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Review

Control of sperm motility and fertility: Diverse factors and common mechanisms

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Abstract. Spermatozoa generated in the testis are immature and incompetent for fertilization. During their journey toward the egg, the sperm acquire fertility and achieving fertilization. These sperm modifications to ensure fertilization are induced by many female or male extra-sperm factors: for example, sperm motility-activating factors from the egg jelly, sperm attractants from the eggs, and decapacitation factors from the seminal plasma. The factors controlling sperm fertility are myriad and species specific; they may be peptides, sugar chains, or small organic compounds. Nevertheless, the fundamental mechanisms underlying fertilization must be common among all animals; increase in $[Ca^{2+}]_i$ triggers all the steps in the process of fertilization, and cAMP plays important roles in many steps. Elucidating the dynamic functional and morphological changes in sperm cells is important for understanding the regulation of fertilization. Here, we introduce the diversity and generality of the control of sperm fertility.

Keywords. Sperm, fertilization, motility, chemotaxis, capacitation, acrosome reaction, female reproductive tract.

Introduction

Sperm cells are generated in the testis after spermatogenesis and spermiogenesis. However, the spermatozoa in the testis are immature and infertile and must undergo many modifications in order to become capable of carrying out fertilization. After spermiogenesis in the testis, mammalian spermatozoa are matured in the epididymis until ejaculation. During this process, the spermatozoa newly acquire cholesterol, proteins, etc., that are secreted from the epididymis. This process, which occurs in the epididymis, is an indispensable first step toward the acquisition of sperm motility and fertility. The epididymis is present only in mammals, but non-mammalian spermatozoa also seem to mature in the spermiduct. Spermatozoa initiate movement after ejaculation and acquire fertility in the female reproductive tract. Finally, the spermatozoa participate in the acrosome reaction on the vitelline coat of the egg and fertilize the egg (Fig. 1). In many invertebrates that show external fertilization, spermatozoa acquire motility after spawning and exhibit chemotactic behavior

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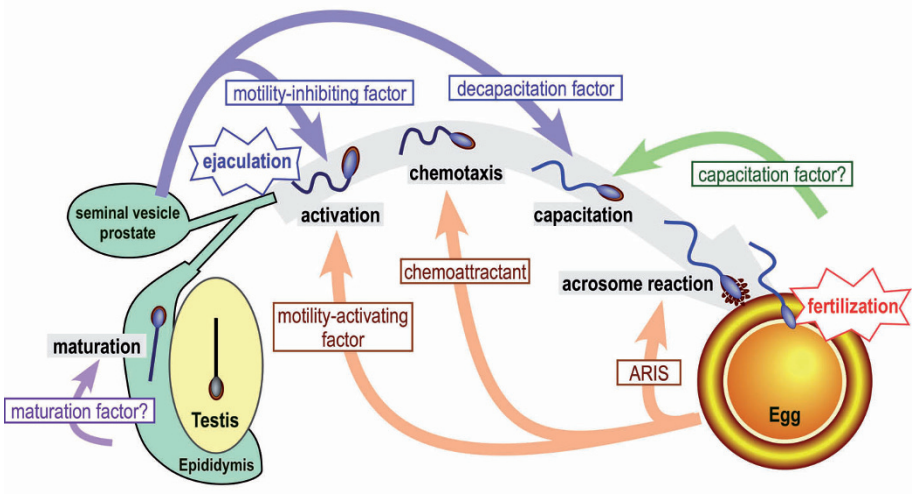

Figure 1. Schematic drawing of sperm journey and events. ARIS: acrosome reaction-inducing substance.

toward the egg (Fig. 1). These sperm modifications required to accomplish fertilization are induced by many female or male extra-sperm factors: sperm motility-activating factors from the egg jelly, sperm attractants from eggs, decapacitation factors from the seminal plasma, etc. (Fig. 1). Thus, the fertility of spermatozoa is controlled by many extracellular factors that operate during their journey from the testis to the egg. Elucidating the dynamic functional and morphological changes that occur in sperm cells is important for understanding the regulation systems of fertilization.

Here, we review the diversity and generality of the control of sperm fertility, with particular focus on the phenomena occurring outside the male body, the initiation and activation of motility, capacitation, and the acrosome reaction. First, we discuss the factors controlling sperm fertility and, next, we focus on the signaling mechanisms in the spermatozoa.

