Reproductive Medicine and Biology 2005; 4: 101–114

Review Article

Activation of motility and chemotaxis in the spermatozoa: From invertebrates to humans

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Activation of the sperm motility and chemotactic behavior of sperm toward eggs are the first communication between spermatozoa and eggs at fertilization, and understanding of the phenomena is a prerequisite for progress of not only basic biology, but also clinical aspects. The nature of molecules derived from eggs by which sperm are activated and attracted towards the eggs and the molecular mechanisms underlying the sperm activation and chemotaxis have been investigated in only a few invertebrate species, sea urchins, ascidians and herring fish. However, knowledge on this phenomena has been ignored in mammalian species including humans. The current review first introduces the studies on the activation and chemotaxis of sperm in marine invertebrates, and the same phenomena in mammals including humans, are described. (Reprod Med Biol 2005; 4: 101–114)

BASIC AND REGULATORY MECHANISMS OF SPERM MOTILITY

SPERM FLAGELLA CONSISTS of the cylindrical 9 + 2 structure of the axoneme with a double row of dynein arms along one side of each outer doublet microtubules. The microtubules are connected to each other with nexin links and to central singlet microtubules with sets of radial spokes.[1] The basic mechanochemical mechanism which defines flagellar motility is fairly well understood: the energy derived from hydrolysis of adenosine triphosphate (ATP) by ATPase activity associated with the dynein arms causes the sliding of the microtubules to produce flagellar wave.[2] Spermatozoa commonly move in a straight forward direction with a symmetrical flagellar wave under the mechanism.

However, change in sperm motility occurs after spawning as a prerequisite process for the accomplishment of fertilization. Spermatozoa, which are almost immotile in the male reproductive tract, initiate their motility upon spawning and ejaculation in the aquatic environment such as sea and reproductive tract of female, respectively. The spermatozoa whose motility is initiated, are activated and attracted towards eggs by some signal substances released from the eggs. The phenomena of initiation and activation of the sperm motility and sperm chemotaxis have been well known. However, mechanochemical regulatory mechanism for the initiation and activation of motility and chemotaxis have been poorly studied until Morisawa and Suzuki[3] and Morisawa and Okuno[4] demonstrated that the changes in extracellular K^+ and osmolarity, and intracellular cyclic adenosine monophosphate (cAMP) participate in the initiation of sperm motility in teleost fish. With regards to the initiation of sperm motility, further extensive studies by Morisawa *et al.* have clarified the cell signaling system for the initiation of sperm motility in the fish.[5–7] Furthermore, recent studies render to increase knowledge on the regulatory mechanism underlying sperm activation and chemotaxis. This is the main subject of the current review.

ACTIVATION OF SPERM MOTILITY IN MARINE INVERTEBRATE

SPERM ACTIVATION IN the vicinity of the egg was first observed in lower marine invertebrates, sea urchin and annelida in the beginning of the last century.[8] Since then several studies have tried to clarify the mechanism of sperm activation. Intensive works on sperm activation by the factors released from the egg coat have been done on sea urchins. Ohtake first found the factor indispensable for activation of respiration and motility from the egg jelly that surround the egg in the

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Received 24 December 2004; accepted 6 January 2005.

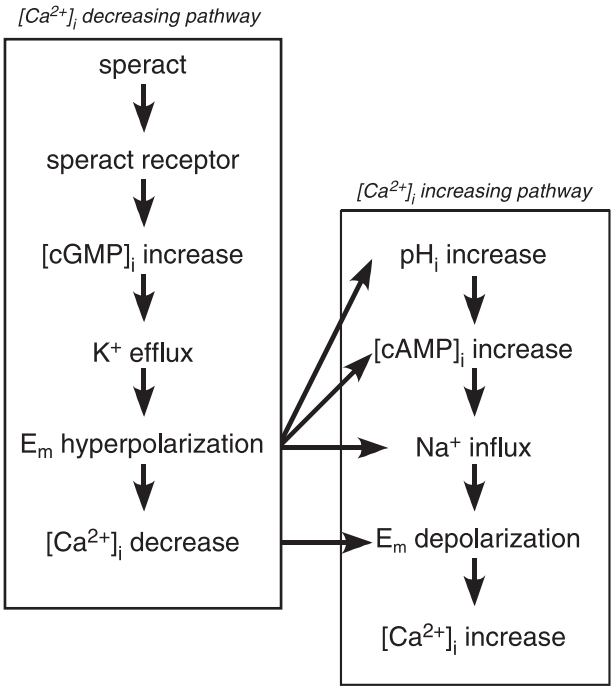

Figure 1 Signaling model for activation of motility in *S. purpuratus* sea urchin sperm induced by speract. (1) Binding of speract to its receptor activates a guanylyl cyclase and increases $[cGMP]_i$, promoting K^+ efflux through a cGMP-dependent K^+ channel. The K^+ efflux causes a decrease in sperm membrane potential (E_m). The E_m hyperpolarization activates Na^+/H^+ exchange, adenylyl cyclase, and a hyperpolarization-activated and cyclic nucleotide-gated K^+ channel, leading to pH_i increase, elevation of $[cAMP]_i$. and Na^+ influx (E_m depolarization), respectively. Em hyperpolarization enhances Na^+/Ca^{2+} exchange to maintain $[Ca^{2+}]_i$ decrease. The increase in $[Na^+]_i$, and Em depolarization could lead to reversal of the Na^+/Ca^{2+} exchange and $[Ca^{2+}]_i$ increase.

