants [33]. Moreover, sperm were found to bind preferentially to oviduct explants from the follicular (or pre-ovulatory) phase compared with the luteal phase of cycle in horses [55]. Unexpectedly in sheep, more sperm bound to OECs from the luteal phase rather than those from the follicular phase of cycle after only 1 hour of co-incubation, whereas after 24 h, an opposite pattern was observed [58]. To our knowledge, only one study in pigs evaluated the capacity of sperm to bind in vivo to the epithelium of UTJ and oviduct out of the periovulatory period [113]. Surprisingly, sperm were found more numerous in the UTJ and oviduct when the intrauterine insemination was performed at mid-luteal than at pre- or post-ovulatory phases of the cycle [113]. However, a higher rate of sperm presented abnormalities or was damaged at the luteal phase, suggesting that this stage did not offer a suitable environment for the maintenance of sperm viability in the oviduct [113]. The oviducts being inaccessible in vivo by current techniques of imaging, the timing of sperm release from the isthmis sperm reservoir related to the time of ovulation was studied after the synchronization or monitoring of ovulation and subsequent collection of oviducts by surgery or post-mortem. A redistribution of spermatozoa from the isthmus toward the ampulla around ovulation time was observed in several mammals including the macaque [114], mouse [59], rabbit [51], and hamster [115]. In pigs, the proportion of sperm in the caudal isthmus was more important at the pre-ovulatory phase, whereas around the time of ovulation, an increasing number of spermatozoa were found in the upper isthmus [13]. In cattle, several compounds increasing in concentrations around the time of ovulation in the OF, including the steroid hormone progesterone, [74], heparin-like sulfated GAGs [109], fibronectin [37], and anandamide [116] have been shown



**Figure 5.** Summarized effects of ovulation time on sperm storage, interactions with luminal epithelial cells, and capacitation in the oviduct. The pre-ovulatory OF [4, 5, 33, 93, 113–115] and its proteins [123] display mostly beneficial effects on sperm physiology and fertilizing ability. Around the time of ovulation, spermatozoa are progressively released from OECs [51, 59, 114, 115] via the action of chemical compounds, including progesterone [37, 74, 109, 116] rising in concentrations in the OF at ovulation time and thanks to sperm hyperactivated motility [109]. The post-ovulatory OF usually promotes capacitation and acrosome reaction [4, 5, 33, 93, 113–115].

to induce sperm release from OECs *in vitro* and were proposed as sperm releasing factors from the sperm reservoir acting alone or in combination (Figure 5).

### Sperm capacitation related to ovulation time

The co-incubation of sperm with OF collected at different times in the cycle has been used to study the regulatory effect of ovulation on sperm physiology. In cattle, the motility, viability [117], tyrosine phosphorylation, and AR [118] of cryopreserved bull sperm were promoted by OF from the follicular/pre-ovulatory phase compared with OF from the luteal phase of the cycle. However, in the same species, another study pointed out that the peri-ovulatory (non-luteal) OF reduced overall sperm motility compared with the luteal OF *in vitro* [88]. In addition, samples of peri-ovulatory and luteal oviduct fluid samples induced similar rates of ARs in fresh bull sperm

[88]. It is likely that these discrepancies are due to differences in pre-capacitation status between cryopreserved and fresh sperm as well as the various sources of OF (cannulation vs. post-mortem collection) used for co-incubation.

In pigs, fresh sperm incubated for 3 h with peri-ovulatory OF (combination of late follicular and early luteal phases) displayed reduced tyrosine phosphorylation levels and protein kinase A activity during capacitation compared with OF from the late luteal phase of cycle [5]. In addition, the peri-ovulatory OF displayed an inhibitory effect on sperm ARs which was not observed with the luteal OF [5]. Differential effects of the OF on sperm physiology were also detected between closer stages around ovulation time (pre vs. post-ovulatory) [119, 120]. A 5-min incubation of frozen-thawed boar sperm with OF collected just before ovulation was sufficient to increase significantly the proportion of motile sperm, whereas the post-ovulatory OF did not affect sperm motility [119]. In cattle,



after 2 h of co-incubation, the proportion of viable sperm with destabilized membrane or acrosome reacted were higher with OF from late follicular compared with OF from early luteal stages [120]. Furthermore, after only 15 to 30 min of incubation, the percentage of tyrosine-phosphorylated sperm was higher in OF collected before rather than after ovulation, and this difference was still visible after 1 h of incubation [119]. These data suggest that at least in pigs and cattle, acute changes in the oviductal environment occur around the time of ovulation in the oviductal environment that may rapidly change sperm motility and process of capacitation.