Factors controlling sperm motility and fertility

Initiation and activation of sperm motility

In the male body, spermatozoa are not motile; they acquire motility after ejaculation or spawning. Usually, motility is initiated by changes in ambient conditions, such as osmotic stimulation or ionic concentrations [1]. For example, spermatozoa of some teleosts acquire motility due to a decrease in K^+ and an increase or decrease in the osmolarity of the fluid surrounding the spawned spermatozoa [1]. It is known that the initiation of motility in chick spermatozoa is temperature dependent [2].

In many invertebrates and vertebrates, sperm motility is activated in response to certain factors released from the eggs. The activation of sperm motility by egg-derived factors is the first communication between the spermatozoa and eggs during fertilization. Sperm activation in the vicinity of eggs was first observed in marine invertebrates almost 100 years ago [3], and many attempts to clarify the mechanism of sperm activation have since been undertaken. Sperm-activating factor was first identified in sea urchins. Hansbrough and Garbers isolated and purified a 10-amino acid-long sperm-activating peptide (SAP) from the egg jelly of the sea urchin *Strongylocentrotus purpuratus* and termed it "speract" [4]. Suzuki et al. continued this work in various sea urchin species and, as a result, 74 SAPs were identified from 17 species [5, 6].

Yanagimachi described the activation of sperm motility during the process of fertilization in herring, a teleost fish [7]. From the eggs of the Pacific herring two types of factors have been isolated: the herring sperm-activating proteins (HSAPs) [8] and a chorion-bound sperm motility-initiating factor (SMIF) [9]. One of the HSAPs was determined to be a 73-amino acid-long protein [10], and SMIF was identified as a 105-kDa glycoprotein [11]. In the bitterling, spermatozoa are activated by a substance released from the egg jelly near the micropyle [12, 13].

Other sperm-activating factors have been identified in corals [14], starfish [15], and ascidians [16]. In corals and ascidians, sperm-activating factors are not proteins but small organic compounds: unsaturated fatty alcohol in the former and sulfated sterol in the latter. Since these molecules have not only sperm-activating but also sperm-attracting activity, we will discuss them subsequently.

Sperm chemotaxis toward egg

Prior to fertilization, the spermatozoa of many animals and plants show chemotactic behavior toward

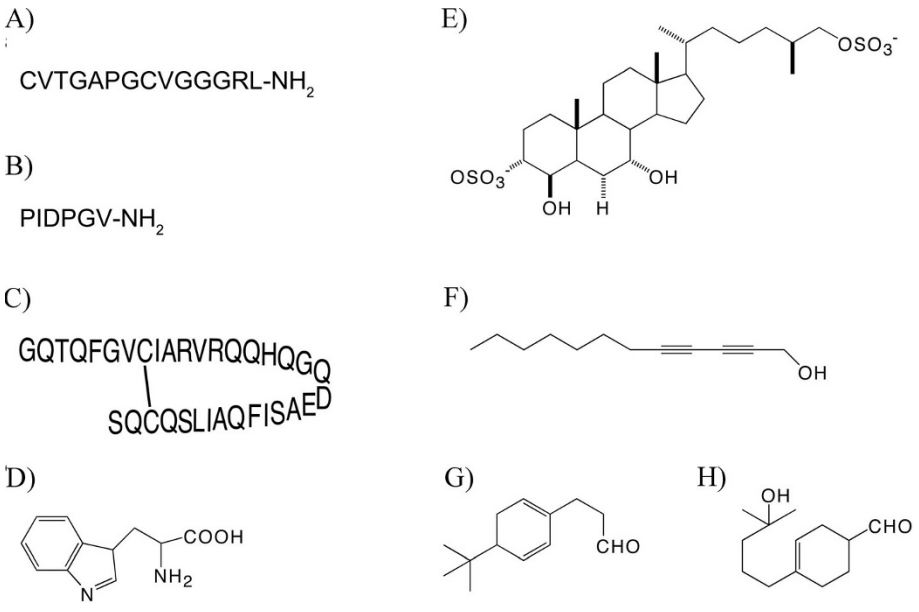

Figure 2. All identified or proposed attractants of animal spermatozoa. (*A*) Resact (sea urchin, *Arbacia punctulata*) [28]; (*B*) peptide (cuttlefish, *Sepia officinalis*) [29]; (*C*) asterosap (starfish, *Asterias amurensis*) [30]; (*D*) tryptophan (abalone, *Haliotis rufescens*) [31]; (*E*) SAAF (ascidian, *Ciona intestinalis*) [16]; (*F*) fatty alcohol (coral, *Montipora digitata*) [14]; (*G*) bourgeonal (human); and (*H*) Lyral (mouse). Allurin, a 21-kDa protein, has also been identified in the amphibian *Xenopus laevis* [32].

eggs. Chemotactic behavior was first described in ferns [17], and the bimalate ion was identified as the attractant [18]. In animals, sperm chemotaxis toward the egg was first observed in the hydrozoan *Spirocodon saltatrix* [19] and is now widely recognized in all species from cnidarians to humans [20–22].