Japanese sea urchins, *Hemicentrotus pulcherrimus*.[9] After the discovery, Hansbrough and Garbers have isolated and purified sperm-activating peptide consisting of 10 amino acids from the egg jelly of the sea urchin, *Strongylocentrotus purpuratus* and named it 'speract'.[10] The work was expanded in the various sea urchin species, and 74 of sperm activating peptides were identified from 17 species.[11]

With regards to cell signalings for sperm activation by the speract (Fig. 1), it was found that the peptide binds to the receptor, a guanylyl cyclase to increase intracellular cyclic guanosine monophosphate (GMP).[12] Cyclic GMP induces hyperpolarization of membrane potential by K^+ efflux through cGMP-activated K^+ channels,[13,14] and then the change in the membrane potential increases intracellular pH, Ca^{2+}, and cAMP.[15] Recently, precise analysis of intracellular Ca^{2+}, $[Ca^{2+}]_i$ using

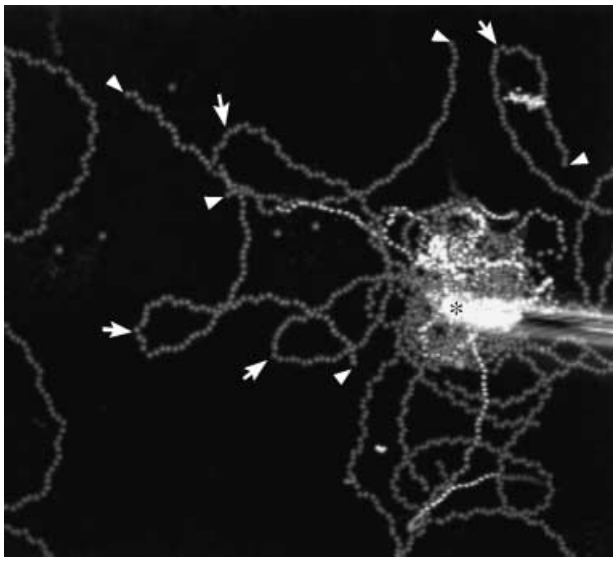

Figure 2 Chemotactic behavior of the sperm in the ascidian, *Ciona intestinalis*. Sperm activating and attracting factor was packed in a tip of glass capillary with agar and then placed in sperm suspension which are preactivated with theophylline, and position of sperm head (white dots) are traced. The photo was integrated from 100 images taken every 20 ms. Sperm show chemotactic behavior toward the capillary tip (*) with chemotactic turn (arrow). Arrowhead show start point of sperm.

caged-speract has showed that the speract initially decreases $[Ca^{2+}]_i$ then induces $[Ca^{2+}]_i$ oscillations in the sperm tail.[16,17] In the starfish, *Asterias amurensis*, the sperm-activating peptide is asterosap and it also binds to the receptor, guanylyl cyclase to increase cGMP, resulting in increase of $[Ca^{2+}]_i$.[18,19]

In the ascidian *Ciona*, spermatozoa are immotile or less motile upon suspending in seawater, and when an unfertilized egg is placed in the sperm suspension, sperm near the egg are intensely activated and showed chemotactic behavior toward the egg. These suggest that sperm activating and attracting factor (SAAF) will be released into the seawater. The activation of sperm motility and sperm chemotaxis are clearly assessed by 'capillary method', which is described later (Fig. 2). With regards to activation of sperm motility, it was shown that intracellular cAMP increases transiently by the factor named SAAF in the presence of external Ca^{2+},[20] suggesting that the second messengers, cAMP and Ca^{2+} are required for the phenomenon. Izumi *et al.* further found that hyperpolarization of the plasma membrane is induced by the SAAF through an increase in K^+ permeability, and the change of the membrane potential causes synthesis of cAMP.[21] The hyperpolarization

was observed in only membrane vesicles prepared from the sperm flagella but not in that from the heads of *Ciona* sperm, suggesting that cell signaling for the SAAF-induced sperm activation occurs in the sperm flagellum. The sperm activation by the SAAF in Ca^{2+}-containing normal seawater was inhibited by flunarizine, a Ca^{2+} channel antagonist, though nitrendipine and verapamil, L-type Ca^{2+} channel specific antagonists, had no effect. Theophylline, a phosphodiesterase inhibitor induced both increases in cAMP and sperm activation in Ca^{2+}-free seawater without SAAF. These results suggest that Ca^{2+} intruded in the cell through the Ca^{2+} channel and stimulates the synthesis of cAMP, and it activates sperm motility.[22] However, it has been unclear whether Ca^{2+} directly participates in cAMP synthesis by activation of adenylyl cyclase or Ca^{2+} participates in membrane hyperpolarization through activation of K^+ channel. Recently, Nomura *et al*. showed that Ca^{2+}/calmodulin system participates in K^+ channel dependent membrane hyperpolarization before cAMP synthesis.[23] Furthermore, it was found that a cAMP-dependent protein kinase (PKA) inhibitor, H-89, inhibited the sperm motility. During *in vivo* and *in vitro* activation of sperm motility by SAAF and cAMP, repectively, phosphorylation of 26 kDa axonemal protein and 21 kDa dynein light chain has been found,[24] suggesting the involvement of PKA-dependent phosphorylation of these proteins in the activation of *Ciona* sperm motility.

In conclusion, the mechanism underlying SAAF-induced activation of sperm motility in ascidians is regulated as shown in Fig. 3b. A sulfate steroid, SAAF, released from unfertilized eggs binds to the receptor on the sperm plasma membrane. The binding may cause opening of the Ca^{2+} channel, resulting in increase in intracellular Ca^{2+}. Ca^{2+}-dependent activation of calmodulin/calmodulin kinase system causes efflux of K^+ via the activated K^+ channel to hyperpolarize the plasma membrane, and the membrane hyperpolarization may activate adenylyl cyclase to synthesize cAMP in the sperm cells. Cyclic AMP dependent phosphorylation of both 21 kDa dynein light chain and the axonemal protein with molecular masses of 26 kDa finally triggers the activation of sperm motility in the ascidian *Ciona*.