The OF was also reported to differentially promote sperm fertilizing ability according to the time relative to ovulation. In cattle, the percentage of fertilized oocytes was greater after 5 h of co-incubation with non-luteal rather than luteal ampullary OF [88]. In pigs, polyspermy is a frequent issue during IVF. In this species, the addition of OF from follicular or early luteal, but not from the late luteal phase, to the IVF medium promoted monospermic fertilization [5]. A favorable effect of the pre-ovulatory OF on the oocyte contributes to this result as the pre-incubation of pig oocytes in peri-ovulatory OF was reported to increase the hardening of the zona pellucida, leading to a reduction of polyspermy and improvement of IVF outcomes in pigs [4, 121].

The horse is another species for which an efficient IVF protocol still needs to be developed. In this species, the pre-incubation of sperm with porcine pre-ovulatory OF succeeded in obtaining oocyte penetration rates between 4 and 12% compared with no penetration at all with post-ovulatory OF [122], showing stage-specific effects on sperm capacitation under heterospecific conditions.

Proteins in the OF may play important roles in the regulation of sperm functions depending on the stage in the estrous cycle. In this regard, proteins extracted from buffalo OF at estrus (pre-ovulatory phase of cycle) maintained higher post-thaw sperm viability, motility, acrosome, and membrane integrity compared with proteins collected at the luteal phase of cycle [123]. Using mass spectrometry, we recently identified 27 sperm-interacting proteins following incubation of bull sperm with OF collected either before or after ovulation, and during the luteal phase of cycle [97]. Among those proteins, eight interacted with sperm only at the post-ovulatory and luteal but not at the pre-ovulatory phases of cycle. Furthermore, 14 proteins were shared between stages but with variable abundance on sperm: this protein abundance was on average twice higher during the luteal phase than at post-ovulatory phase of cycle, and again twice higher at the post-ovulatory than at the pre-ovulatory phase of cycle [97]. On the other hand, only one protein, the oviduct-specific glycoprotein 1 or OVGPI, was more abundant on sperm at the pre-ovulatory than at other phases of cycle. This suggests that ovulation triggers changes in sperm-OF protein interactions that may modulate the time-specific release of sperm from the oviductal reservoir as well as the initiation of sperm capacitation.

### **Steroid hormones locally regulate sperm migration, capacitation, and release from the oviduct reservoir**

#### **Sperm exposure and receptivity to steroid hormones in the oviduct**

In most mammals, the circulating concentrations of estrogens, in particular of estradiol (E2), are elevated during the pre-ovulatory period and drop after ovulation, whereas the levels of progesterone (P4) rise locally in the pre-ovulatory follicle after the Luteinizing Hormone