The species specificity of sperm chemotaxis was observed in hydrozoa [23] and echinoderms [24]. The existence of genus specificity has been shown in ascidians [25]. Thus, sperm chemotaxis may participate in the prevention of crossbreeding, especially in animals that show external fertilization.

In plants, sperm chemoattractants are low-molecular-weight organic compounds, e.g., bimalate ions in bracken fern [18, 26] and unsaturated cyclic or linear hydrocarbons, such as ectocarpene, in algae [27]. In animals, sperm chemoattractants have been identified in only seven species, and most of these are proteins or peptides. In the sea urchin *Arbacia punctulata* [28], the cuttlefish *Sepia officinalis* [29], and the starfish *Asterias amurensis* [30], sperm chemoattractants are peptides (Fig. 2). An amino acid, tryptophan and a 21-kDa protein, allurin, which belongs to the CRISP family and is implicated in sperm-egg interaction in mammals, also act as sperm chemoattractants in the abalone *Haliotis rufescens* [31] and the amphibian *Xenopus laevis* [32], respectively. Furthermore, the sperm-attracting activities of some hydrozoans are lost following treatment with some proteases [33, 34]. On the other hand, chemoattractants derived from the eggs of the coral *Montipora digitata* [14] and the ascidian *Ciona intestinalis* [16] were determined to be low-molecular-weight organic compounds: an unsaturated fatty alcohol and a sulfated hydroxysterol, respectively (Fig. 2).

Among mammals, sperm chemotaxis toward the follicular fluid is observed in humans [35], and many candidate sperm attractants in the follicular fluid have been proposed: N-formylated peptides [36], atrial natriuretic peptide [37], progesterone [38], etc. However, the effects of these factors remain obscure because, in experiments for the determination of these effects, the condition of spermatozoa varies, resulting in low reproducibility and reliability.

Sperms are known to contain many G-protein-coupled odorant receptors [39, 40], and hOR17–4, one of these receptors, seems to be involved in the chemotaxis of human spermatozoa. Bourgeonal, an aromatic aldehyde used in perfumery, is a potent ligand of hOR17–4 and acts as a chemoattractant [41] (Fig. 2). Similar results have been reported regarding mouse sperm chemotaxis: the odorant Lyral acts as an attractant of mouse spermatozoa [42]. However, only 36% of human spermatozoa [41] and ≈10% of mouse spermatozoa [42] show an increase in Ca^{2+} levels in response to bourgeonal and lyral, respectively. MOR23, the odorant receptor of lyral, is expressed in only ≈30% of the seminiferous tubules in the testis. Therefore, all spermatozoa cannot respond to attractants even if hOR17–4 or MOR23 do mediate sperm chemotaxis. Further studies are required to determine whether the heterogeneity of sperm response is normal and helps in the selection of spermatozoa or whether another unknown odorant receptor mediates mammalian sperm chemotaxis. Furthermore, odorants such as bourgeonal and lyral are artificial com-