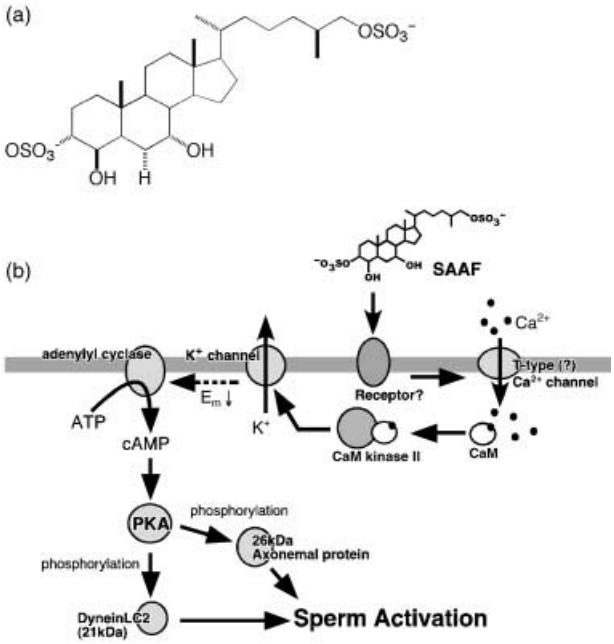

Figure 3 Structure of sperm activating and attracting factor (SAAF) and cell signaling for the activation of sperm motility in the ascidian *Ciona intestinalis*. (a) SAAF is identified as a sulfated steroid (3*R*,4*R*,7*R*,25*S*)-3,4,7,26-tetrahydroxycholestane-3,26-disulfate. (b) SAAF may bind to its receptor that is still unknown and induces Ca^{2+} influx via a T-type voltage-dependent Ca^{2+} channel, which activates CaM/CaM kinase II. CaM kinase II promotes K^+ efflux through a K^+ channel, causing a hyperpolarization of sperm plasma membrane ($E_m \downarrow$). The hyperpolarization may activate adenylyl cyclase, resulting in $[cAMP]_i$ increase, which activates a cAMP-dependent protein kinase (PKA). The PKA phospholylates of 21 kDa dynein light chain protein and 26 kDa axonemal protein, resulting in activation of sperm motility.

SPERM CHEMOTAXIS IN MARINE INVERTEBRATES

Nature of sperm chemoattractants

SPERM CHEMOTAXIS TOWARD an egg in the animal kingdom was first described by Dan in the hydrozoa, *Spirocodon saltatrix*.[25] Since then the phenomenon has been extensively studied in many animal phyla: Cnidaria, Urochordata, Mollusca and Echinodermata.[26–29] Even though the phenomena of sperm chemotaxis are known in many animal species nowadays, the chemical nature of sperm chemoattractants has been identified in several species (Table 1).

Most of the known sperm chemoattractants and candidates of chemoattractants are considered to be amino acids, peptides or proteins. The chemoattractant in the abalone *Haliotis rufescens* was reported as tryptophan,[30] and the chemoattractants in the cuttlefish, *Sepia officinalis*, were identified as peptides.[31] In the sea urchins, among the small peptides which were isolated from eggs,[11] resact from *Arbacia punctulata* has both sperm-activating

Table 1 Sperm activators and chemoattractants of which the chemical structures were identified in animals

Animals	Trivial name	Chemical nature	Function	Reference number
Coral	dodeca-2,4-diynol	Fatty alcohol	Chemotaxis	33
Abalone	L-triptophan	Amino acid	Chemotaxis	30
Cattle fish		Peptide	Chemotaxis	31
Sea urchin	Speract	Peptide	Activation	10
	Resact	Peptide	Chemotaxis	27
Starfish	Asterosap	Peptide	Activation	18
Ascidians	SAAF	Steroid	Activation and chemotaxis	36
Pacific herring	HSAP	Proteins	Activation	117
Xenopus	Allurin	Protein	Chemotaxis	35

HSAP, herring sperm-activating proteins; SAAF, sperm activating and attracting factor.

and -attracting activities.[27] Sperm chemoattractants of the hydrozoa *Hippopodius hippopus*[32] with molecular masses of 25 kDa and the starfish *Picnopodia helianthoides*[28] are considered to be proteins. However, chemoattractants from eggs of the coral, *Montipora digitata*, is considered to be the unsaturated fatty alcohol, dodeca-2,4-diynol.[33] The sperm chemoattractant was found in several non-mammalian vertebrates such as the Pacific herring *Clupea pallasi*[34] and the amphibian *Xenopus laevis*.[35]

Recently, a novel steroid was identified as a sperm chemoattractant in the ascidian, *Ciona*. Egg seawater (ESW) in ascidians which is obtained as a supernatant of seawater incubated with the eggs, has both sperm-activating and sperm-attracting activities, indicating that some sperm-activating and -attracting factors are released from the eggs.[20] During the purification process of the SAAF from ESW using column chromatographies, sperm-activating and -attracting activities always comigrated, suggesting that both activities are derived from a single molecule, thus the substance was named SAAF (sperm-activating and -attracting factor).[22] The chemical nature of SAAF was determined to be the novel sulfated steroid, 3,4,7,26-tetrahydroxycholestane-3,26-disulfate,[36,37] (Fig. 3a). Synthetic SAAF has both sperm-activating and sperm-attracting activities (Fig. 2) at <10 nM.[38]

Cell signalings for sperm chemotaxis in hydrozoans and sea urchins

In the hydrozoan, *Siphonophore*, the radius of curvature of the sperm trajectory reduces as the spermatozoa gets close to the source of sperm attractant, cupule.[29] However, a quick-turning movement termed a 'chemotactic turn' was observed in spermatozoa of other hydrozoans, as the sperm exhibits chemotactic behavior.[39] During the turning movement, sperm demonstrate a temporary asymmetrical flagellar beating.[39,40] These suggest that two ways: reduction of radius of curvature of sperm trajectory by moderate occurrence of asymmetrical flagellar wave form and 'chemotactic turn' by occurrence of the drastic flagellar asymmetry may induce sperm chemotaxis. Ishikawa *et al*. have recently conducted the numerical analysis on the chemotactic behaviors in the ascidian and hydrozoa.[41]

The requirement of extracellular Ca^{2+} for chemotaxis has been known in the hydroids,[29] sea urchin,[27] and ascidians:[20,40,42] Ca^{2+}-chelating agents, for example, ethylenediaminetetraacetic acid (EDTA) suppresses chemotactic behavior of the sperm. The reduction of the diameters of the trajectories that are observed in the chemotaxis of hydrozoa could not be observed in the presence of ethylene glycol-bis (2-aminoethylether)-N, N, N′, N′-tetraacetic acid (EGTA), suggesting that Ca^{2+} regulates the motility pattern of the flagellum by changing radius of sperm trajectory.[29] In the sea urchin, resact can induce Ca^{2+}-dependent asymmetry of the flagellum waveform. The same role of extracellular Ca^{2+}-induced flagellar asymmetry on induction of sperm chemotaxis has been reported in hydrozoa.[39]