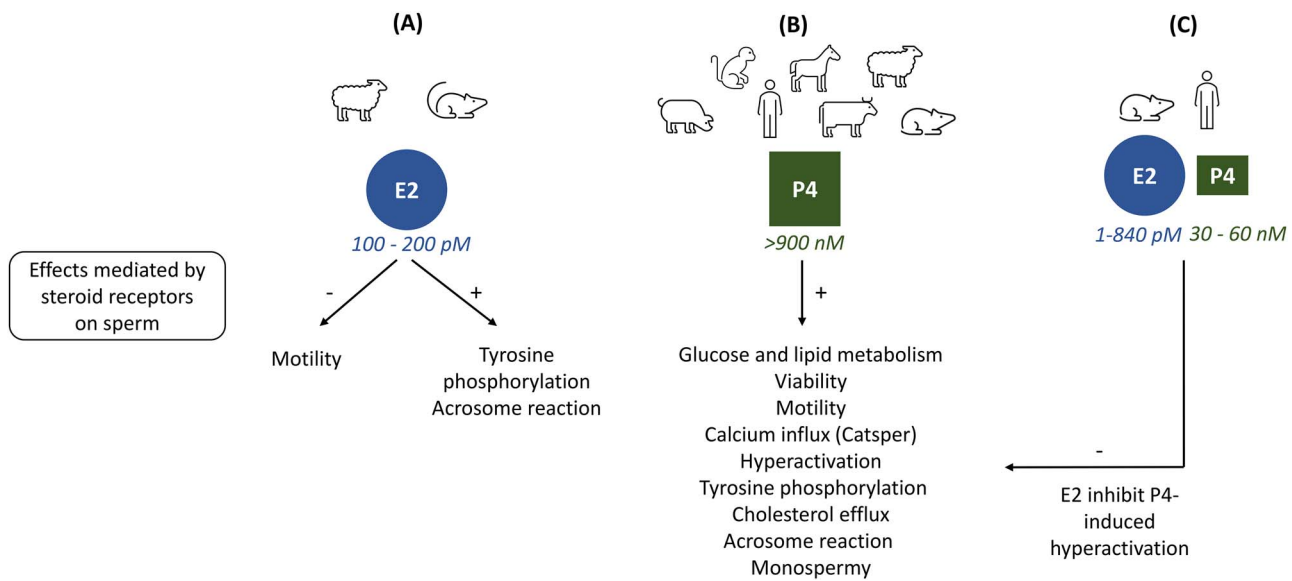
(LH) peak and become detectable in the circulating blood a few days after ovulation [124]. Ovarian steroid hormones diffuse locally and may be transported by vascular transfer to various parts of the genital tract [125]. In vivo, both P4 and E2 are present in the OF around the time of ovulation but at species-specific concentrations. Nanomolar concentrations of P4 were found in the OF ipsilateral to ovulation in mares [126] and cows [127] and in both oviducts of sows [4] around the time of ovulation. Levels of E2 in the OF were highest at the pre-ovulatory stage and measured at picomolar concentrations in cows [127] and sows [4], and at nanomolar concentrations in mares [126]. In monovular species such as cattle and horses, steroid hormones were also found at much higher levels in the side of ovulation than in the contralateral side [126, 127], showing that there is an important diffusion of steroid hormones from the ovary to the proximal oviduct. Therefore, although little is known about the biological activity of steroid hormones in the OF, spermatozoa may be exposed to relatively high concentrations of P4 and E2 during their migration in the oviduct. Atypical P4 receptors were detected in acrosomal and equatorial regions of sperm head as well as in the midpiece of sperm in pigs [128] and sheep [129], suggesting a non-genomic effect of this hormone on spermatozoa. Moreover, estrogen receptors were identified on sperm acrosomal region, midpiece, and tail in humans [130], pigs [131], and mice [132].

#### **Sperm migration according to the steroid hormonal environment**

Experiments conducted by Hunter *et al.* in pigs [3, 133] provided evidence that P4 plays a role in sperm transport toward the site of fertilization. The surgical microinjection of nanomolar concentrations of exogenous P4 or of P4-rich follicular fluid into the isthmus of inseminated gilts significantly increased the number of sperm around the oocytes and the rate of polyspermy compared with control animals [3, 133]. It was suggested that P4 inhibits oviductal contractions and enlarged the oviductal lumen, leading to less selective sperm migration. More recently, P4 was proposed as a chemoattractant for sperm cells in vivo. The cumulus cells surrounding the oocyte produce and secrete P4 [134, 135]. Co-incubation of rabbit sperm with oocyte-cumulus complexes (COCs) revealed that sperm migrate preferentially toward the cumulus cells [136]. In addition, the suppression of P4 action inhibited sperm chemoattractive response [136]. Similarly, human spermatozoa subjected to a picomolar gradient of P4 orient themselves toward the highest concentration of P4 [137, 138]. Moreover, a higher percentage of capacitated sperm with intact DNA and low levels of oxidative stress were found among human spermatozoa oriented toward the P4 gradient compared with non-attracted sperm [139], suggesting that P4 positive chemotaxis selects a subpopulation of high-quality spermatozoa. The chemotactic response of human sperm toward this hormone was optimal at P4 picomolar concentrations (1–100 pM), but a small population of sperm was also able to chemotactically respond to higher P4 levels up to 1  $\mu$ M [138]. To our knowledge, the concentration of P4 in the human OF has not been reported to date. However, since P4 was measured in the OF of several mammals at nanomolar concentrations [4, 126, 127], the existence of a sperm chemotaxis toward picomolar levels of P4 in vivo remains questionable.