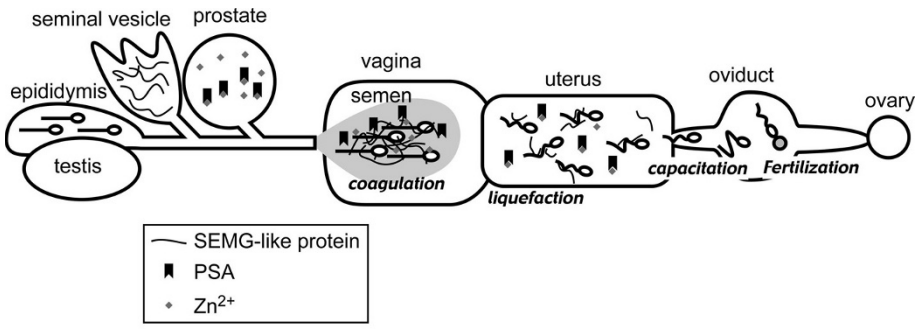

Figure 3. Simplified diagram of capacitation of mammalian spermatozoa in the male and female reproductive tracts. Proteins homologous to human SEMG (SEMG-like proteins) in the seminal vesicles and prostate-specific antigen (PSA) and Zn^{2+} in the prostate are mixed at ejaculation. SEMG-like proteins coagulate and trap the spermatozoa. Inside the coagulum, the spermatozoa are immobilized. Decrease in the $[Zn^{2+}]$ in the coagulum may activate PSA, which is a chymotrypsin-like protease and digests SEMG-like proteins, resulting in the liquefaction of semen. The spermatozoa released from coagulum become motile. In the uterus, fragmented SEMG-like proteins still bind to the spermatozoa and inhibit sperm capacitation. Removal of the fragmented SEMG-like proteins from the sperm in the oviduct may trigger sperm capacitation.

pounds, and native sperm attractants are as yet unknown.

Capacitation and decapacitation

Mammalian spermatozoa are ejaculated into the vagina or uterus and swim toward the ampulla of the oviduct (Fig. 3). The spermatozoa are incapable of fertilizing oocytes immediately after ejaculation and acquire fertility after remaining in the uterus for an appropriate time period [43, 44]. The series of biochemical modifications that confers fertility on the spermatozoa is called "capacitation." The capacitated spermatozoa show highly activated flagellar beating (hyperactivation), undergo the acrosome reaction, penetrate the zona pellucida (ZP), and finally bind and fuse with oocytes. In contrast, non-mammalian spermatozoa are usually capable of fertilization immediately after the acquisition of motility. Therefore, we concentrated on mammalian spermatozoa in this section.

Capacitation is a reversible process mediated by extracellular factors that supposedly exist in the fluids surrounding the spermatozoa: secretions from the uterus, ovarian fluid, testicular tissue fluid, epididymal fluid, and secretions from the male accessory reproductive glands such as the seminal vesicles, prostate, and bulbourethral glands (Fig. 3). In almost all mammalian species, the seminal plasma is considered as one of the main sources of the factors mediating capacitation. Seminal vesicle fluid is the major component of seminal plasma and contains many factors: the activators of sperm motility; fructose and citric acid, which act as the main energy sources of the spermatozoa; prostaglandins that suppress the female immune response; and a semen-coagulating factor that saves sperm metabolism [45–49]. Peitz and Olds-Clarke reported that the removal of the seminal vesicle in the house mouse decreased the pregnancy ratio and delayed the time of birth [50]. Thus, the seminal vesicle fluid was considered to produce an optimal environment that ensured sperm motility and their journey to the ampulla of the oviduct. On the other hand, seminal plasma is also known to abolish sperm fertility [51], a phenomenon termed "decapacitation" [52]. Despite many attempts, few decapacitation factors have been identified. Of these, two are proteins secreted by the seminal vesicles in mice: seminal vesicle autoantigen (SVA) [53] (GenBank: NM009299) and seminal vesicle secretion 2 (SVS2) [54] (GenBank: NM017390). SVS2, in particular, the homolog of human semenogelin (SEMG), seems to be a native regulator of sperm fertility. SVS2 is a major component of the copulatory plug and, after copulation, part of it intrudes into the uterus and interacts with the ejaculated sperm heads [54]. SVS2 reduces the fertility of epididymal spermatozoa, and the fertility of ejaculated spermatozoa is associated with the distribution of SVS2 in the female reproductive tract [54]. SEMG also prevents the protein tyrosine phosphorylation of human spermatozoa, the induction of the acrosome reaction, and the capacitation of ejaculated spermatozoa upon treatment with fetal cord serum ultrafiltrate – an inducer of the acrosome reaction [55]. In humans, a glycoprotein [56] and some glycopeptide [57] decapacitation factors have been purified; however, their identities have not been confirmed.

During *in vitro* incubation for capacitation, some researchers have observed that decapacitation factors originate from the spermatozoa themselves. Although the molecular mechanisms underlying sperm capacitation are poorly understood, it is known that the *in vitro* incubation of spermatozoa extracted from the cauda epididymis (epididymal spermatozoa) in de-