The cell signaling underlying sperm activation and chemotaxis by resact has been proposed in the sea urchin, although the sperm activation and chemotaxis could not be discussed as the separated event. Resact binds to the receptor guanylyl cyclase,[43] and seems to induce an increase in $[Ca^{2+}]_i$ through cGMP and cAMP[44] to induce flagellar asymmetry, resulting in sperm chemotaxis. The $[Ca^{2+}]_i$ elevation may be controlled by a channel such as the sperm-specific cyclic nucleotide-gated and voltage-dependent Ca^{2+} channels.[45–47]

Cell signaling for induction of sperm chemotaxis in ascidians

Spermatozoa of the ascidians *Ciona intestinalis* and *C. savignyi* are immotile or less motile upon suspension in seawater. When an unfertilized egg of the same species is placed in the sperm suspension, sperm near the egg are intensely activated and show chemotactic behavior toward the egg. These suggest that SAAF is released from the egg into the seawater. The SAAF has been purified from the ESW, and the molecular structure has been identified to be a novel steroid as described above. For an activity assay of sperm attraction, 'micropipette assay'[20] is used as the most common technique. In the method, samples are enclosed in the tip of a glass micropipette, and the micropipette is placed in the sperm suspension and then the sperm trajectories around the micropipette tip are observed. When a micropipette containing ESW or purified SAAF is inserted in the sperm suspension, spiral trajectories exhibiting the chemotactic turn of sperm toward the micropipette tip are seen (Fig. 2).

As a result of sperm activation and attraction occuring simultaneously in many species, for example, sea urchins and ascidians, it seems to be difficult to separate each other. Quantitative evaluation of sperm chemotaxis therefore has not been well established, although molecular structures of the attractants have been proposed in several species including sea urchins. In the ascidians *Ciona*, however, sperm chemotaxis can be separated from sperm activation. Sperm chemotaxis does not require intracellular cAMP, but Ca^{2+} is essential in the species.[22] In order to distinguish the chemotactic behavior from the cAMP-dependent activation of motility in *Ciona* sperm, spermatozoa are treated first with 1 mM theophylline for 1 min, in which increase in intracellular cAMP causes sperm activation with a circular movement.[22] Using the activated sperm and 'micropipette assay', a new method for quantitative evaluation of sperm chemotaxis has been established.[36] In the method, linear equation chemotaxis index (LECI) was established as a parameter that is derived from the negative value of the coefficient ($-a$) in a linear equation ($y = ax + b$) of time (abscissa in Fig. 4a) versus the distance between the micropipette tip and the sperm head (D) (ordinate in Fig. 4a). When the trajectories of sperm around the micropipette containing ESW are analyzed, D decreases with oscillation. On the contrary, the parameter does not decrease by the addition of artificial seawater. The parameter, LECI, can represent the strength of sperm-attracting activity and will offer reliable aspects of the quantification of sperm chemotaxis (Fig. 4).

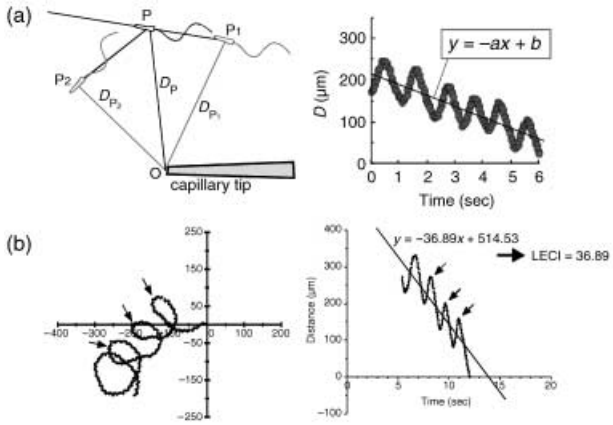

Figure 4 (a) Definition of the linear equation-based chemotaxis index (LECI). D_p is defined as the distance between the micropipette tip and the sperm head on the point P (left). The tip of the micropipette was set as the origin of coordinates (0). P_1 and P_2 represent the points of the sperm before and after Δt time, respectively. The right graph is typical plots of D against time. Line and formula represent the linear equation and the coefficient of time versus D, respectively. Negative value of the coefficients of the equation (a) indicates the chemotaxis index (LECI). (b) Trajectories of the sperm (left) and plots of D against time around the tip of micropipette (right) containing SAAF. Arrows indicate points of the chemotactic turn. Line on the graph and formula represent the linear equation and the coefficient of time versus D, respectively.

Store-operated Ca^{2+} channel mediates the asymmetrical flagellar waveform of the ascidian sperm in the Ca^{2+}-essential *Ciona* chemotaxis.[48] The modulators of store-operated Ca^{2+} channel, SKF96365, Ni^{2+}, 2-APB, and thapsigargin, inhibited sperm chemotactic behavior of the ascidian sperm, but any blocker for voltage-dependent Ca^{2+} channel did not,[48] while the sperm activation operated by voltage-dependent Ca^{2+} channel did so.[22] These blockers of store-operated Ca^{2+} channel also inhibited asymmetrical flagellar wave form and turning movement of sperm that is a typical sign of sperm chemotaxis. It is known that depletion of intracellular Ca^{2+} stored in the endoplasmic reticulum by thapsigargin induced the capacitative Ca^{2+} entry in the cytoplasm of the sperm cells from the outside of the cells, and the Ca^{2+} entry was blocked by SKF96365. From the evidence, it was suggested that increase in $[Ca^{2+}]_i$ through the store-operated Ca^{2+} channel causes asymmetrical flagellar movement which establishes the chemotactic behavior of *Ciona* spermatozoa (Fig. 5).