#### **Sperm physiology according to the steroid hormonal environment**

The effects of physiological (i.e. intra-oviductal) concentrations of P4 and E2 on spermatozoa were studied in various mammals (Figure 6).



**Figure 6.** Effects of intra-oviductal concentrations of 17 $\beta$ -estradiol (E2) and progesterone (P4) on sperm physiology in vitro. (A) Treatment with picomolar concentrations of E2 inhibits sperm motility in sheep [129], promotes capacitation in mice [132], and acrosome reaction in sheep [129]. (B) Nanomolar concentrations of P4 found after ovulation have beneficial effect on sperm capacitation in pigs [4, 128, 142, 145], human [140, 143, 144, 148], cattle [105], sheep [129], in horse [129], in macaques [147], and in hamster [141]. (C) A suppressive effect of picomolar levels of E2 on P4-induced hyperactivation was reported in hamster [149, 150] and humans [148].

Exposure of boar sperm to P4 concentrations ranging from 10 to 100 nM was reported to rapidly enhance sperm viability and sperm glucose and lipid metabolism compared with untreated controls [128]. In addition, P4 at 50 nM was found to stimulate within 30 min the progressive motility of human sperm after cryopreservation [140], suggesting a significant role of P4 on sperm motility. Accordingly, sperm hyperactivated motility was shown to be promoted by nanomolar concentrations of P4 in cattle [105] and hamster [141]. The depletion of extracellular calcium in the medium or the inhibition of the sperm-specific calcium channels CatSper (Cation channel of Sperm) suppressed the promoting effect of P4 on sperm hyperactivated motility, indicating that the P4-mediated hyperactive motility relies on calcium influx via CatSper [105, 142]. Furthermore, in several mammals including pigs, sheep, and humans, P4 up to 900 nM in concentrations induced calcium influx [142–144], cholesterol efflux [128], and tyrosine phosphorylation [128, 129, 145], indicating a promoting role of P4 on sperm capacitation. P4 was also shown to promote AR in several mammals including pigs [128, 145], horse [146], sheep [129], macaque [147], and humans [148]. In pigs, the addition of P4 at concentrations found in the pre-ovulatory OF (8 nM) in the fertilization medium was shown to favor monospermic fertilization compared with the control group [4].