fined media leads to capacitation [58]. The most well investigated decapacitation factor derived from spermatozoa is designated DF and was reported by Fraser and colleagues [59–61]. DF, whose molecular structure has not been completely elucidated, is a 40-kDa glycoprotein that binds to a glycosylphosphatidylinositol (GPI)-anchored membrane receptor located on the postacrosomal region of incapacitated epididymal spermatozoa [61]. DF is believed to positively regulate plasma membrane Ca^{2+}-ATPase activity, resulting in an increase in intracellular Ca^{2+} levels and concomitantly stimulating capacitation [62]. Recently, two laboratories independently identified Raf kinase inhibitor protein-1 (RKIP-1) as a sperm surface protein acting as either a decapacitation factor [63] or a decapacitation factor receptor [64]. Moreover, a study on RKIP-1-deficient mice indicated that RKIP-1 modulated capacitation [65]. In primates and humans, another factor is released from ejaculated-washed spermatozoa during *in vitro* incubation – platelet-activating factor (PAF) (see review [66]). Primate spermatozoa produce PAF, and this ligand binds to its membrane receptor. The binding of PAF to spermatozoa results in increased sperm motility, acrosome reaction, and fertilization [66].

As described above, despite the availability of many studies on sperm capacitation and decapacitation, these phenomena remain obscure. This may be due to the fact that the "capacitation" state, which itself exhibits no visible change, is examined by means of the acrosome reaction, hyperactivation, or tyrosine phosphorylation, which are the results of capacitation. Furthermore, most decapacitation factors have been found using spermatozoa incubated *in vitro,* since decapacitation is determined by the reversibility of the capacitation state. Although all these factors seem to be effective, the essential common system is as yet obscure. Reversible capacitation may finally be altered to "irreversible" capacitation immediately prior to the fertilization of the egg. Therefore, *in vivo* studies on fertilization in the female reproductive tract and the consideration of female factors are required to understand sperm capacitation.

Acrosome reaction in non-mammalian spermatozoa

Except in teleosts and some protostomes, the acrosome reaction is the final step that spermatozoa undergo prior to fertilization. The acrosomal vesicle, which covers the anterior of the sperm head like a cap, is derived from the Golgi apparatus. The acrosomal vesicle is exocytosed when the spermatozoon approaches the egg (echinoderms, etc.) or when it attaches to the vitelline coat (mammals, frogs, etc.). This vesicle contains enzymes that facilitate the penetration of the vitelline coat, and the inner membrane of the acrosomal vesicle seems to contain the essential molecule required for fusion to the egg plasma membrane. Furthermore, some animals form an acrosomal process by actin polymerization in the acrosome, which helps sperm-egg fusion. Thus, the acrosome reaction is an indispensable step for fertilization.

Inducers of the acrosome reaction have been well investigated in the starfish *Asterias amurensis.* In starfish, these inducers have been identified as the three components of the egg jelly: a highly sulfated high-molecular-weight ($>10^4$ kDa) glycoprotein termed acrosome reaction-inducing substance (ARIS), steroid saponins termed Co-ARISs, and the peptide asterosap [67]. ARIS is the indispensable main component of the inducers, but ARIS alone cannot induce the acrosome reaction; high Ca^{2+} levels or high pH is required [68]. ARIS contains the pentasaccharide repeat tract (4-β-D-Xyl*p*-1 > 3-α-D-Gal*p*-1 > 3-α-L-Fuc*p*-4(SO_3^-)-1 > 3-α-L-Fuc*p*-4(SO_3^-)-1 > 4-α-L-Fuc*p*-1), and a polymer containing 10–11 repeat tracts has biological activity [69]. Under normal seawater conditions, ARIS can induce the acrosome reaction in starfish spermatozoa in the presence of Co-ARIS [70]. Three steroid saponins have been identified as Co-ARISs [71], and their action does not seem to be species specific [72]. Asterosap, the third factor required for the acrosome reaction, itself induces sperm activation and accelerates the acrosome reaction induced by ARIS and Co-ARIS [73].

The acrosome reaction in sea urchin spermatozoa is also induced by the egg jelly, and fucose sulfate polymer, a component of egg jelly, is the acrosome reaction-inducing substance [74]. Sulfation patterns of the fucose sulfate polymer are species specific, and the variations in the sulfate residues seem to be responsible for this specificity [75].

In *Xenopus laevis*, the acrosome reaction is induced by a 300-kDa glycoprotein located in the vitelline membrane [76]. Glycans of this glycoprotein seem to be involved in the induction of the acrosome reaction [76].