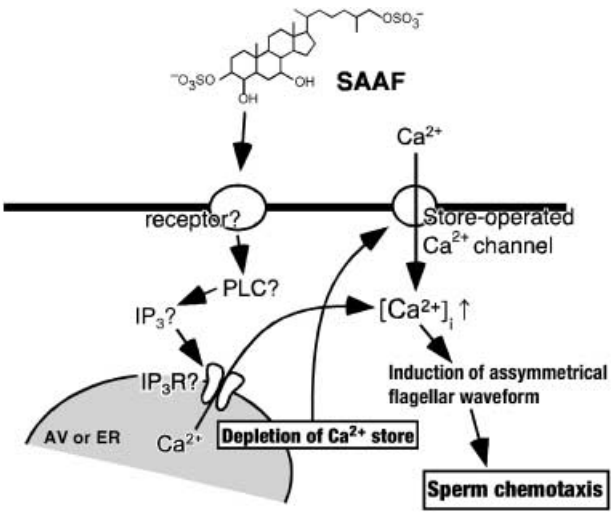

Figure 5 Working hypothesis of cell signaling for sperm chemotaxis in the ascidian *Ciona intestinalis* induced by sperm activating and attracting factor (SAAF). Binding of SAAF to its receptor may activates phospholipase C (PLC), which increases inositol 1,4,5-trisphosphate (IP_3), resulting in Ca^{2+} release via IP_3 receptor from internal Ca^{2+} store, e.g. endoplasmic reticulum (ER) or acrosomal vesicle (AV). Depletion of the internal Ca^{2+} store and induce Ca^{2+} influx via a store-operated Ca^{2+} channel. The elevation of $[Ca^{2+}]_i$ induces asymmetrical flagellar waveform and chemotactic turn, causing sperm chemotaxis.

REGULATION OF SPERM MOTILITY IN MAMMALS

SPERM ACTIVATION AND chemotaxis is well known in the species with external fertilization as described above. However, in internal fertilization mammalian species, the phenomena are enigmatic for many years, although numerous studies suggest that sperm motility is regulated by factors included in fluids which derive from male and female reproductive organs or cells (Table 2).

Factors from male reproductive glands affecting sperm motility

In humans, ejaculated seminal plasma contains fluids which are secreted from the seminal vesicle, prostate, epididymis, testes, Cowper's glands and Littre's glands. It has been proposed that the fluids in the epididymis, seminal vesicles and prostate as well as the female genital tract contain factors affecting sperm motility. It has been reported that the epididymal fluid of hamster contains factors that could stimulate the motility of hamster spermatozoa.[49,50] The presence of progressive motility sustaining factor in the epididymis was also reported in humans.[51] Forward motility protein has also been reported in bovines.[52] However, epididymal factors having detrimental effect for sperm motility have been mentioned. The viscoelastic factor in rat epididymal fluid appears to be responsible for the latency state of epididymal rat spermatozoa.[53] In the bull, a low extracellular pH appears to be responsible for the dormancy state of epididymal bull spermatozoa.[54]

Table 2 Substances proposed as chemoattractant for mammalian sperm

Substance	Sperm used for assay and reference number
N-formylated peptides	Bull,[123] human[124]
Atrial natriuretic peptide	Human[127]
Heparin	Human[125]
Progesteron	Human[126]
Folicular fluid	Human,[129,131] mouse[122]
Ligand for odorant receptor	Human,[134] mouse[135]

The seminal plasma, a mixture of the fluids from the male reproductive glands, has been considered to contain factors with both beneficial and detrimental effects on sperm motility. The semen containing prostatic secretion predominantly enhances human sperm motility,[55] while the prostatic-inhibiting factors are reported.[56] The seminal plasma from men whose sperm show normal motility improves the motility from oligoasthenozoospermic patients, and conversely, the seminal plasma from the patients has deterimental effect on the motility of sperm from normal men.[57] It has been also announced that macromolecular components in human seminal plasma initiate progressive motility.[58] Placental protein 5, a normal secrete of placentral trophoblast[59] and pregnancy-associated plasma protein A were found in human seminal plasma, both of them enhance sperm motility.[60,61] Prostaglandins are present at remarkably high concentrations in the seminal, but their effect on sperm motility has been unclear.[62]

It was observed in the mouse that ejaculated sperm after removal of the seminal vesicle exhibited less progressive wider head lateral movement associated with a net decrease in linearity,[63] while it has been also reported that the same fluid contains factors for inhibiting sperm motility.[64] These suggest that the balance between detrimental and beneficial factors which are present in the semen may play an important role for sperm motility at ejaculation. According to Ashizawa and Okauchi, the addition of fowl seminal plasma to washed fowl sperm

stimulate sperm motility.[65] The stimulatory factor is considered to be a molecule smaller than 1 kDa. Motility of bull spermatozoa, which drastically decreases by washing is restored by the addition of the seminal plasma.[66,67] Dulation of the sperm motility is dependent on the concentration of the seminal plasma, and the molecular mass of the beneficial factor is below 500 Da, and that of the detrimental one is considered to be a macromolecule.[68] Furthermore, it was suggested that the bull seminal plasma can stimulate motility of hamster spermatozoa.

Mechanism for regulation of sperm motility by male factors

The first function of the seminal plasma that consisted of secretions from the various glands, may be dilution of the concentrated epididymal sperm. The dilution may decrease concentration of viscoelastic component, immobilin in rat[53] and may increase in intracellular and extracellular pH in bull spermatozoa during ejaculation.[54] It may be possible to consider that the process triggers the initiation of sperm motility.

Zinc ion has been noted to be present in the sperm and concentrated in the seminal plasma in mammals,[69–71] and the various roles for the reproductive events,[72] including sperm motility were reported[73] in variety of mammalian species. For example, albumin which is produced by the prostate and may play a role as a detoxicant, binds zinc ions in the seminal plasma, and the removal of zinc may promote sperm motility.[74,75]

Studies on the effect of prolactin, a main source of the seminal plasma[76] on sperm motility have yielded contradictory results: a high level of the prolactin in the seminal plasma enhances sperm motility,[77,78] whereas opposite results have been reported.[79,80] Prolactin increases cAMP level and ATPase activity,[81,82] both of which cause the increase in sperm motility.[83,84]

Recently, two factors, seminal plasma motility inhibitor (SPMI) and relaxin, derived from male reproductive organs have been investigated in special reference to their inhibitory and accelelative effects on sperm motility, respectively. It is well known that human semen spontaneously coagulates following ejaculation and liquefies within 5–20 min. The major coagulum components are originated from the seminal vesicle secretions.[85] The molecular structure of the seminal component in humans was identified as disulfide-linked polypeptide with molecular masses of 52 kDa and 71–76 kDa which are named semenogelin I (Sg I) and semenogelin II (Sg II), respectively.[86] The structure of genes and proteins of the Sg I and II have been investigated.[86,87] Prostate-specific antigen (PSA) with chimotrypsin-like proteolytic activity degradates the semenogelins during liquefaction of the coagulum.[88] However, the seminal plasma from various mammalian species contains a factor inhibiting the reactivated motility of demembranated spermatozoa.[89] Iwamoto and Gagnon isolated a protein of 18–22 kDa associated this activity from human seminal plasma and named it SPMI.[90] The purified SPMI interferes the motility of intact sperm, although 1000 times concentration is required for inhibition of motility.[91] It has been suggested from several resons such as identical glandular origin, processing by PSA, association with semen coagulation, similarity of cDNA sequence, that the SPMI is the same molecular entity as semenogelin I.[92]