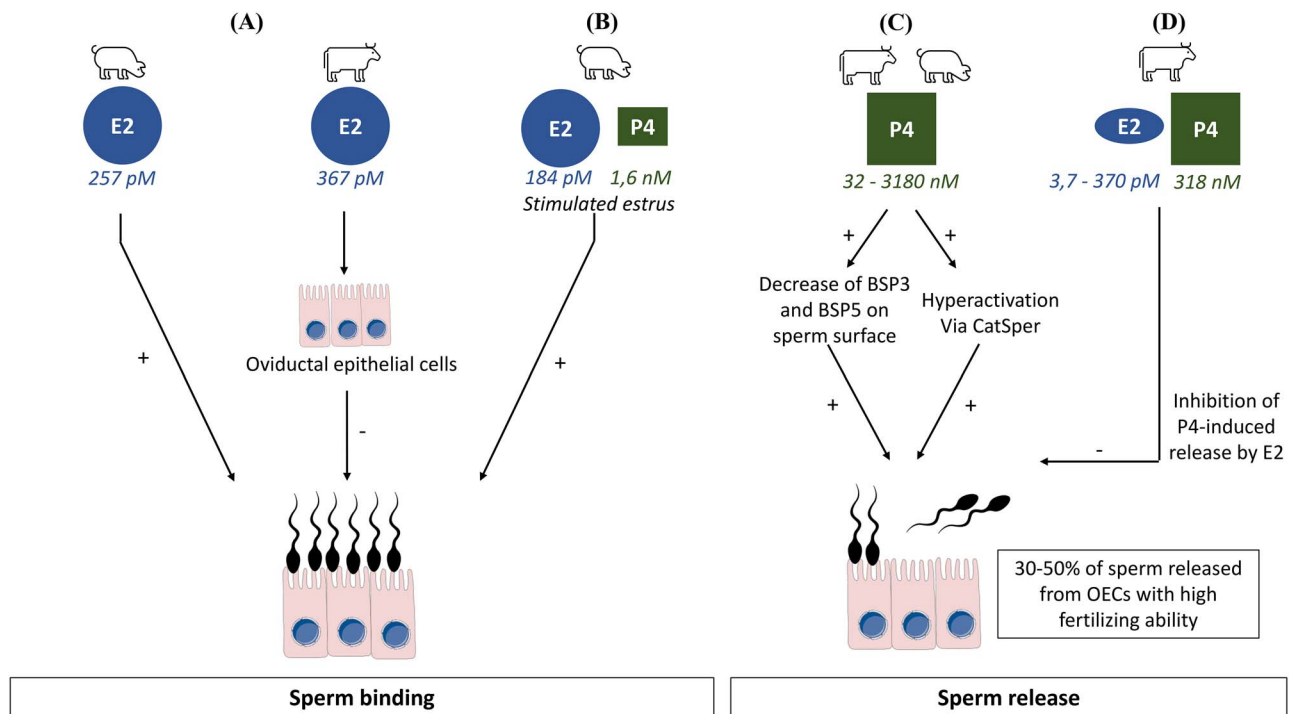
The reported effects of intra-oviductal concentrations of E2 on sperm physiology were less numerous and consistent. Exposure to picomolar concentrations of E2 had no effect on sperm motility in hamster [149] but reduced total sperm motility in sheep [129] compared with controls. At 200 pM, E2 promoted tyrosine phosphorylation on sperm head in mice [132]. Incubation of ram sperm with 100 pM of E2 increased the percentage of acrosome reacted sperm compared with controls [129]. This suggests positive but species-specific effects of E2 on mammalian sperm capacitation. Furthermore, in vitro studies reported a suppressive effect of E2 on P4 action on sperm. For instance, in hamster, the addition of

picomolar concentrations of E2 to an incubation medium containing nanomolar concentrations of P4 inhibited sperm hyperactivation compared with P4 alone [148–150] (Figure 6). As both hormones are present at the fertilization site in vivo, the combined effects of E2 and P4 at physiological concentrations on sperm capacitation require further investigation.

### Sperm interactions with oviductal cells according to the steroid hormonal environment

Domestic mammalian females become receptive to mating during estrus, in parallel with increasing picomolar concentrations of E2 in the oviduct lumen [4, 126, 127]. Accordingly, hormonal treatment of porcine OECs with physiological concentrations of P4 and E2 mimicking the estrous phase of the cycle led to an increase in sperm binding to OECs compared with a simulated luteal phase of the cycle [151]. Some studies examined the specific effects of steroid hormones on sperm-oviduct interactions, with divergent results among species (Figure 7). In pigs, a 48-h pre-exposure of OEC explants to 100 ng/mL (i.e. 367 nM) of E2 did not affect numbers of sperm bound and released from oviductal cells over time [152]. However, another study reported a rapid increase in the number of spermatozoa bound to oviductal explants after the addition of 70 pg/mL (257 pM) of E2 to the incubation medium [34], suggesting that E2 may promote the ability of OECs to bind sperm cells in a short-term manner. On the other hand, in cattle, a pretreatment of OECs monolayers with 100 pg/mL or 100 ng/mL of E2 temporally reduced the numbers of sperm able to bind to OECs compared with untreated controls but this effect lasted no more than 1 h [74] (Figure 7). These data suggest that E2, although highly present in the OF before ovulation, may not have a strong impact on sperm binding to the sperm reservoir.