Acrosome reaction in mammalian spermatozoa

In contrast to non-mammalian animals, the inducer(s) of the acrosome reaction in mammalian spermatozoa remains obscure. The acrosome reaction in mammalian spermatozoa occurs on the vitelline membrane termed the ZP. This reaction has been thought to be induced by binding to ZP3 – one of the glycoprotein components of the ZP [77, 78]. In mouse spermatozoa, the acrosome reaction seems to be induced by *O*-glycans of ZP3 [79], and the *N*-acetylglucosamine of the sugar chains and β 1,4-galactosyltransferase

(GalT) of the spermatozoa act as receptors for ZP3 [80, 81]. Furthermore, ZP3-null mice are infertile [82, 83], and human ZP3 restores fertility in ZP3-deficient mice [84]. These results support the model that glycans of ZP3 are the inducers of the acrosome reaction. However, GalT-null spermatozoa [85] and *O*-glycans-deficient spermatozoa [86] are fertile, even though soluble ZP3 does not induce the acrosome reaction in GalT-null spermatozoa, and 93 % of these spermatozoa could not penetrate the ZP [87]. Furthermore, Baibakov et al. reported that sperm binding to the ZP was not sufficient to induce the acrosome reaction [88].

On the other hand progesterone – one of the major components of follicular fluid – released from cumulus cells has been shown to induce the acrosome reaction in a presumably physiological manner [89–91]. Progesterone is also proposed as a candidate motility activator, chemoattractant, and inducer of capacitation in mammalian spermatozoa [92]. Progesterone canonically binds to intranuclear receptors and induces gene expression, but the response of spermatozoa to progesterone must be non-genomic. Some membrane receptors for progesterone have been identified [93] or estimated [94]. Furthermore, glycine [95], prostaglandin E [96], cholesterol sulfate [97], acetylcholine [98], nicotine [98], γ-aminobutyric acid (GABA) [99], ATP [100], epidermal growth factor (EGF) [101], PAF [102], and other glycans [103] have been shown to act as inducers of the acrosome reaction. However, it remains unclear whether these factors are actually involved in the acrosome reaction *in vivo*.

Though inducers of the acrosome reaction remain disputed, the sugar chains of the ZP and/or the matrix surrounding the eggs appear to play important roles in the induction of the acrosome reaction in mammalian spermatozoa, as in the case of non-mammalian spermatozoa. Furthermore, mammalian spermatozoa often show a spontaneous acrosome reaction. Since a sustained $[Ca^{2+}]_i$ increase is sufficient to induce the acrosome reaction [104, 105], all the candidates described above may cooperatively induce a sustained $[Ca^{2+}]_i$ increase. Thus, all these candidates may act as native inducers of the acrosome reaction.

Signaling mechanisms in spermatozoa

The question arises as to how such various factors regulate sperm motility and fertility. Since methods for the manipulation of molecules (expression, RNA_i, etc.) in spermatozoa and unfertilized oocytes are not yet established, the molecular mechanisms underlying these phenomena are scarcely known despite many pharmacological studies being performed. However, it is known that Ca^{2+} and cyclic nucleotides are the key factors in these phenomena.

Signaling mechanisms involved in the activation and chemotaxis of spermatozoa

Even though many sperm activators and attractants have been identified, the receptors for these factors have only been identified in echinoderms. In sea urchin spermatozoa, a transmembrane-type guanylyl cyclase has been identified as the receptor for resact – a sea urchin SAP [106]. The receptor for speract, another SAP, is not a guanylyl cyclase [107] and associates with guanylyl cyclase [108]. Cyclic GMP, synthesized by the receptor guanylyl cyclase, induces the hyperpolarization of the membrane potential by K^+ efflux through cGMP-activated K^+ channels [109, 110]. The change in the membrane potential in turn increases the intracellular pH and the Ca^{2+} and cAMP levels [109, 111]. In the starfish *Asterias amurensis*, the receptor for the SAP asterosap is also a guanylyl cyclase, and increasing cGMP levels result in an increase in $[Ca^{2+}]_i$ [112,113].

Mammalian sperm chemotaxis seems to be mediated by odorant receptors and results in an increase in $[Ca^{2+}]_i$, as described above [41, 42]. The odorant receptors are coupled with trimetric G_{olf} protein and activate membrane-associated adenylyl cyclase (mAC) [114]. In both cases, the cyclic nucleotide is the first signal, and $[Ca^{2+}]_i$ increase follows.