Zinc ions are concentrated in the prostate gland in which PSA and zinc ions are secreted in the semen at ejaculation. The secreted zinc is tightly bind with Sg I.[93] Protein C inhibitor (PCI), a serine protease inhibitor which is present in the seminal vesicle together with semenogelins, binds to both PSA and semenogelin, and inhibits PSA-mediated degradation of semenogelin. Zinc ions reduce binding of semenogelin to both PCI and PSA.[94] Semenogelin and PCI from the seminal vesicle, and PSA whose activity is inhibited by Zn^{2+} in the prostate, are mixed with spermatozoa at ejaculation. Coagulum established by the binding of Zn^{2+} to semenogelin, spontaneously traps the spermatozoa inside in it. It may be possible to consider that sperm motility is suppressed by SPMI which is suggested to be identical to semenogelin. A sudden decrease in Zn^{2+} activated PSA by release from inhibitory effect of the heavy metal ions, and the PSA cleaves the coagulum by gradual proteolysis of semenogelin. The spematozoa which is allowed to move by release from tight trapping by coagulum becomes motile during the process of liquefaction of the semen. The PSA activity will decrease by the inhibition of the enzyme, PCI. From these studies, a possible mechanism for semen coagulation and requefaction was proposed (Fig. 6).

Relaxin, a peptide with molecular masses of 6 kDa and originally described as a hormone of pregnancy,[95] has been detected in the seminal plasma of human, boar, bull, ram and hi-goat.[96–101] In the boar, relaxin is produced in the male accessory glands[102] and is secreted in the seminal plasma.[98–100] Receptors of the relaxin are present on spermatozoa,[103,104] distributing at the acrosomal cap surface, midpiece and tail in boar sperm.[104] Binding between the relaxins and their receptors is thought to promote sperm motility through cAMP

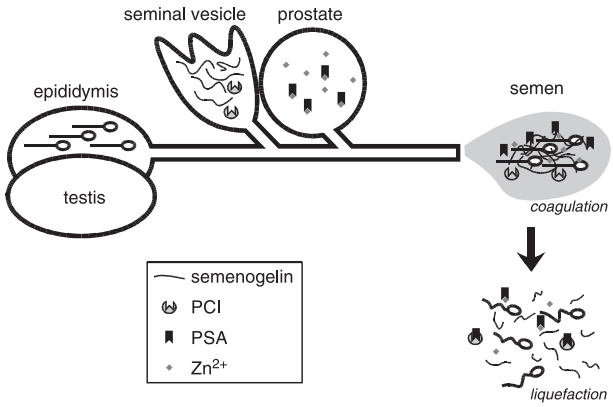

Figure 6 Coagulation and liquefaction of human semen by interaction of Sg, Zn^{2+} and PSA. A simplified diagram of the components of the male reproductive tract is shown. Semenogelin (Sg) and protein C inhibitor (PCI) in the seminal vesicles and prostate specific antigen (PSA) and zinc ions (Zn^{2+}) in the prostate are mixed at ejaculation. Binding of the Sg and zinc ions makes semen coagulation and traps the ejaculated sperm inside of the coagulum, in which the sperm are immobilized. Decrease in the concentration of Zn^{2+} in the semen by its binding to Sg, releases Zn^{2+}-dependent suppression of proteplytic activity of PSA. Activated PSA digests the Sg, resulting in liquefaction of the semen. The sperm released from coagulum become motile. PCI inactivates PSA to prevent further proteolysis of Sg.

synthesis in boar.[100,104,105] Promoted sperm motility by relaxin is reported in bovine sperm.[101]

Regulation of sperm motility by female factors

Hyperactivation of sperm motility with high-amplitude and asymmetric flagellar bending is one of the events of capacitation that occurs during transit of sperm through the female reproductive tract.[106] The capacitation is induced by only folicular fluid among follicular components.[107] Modification of such as plasma membrane lipid, membrane potential, intracellular Ca^{2+} and cAMP, and protein phosphorylation, are considered to be involved in the process of the sperm capacitation.[108–111] Cell signaling for induction of hyperactivating sperm motility is partially understood[15] as well as one for acrosome reaction capacity. In the course of hyperactivation of sperm motility, cholesterol may partially be removed from the plasma membrane, causing increase in intracellular pH and influx of HCO_3^- which activates a bicarbonate-dependent adenylyl cyclase.[112] Intracellular Ca^{2+} concentration increases through an unknown pathway. Phosphorylation of proteins by cAMP-dependnet protein kinase and Ca^{2+} may cause the hyperactivated sperm motility.[15]

Porcine follicular fluid contains proteinase inhibitors and the inhibitor increases the motility of porcine spermatozoa.[113,114] However, acrosin inhibitors exist in the mammalian seminal plasma, are considered to have significance in the fertilization through the inhibition of trypsin-like protease, acrosin,[115] although their biological roles are still remained unclear. The eggs of fish, the Pacific herring *Clupea pallasii* release proteinaceous sperm motility activation-factor into the surrounding seawater.[116] The factor, herring sperm-activating proteins (HSAP) with molecular mass of 8084 consisting of 73 amino acids has striking homology with the Kazal-type trypsin inhibitors.[117] The enzyme, prolylendopeptidase present in the herring sperm may be the receptor of HSAP, suggesting that binding of the protease and the protease inhibitor occurs in the activation of the herring sperm motility.[118] The fact that abundant proteinase inhibitors that are homologs of HSAP exist in the female and male reproductive tracts, and the inhibitors and HSAP participate in sperm motility and fertilization, suggests new physiological function of the proteinase inhibitors in vertebrate fertilization. When these mammalian sperm-activating factors from follicular fluid are identified, the relationship of sperm-activating factors of mammals and fish will be clarified, and the mechanism of establishment of sperm activation by eggs in vertebrates will be discussible.