Once spermatozoa bind to OECs in the isthmus in vivo, a progressive release of sperm has been observed around the time of



**Figure 7.** Regulation of sperm interactions with oviductal epithelial cells (OECs) by physiological concentrations of 17 $\beta$ -estradiol (E2) and progesterone (P4). (A) Treatment with E2 shortly increases in pigs [34] or reduces in cattle [74] the ability of OECs to bind spermatozoa. (B) Pretreatment of OECs with P4 and E2 at concentrations mimicking estrus promotes sperm binding in pigs [151]. (C) Nanomolar concentrations of P4 induce sperm release from OECs in cattle [25, 74, 104, 105] and pigs [142]. (D) E2 has no direct effect on sperm release but inhibits P4-induced release in cattle [74].

ovulation *in vivo* [66, 153]. Therefore, it is assumed that sperm release from isthmic cells occurs in parallel with increasing local concentrations of P4. A specific role of P4 on sperm release from OECs was recently shown in the bovine [74, 105] and porcine [142] species. The addition of nanomolar concentrations of P4 to co-cultures induced the release of 30–50% of bull sperm [74] and around 50% of boar sperm bound to oviductal cells [142]. It was shown that P4 increases sperm intracellular calcium levels and induces hyperactivated motility at the time of sperm detachment from OECs within minutes in cattle [105] and pigs [142]. In addition, P4 was shown to induce an increase in sperm membrane fluidity and significant changes in bull sperm lipidomic and proteomic composition at the time of sperm release from bovine OEC monolayers [104]. Among those proteomic changes, a significant decrease in the abundance of BSPs 3 and 5, two seminal proteins involved in sperm binding to OECs and capacitation, were demonstrated [25, 104]. In this regard, the sperm population collected after the sequential binding to OEC and subsequent P4-induced release displayed higher fertilizing ability than controls in cattle [74]. Therefore, P4 seems to play important roles in the induction of sperm release from OECs at ovulation time, which in turn induces sperm hyperactivated motility and capacitation leading to improved fertilizing ability.

Although E2 showed no direct effect on bull sperm release from bovine OEC monolayers, this hormone was shown to inhibit the P4-mediated release of sperm from OECs in a dose-dependent manner [74]. Thus, it is likely that both P4 and E2 regulate sperm release from the sperm reservoir around the time of ovulation. It is to note that in pigs and cattle, up to 70% of spermatozoa remained bound to

OECs after the action of physiological concentrations of P4 *in vitro* [74, 142], suggesting that only a subpopulation of sperm are P4-responsive and able to be released from the sperm reservoir toward the oocyte.

## Conclusion

After uterine migration, spermatozoa are sequentially exposed to the UTJ, isthmus, and ampulla, each region having specific properties in terms of contractions, ability to bind sperm, and molecular secretions. The mice KO models have provided valuable data on requirements for sperm to enter the oviduct, yet, a more precise anatomical and molecular characterization of the UTJ and of its selective role on sperm progression is still needed in domestic mammals. The behavior of spermatozoa within the oviduct has become clearer in mice thanks to *ex vivo* live-cell imaging but these pictures are rather scarce in domestic species. Therefore, the exact roles played by each part of the oviduct in the selection of a subpopulation of top quality spermatozoa around the oocyte still remain to be elucidated. Sperm are also exposed to time-specific changes during their journey. Recent data demonstrated important changes in the oviductal microenvironment triggered by ovulation, which in turn may regulate sperm lifespan and ability to fertilize. However, it is still difficult to figure what is really going on in the isthmus when spermatozoa enter the oviduct either days before or just after ovulation. Because of the limited access to oviducts in live animals, *in vitro* studies were mostly used to dissect sperm-oviduct interactions. We pointed out multiple discrepancies between studies, which could

be due to the partial loss of the morphological and molecular characteristics of OECs in vitro. More physiological models able to mimic the oviduct microenvironment throughout the estrous cycle are being developed and will be helpful to proceed further on those unsolved questions.

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