In the ascidian *Ciona intestinalis*, the cAMP level in spermatozoa is increased by sperm-activating and attracting factor (SAAF), but this requires extracellular Ca^{2+} [115]. Ca^{2+} influx appears to activate the calmodulin/calmodulin kinase II systems, resulting in the hyperpolarization of the plasma membrane [116], which in turn induces cAMP synthesis [117]. Furthermore, theophylline, a phosphodiesterase inhibitor, increases intracellular cAMP levels and activates sperm motility even in the absence of external Ca^{2+} [115]. Thus, $[Ca^{2+}]_i$ increase triggers the cAMP signal in ascidians. Moreover, cAMP-dependent protein kinase (PKA) appears to phosphorylate both a 26-kDa axonemal protein and Tctex2-related dynein light chains, resulting in the activation of sperm motility in the *Ciona* species [118, 119]. On the other hand, the increase in cAMP seems to be independent of chemotaxis of the ascidians sperm [115]. Thus, the signaling cascades of sperm activation and chemotaxis are independent phenomena, even though SAAF controls both sperm activation and chemotaxis.

In many species, sperm chemotaxis requires extracellular Ca^{2+} [25,28,115,120,121]. Store-operated Ca^{2+} channels seem to mediate the asymmetric flagellar waveform of spermatozoa and result in chemotactic

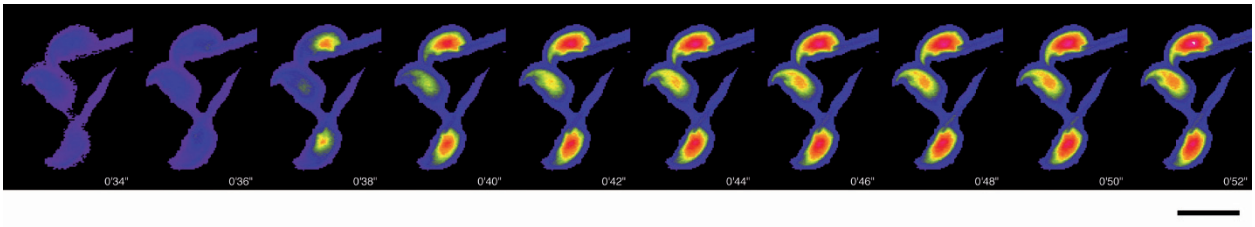

Figure 4. $[Ca^{2+}]_i$ dynamics in mouse spermatozoa treated with solubilized ZP. Capacitated spermatozoa were loaded with 4 μM fluo4-AM for 15 min, and $[Ca^{2+}]_i$ mobilization in a single sperm was monitored. The sperm was stimulated by solubilized ZP. Images were collected every 2 s. The bar indicates 10 μm.

behavior [122]. In sea urchins, the Ca^{2+} level in the sperm appears to be correlated with its flagellar asymmetry [123, 124], and speract increases the $[Ca^{2+}]_i$ in the spermatozoa [125, 126]. $[Ca^{2+}]_i$ fluctuations have been observed in the swimming spermatozoa of sea urchins, and these fluctuations were related to changes in the direction of sperm movement [30, 127]. However, no information is available on the Ca^{2+} dynamics in a single spermatozoon during chemotaxis, and the role of Ca^{2+} in this phenomenon remains unclear, although it is accepted to be an important factor in sperm chemotaxis.

Molecular mechanism of capacitation

During sperm capacitation, the membrane fluidity increases due to the efflux of cholesterol [128]. Cholesterol efflux requires bicarbonate and albumin, which are present in abundance in the fluids of the female reproductive tract. On the other hand, when spermatozoa are decapacitated by seminal plasma or by decapacitation factors, a decrease in membrane cholesterol is prevented [129, 130]. In boar spermatozoa, bicarbonate appears to first induce lateral redistribution in the low cholesterol-containing spermatozoa, which in turn facilitates cholesterol extraction by albumin [131]. Bicarbonate induces the activation of soluble adenylyl cyclase (sAC), resulting in cAMP synthesis [132], and the PKA-signaling pathway promotes the tyrosine phosphorylation of sperm proteins [133, 134]. The unusual protein kinase pathway – cAMP/PKA activates some tyrosine kinase resulting in the tyrosine phosphorylation – seems to be mediated by cytoplasmic tyrosine kinase Src [135]. Furthermore, PKA mediates bicarbonate-induced hyperactivation and $[Ca^{2+}]_i$ increase [136]. The PKA-activated phosphorylation of apolipoprotein AI-binding protein (AIBP) during sperm capacitation has recently been reported [137]. Since AIBP interacts with apolipoprotein AI that is involved in cholesterol transport, it may mediate cholesterol efflux.