Sperm activation and chemotaxis induced by factors included in the follicular fluid

Mammalian sperm ejaculated into the female reproductive tract remain motionless in the storage sites. Their motility is activated in order to reach the ovulated egg at fertilization. The nature of signaling for sperm activation and attraction is not known, but the phenomena may be caused by a factor released from the egg. Follicular fluid, granulosa cells and oviductal fluids surrounding the egg have been extensively studied in rats, humans and mice.[119–122] The surroundings of the egg influence sperm motility for example to cause accumulation and chemotactic behavior. There have been many proposed candidates of chemoattractants of mammalian sperm (Table 2). For humans as well as bulls, synthetic N-formylated peptides,[123,124] heparin,[125] progesterone[126] and atrial natriuretic peptide (ANP)[127] were suggested as a sperm chemoattractant. Progesterone has been thought to be a major candidate, as it is one of the main ingredients of follicular fluid, and it really causes

sperm accumulation. However, counter arguments of these ideas were shown as progesteron and ANP causes only a few changes in the direction of human sperm swimming that are characteristic of chemotaxis.[128] Ralt *et al*. proposed a possible occurrence of sperm chemotaxis to follicular fluid in humans.[129] However, the results of later studies were not consistent: sperm showed no chemotactic response to follicular fluid.[130] Seventy percent of sperm has a response to follicular fluid,[131] only 2–12% of the sperm population is responsive to follicular fluid.[132] Thus, the mammalian sperm attractants including human are still not identified, and the molecular mechanism of the chemotaxis is not known in mammalian including human.[133]

Testicular odorant receptor has been suggested to mediate chemotaxis of human sperm.[134] When the odorant receptor, hOR17-4, expressed in testicular tissue, was transfected into HEK293T cells, some odorants, cyclamal and bourgeonal, could induce increase in intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$ in the transfected cells. Bourgeonal could furthermore, induce $[Ca^{2+}]_i$ increase in human sperm, and the sperm showed chemotaxis toward the bougeonal. Recently, Fukuda *et al*. showed that another odorant, lyral, a cognate ligand of an olfactory receptor increases intracellular Ca^{2+} and induced sperm chemotaxis in mouse.[135] These suggest that mammalian sperm chemotaxis is mediated by some odorants and odorant receptor.

Our recent studies have found that follicular fluid contains factors activating sperm motility in human. The follicular fluid caused an increase in hyperactivation-like motility in the non-capacitated sperm within a short period after contact with the sperm. Percentages of motile sperm, velocity and amplitude of lateral head displacement increase, but linearity and beat/cross frequency did not change in the measurement of sperm behavior with CellSoft (Cryo Resources, NY, USA). These changes in parameter of hyperactivation-like sperm behavior were seen both in the sperm donated from young volunteers and in those from men visiting infertility clinic, but were conspicuous in the sperm from young healthy donors. The molecular mass of a factor in the follicular fluid that activates sperm motility may be less than 5 kDa. Further studies on purification and identification of the factor may open a new insight on the improvement of artificial insemination in humans.

CONCLUSIONS

THE CELL SIGNALINGS underlying regulation of sperm motility are various. Among them, mechanisms for the initiation of sperm motility have been well investigated in various species, although the subject is excluded from the current review. The external signals for the initiation of sperm motility may basically be changed in environmental inorganic atmosphere in the reproductive ground. Most reliable factors are the decrease in K^+ and increase or decrease in osmolality surrounding spawned teleost sperm, and the cell signaling for the initiation of sperm motility is well established.[5–7] The osmoregulatory system also contributes to the initiation of sperm motility in frog,[136] and osmolality and egg-jelly derived factor play key roles for the phenomenon in newt.[137] The effect of temperature on the initiation of sperm motility in chicken is obvious.[138]

Activation of sperm motility and chemotactic behavior of sperm, in contrast, are mainly caused by organic factors, amino acid, peptides and proteins, released from the eggs. Cell signalings for sperm activation and chemotaxis induced by peptides, speract and resact, are well investigated in sea urchins. The roles of proteins, HSAP and SMIF, for sperm activation and chemotaxis in the herring have been understood.[117,139]

However, SAAF in the ascidians is the non-protenaceous small molecule, a steroid. The SAAF-induced cell signaling being well established, is very unique as one molecule induces two cell signaling pathways, i.e. cAMP-dependent sperm activation and cAMP-independent chemotaxis. The problem, how one SAAF molecule can render stimuli for two different responses, activation and chemotaxis to a spermatozoon, remains to be solved.

Hyperpolarization of the plasma membrane through efflux of K^+ and subsequent cAMP synthesis fundamentally and largely contribute to the initiation of teleost sperm motility and sperm activation in the ascidian, sea urchin and mammals. In the ascidians increased Ca^{2+} in the sperm cell contributes to the membrane hyperpolarization through calmodulin/calmodulin kinase system. Roles of increased intracellular Ca^{2+} also fundamentally and largely contributes in the motility activation and chemotaxis of sperm in marine invertebrates, that is, sea urchins, ascidians, and in vertebrates, for example, fish[139] and mammals. The fact that chemoattractants modify the flagellar asymmetry in a Ca^{2+}-dependent manner in siphonophores and *Ciona*, suggests a role of the attractants in regulation of intracellular Ca^{2+}. Recent studies in the author's laboratory showed the contribution of store-operated Ca^{2+} channel in the phenomenon. The identification of target protein of the increased Ca^{2+} in the cell signaling underlying sperm chemotaxis is important for understanding the phenomenon.

Much evidence has accumulated in the regulatory mechanisms for motility activation and attraction of spermatozoa in marine invertebrates. However, our knowledge for understanding the regulatory mechanism of the phenomena in mammals especially in humans is quite limited, in spite of the announcement of many factors correlated to sperm motility. Semenogelin and relaxin has been proposed as the factors indispensable for suppression and stimulation of sperm motility, respectively, and roles of the factors for regulation of sperm motility are under investigation in humans and boar.