Moreover, it is known that the K^+ permeability of the sperm plasma membrane is enhanced, resulting in the hyperpolarization of membrane potential and the activation of voltage-dependent Ca^{2+} channels [109, 138]. Recently, a sperm-specific Ca^{2+}-selective channel termed CatSper was discovered [139]. CatSper is similar to cyclic nucleotide-gated (CNG) channels [139] and is activated by alkaline stimulation [140]. CatSper consists of 4 isomers, and all of them are indispensable for capacitation and hyperactivation [139, 141, 142]. The relation between CatSper/Ca^{2+} influx and the cAMP/PKA pathway remains unknown.

Signaling mechanisms involved in the acrosome reaction

Sperm receptors for acrosome reaction inducers have not yet been identified. In sea urchins, fucose sulfate polymer shows affinity to the sperm protein receptor for egg jelly 1 (REJ-1) [143], which is a homologue of human polycystic kidney disease 1 protein (PKD1) [144]. However, it is currently unknown whether REJ-1 is the receptor for fucose sulfate polymer. Moreover, a receptor for the inducers of the acrosome reaction in mammalian spermatozoa is under dispute, as described above.

Though little is known regarding the receptors on spermatozoa, the mechanism of the acrosome reaction appears to be similar to that of exocytosis in other cells. In fact, soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) that regulate exocytosis have been found in acrosomal vesicles [145, 146]. Moreover, synaptotagmin, which is a Ca^{2+}-binding protein and controls SNARE-mediated exocytosis, is located on the outer membrane of the acrosomal vesicle [145, 147]. $[Ca^{2+}]_i$ increase also plays a significant role in the induction of the acrosome reaction [109, 148, 149], and synaptotagmin VI appears to regulate the Ca^{2+}-mediated acrosome reaction in human spermatozoa [147].

With regard to $[Ca^{2+}]_i$ regulation during the acrosome reaction, ZP3 initially induces transient Ca^{2+} influx in mammalian spermatozoa through voltage-dependent cation channels, which in turn leads to the activation of phospholipase C (PLC) [109, 138, 149] (Fig. 4).

Progesterone, another inducer of the acrosome reaction, also induces sustained $[Ca^{2+}]_i$ increase [105]. Among the PLCs, PLCδ4 has been described as essential for the acrosome reaction in mouse spermatozoa [105, 150]. Moreover, PLCδ4 appears to affect Ca^{2+} influx, since the $[Ca^{2+}]_i$ responses were altered in the spermatozoa of PLCδ4-deficient mice [105]. The activation of PLC seems to generate inositol triphosphate (IP_3), which in turn induces Ca^{2+} release from intracellular Ca^{2+} stores [151], resulting in sustained Ca^{2+} influx via store-operated channels (SOC), which ultimately induces the acrosome reaction [104, 148, 149, 152]. Transient receptor potential protein (Trp) channels, which are candidate SOCs, are expressed in spermatozoa [153], and Trp2 reportedly plays a role in the ZP3-induced acrosome reaction in mouse spermatozoa [154]. Furthermore, a $GABA_A$-like progesterone receptor/Cl^- channel is thought to mediate the Ca^{2+} influx induced by progesterone [94], although the mechanism of action of progesterone on spermatozoa is not yet fully understood.

Conclusions

Since the specificity of the interactions between the spermatozoa and eggs dictates successful fertilization, fertilization is an intrinsically species-specific phenomenon. In fact, factors controlling sperm fertility are myriad and species specific; they may be peptides, sugar chains, or small organic compounds. Furthermore, different molecules usually control sperm fertility at different steps in the fertilization process. Numerous factors control sperm fertility, and the recognition of all these factors is difficult.

Nevertheless, the fundamental mechanisms of intracellular signaling underlying fertilization must be common among all animals, since Ca^{2+} and cyclic nucleotides seem to act as the common key factors for the regulation of sperm fertility. Increase in $[Ca^{2+}]_i$ triggers all the steps, and cAMP plays important roles in many steps. It is very interesting that such myriad factors induce similar signal cascades in many different animals.

Unfortunately, knowledge regarding the control of sperm fertility is limited because numerous factors are active in different animals and because of technical difficulties. Furthermore, the available knowledge is occasionally disputed. This may be because many studies that focused on mammalian species, which show internal fertilization with many interactions between the spermatozoa and the female reproductive tract, made us of *in vitro* experiments, which provide different conditions from those in the female reproductive tract. The acrosome reaction in starfish is well known, and ascidian is one of the best models for sperm chemotaxis. We consider that further experiments on invertebrates that show external fertilization will provide useful and suggestive knowledge regarding mammalian sperm fertility.

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