With regards to human sperm chemotaxis, several factors have been proposed as chemoattractants, however none have been identified. Despite that, ligands for sperm chemotaxis is not known in humans as well as other mammals. Interaction between odorants and the receptor was recently suggested to mediate Ca^{2+}-induced chemotaxis in human and mouse spermatozoa. Identification of ligand molecules for regulation of sperm chemotaxis in mammalian species, especially in humans, will open the future insight for understanding cell signaling for mammalian sperm chemotaxis.

REFERENCES

[1] Afzelius B. Electronmicroscopy of the sperm tail; results obtained with a new fixative. *J Biophys Biochem Cytol* 1959; **5**: 269–278.

[2] Gibbons IR. Cilia and flagella of eukaryotes. *J Cell Biol* 1981; **91**: 107S–124S.

[3] Morisawa M, Suzuki K. Osmolality and potassium ions: their roles in initiation of sperm motility in teleosts. *Science* 1980; **210**: 1145–1147.

[4] Morisawa M, Okuno M. Cyclic AMP induces maturation of trout sperm axoneme to initiate motility. *Nature* 1982; **295**: 703–704.

[5] Morisawa M. Initiation mechanism of sperm motility at spawning in teleosts. *Zool Sci* 1985; **2**: 605–615.

[6] Morisawa M. Cell signaling mechanisms for sperm motility. *Zool Sci* 1994; **11**: 647–662.

[7] Morisawa M, Oda S, Yoshida M, Takai H. Transmembrane signal transduction for the regulation of sperm motility in fishes and ascidians. *The Male Gamet from Basic Science to Clinical Application*. Cache River Press, Vienna, 1999; 149–160.

[8] Lillie FR. *Problems of Fertilization*. University of Chicago Press, Chicago, 1919.

[9] Ohtake H. Respiratory behavior of sea-urchin spermatozoa II. Sperm-activating substance obtained from egg jelly coat of sea-urchin eggs. *J Exp Zool* 1976; **198**: 313–322.

[10] Hansbrough JR, Garbers DL. Speract. Purification and characterization of a peptide associated with eggs that activates spermatozoa. *J Biol Chem* 1981; **256**: 1447–1452.

[11] Suzuki N. Structure and function of sea urchin egg jelly molecule. *Zool Sci* 1990; **7**: 355–370.

[12] Garbers DL. Molecular basis of fertilization. *Ann Rev Biochem* 1989; **58**: 719–742.

[13] Babcock DF, Bosma MM, Battaglia DE, Darszon A. Early persistent activation of sperm K^+ channels by the egg peptide speract. *Proc Natl Acad Sci USA* 1992; **89**: 6001–6005.

[14] Galindo BE, Beltran C, Cragoe EJ Jr, Darszon A. Participation of a K^+ channel modulated directly by cGMP in the speract-induced signaling cascade of *Strongylocentrotus purpuratus* sea urchin sperm. *Dev Biol* 2000; **221**: 285–294.

[15] Darszon A, Beltrán C, Ricardo F, Nishigaki T, Treviño CL. Ion transport in sperm signaling. *Dev Biol* 2001; **240**: 1–14.

[16] Wood CD, Darszon A, Whitaker M. Speract induces calcium oscillations in the sperm tail. *J Cell Biol* 2003; **161**: 89–101.

[17] Nishigaki T, Wood CD, Tatsu Y *et al.* A sea urchin egg jelly peptide induces a cGMP-mediated decrease in sperm intracellular Ca^{2+} before its increase. *Dev Biol* 2004; **272**: 376–388.

[18] Nishigaki T, Chiba K, Hoshi M. A 130-kDa membrane protein of sperm flagella is the receptor for asterosaps, sperm-activating peptides of starfish *Asterias amurensis*. *Dev Biol* 2000; **219**: 154–162.

[19] Matsumoto M, Solzin J, Helbig A *et al.* A sperm-activating peptide controls a cGMP-signaling pathway in starfish sperm. *Dev Biol* 2003; **260**: 314–324.

[20] Yoshida M, Inaba K, Morisawa M. Sperm chemotaxis during the process of fertilization in the ascidians *Ciona savignyi* and *Ciona intestinalis*. *Dev Biol* 1993; **157**: 497–506.

[21] Izumi H, Marian T, Inaba K, Oka Y, Morisawa M. Membrane hyperpolarization by sperm-activating and attracting factor increases cAMP level and activates sperm motility in the ascidian *Ciona intestinalis*. *Dev Biol* 1999; **213**: 246–256.

[22] Yoshida M, Inaba K, Ishida K, Morisawa M. Calcium and cyclic AMP mediate sperm activation, but Ca^{2+} alone contributes sperm chemotaxis in the ascidian, *Ciona savignyi*. *Dev Growth Differ* 1994; **36**: 589–595.

[23] Nomura M, Yoshida M, Morisawa M. Calmdulin/calmodulin dependent protein kinase II mediates SAAF-induced motility activation of ascidian sperm. *Cell Motil Cytoskelt* 2004; **59**: 28–37.

[24] Nomura M, Inaba K, Morisawa M. Cyclic AMP- and calmodulin-dependent phosphorylation of 21 and 26 kDa proteins in axoneme is a prerequisite for SAAF-induced motile activation in ascidian spermatozoa. *Dev Growth Differ* 2000; **42**: 129–138.

[25] Dan JC. Fertilization in the medusan, *Spirocodon saltatrix*. *Biol Bull* 1950; **99**: 412–415.

[26] Miller RL. Sperm chemo-orientation in metazoa. *Biology of Fertilization*. New York, Academic Press, 1985; 257–337.

[27] Ward GE, Brokaw CJ, Garbers DL, Vacquier VD. Chemotaxis of *Arbacia punctulata* spermatozoa to resact, a peptide from the egg jelly layer. *J Cell Biol* 1985; **101**: 2324–2329.

[28] Punnett T, Miller RL, Yoo B-H. Partial purification and some chemical properties of the sperm chemoattractant

from the forcipulate starfish *Pycnopodia helianthoides* (Brandt, 1835). *J Exp Zool* 1992; **262**: 87–96.

[29] Cosson MP, Carré D, Cosson J. Sperm chemotaxis in siphonophores. II. Calcium-dependent asymmetrical movement of spermatozoa induced by attractant. *J Cell Sci* 1984; **68**: 163–181.

[30] Riffell JA, Krug PJ, Zimmer RK. Fertilization in the sea: the chemical identity of an abalone sperm attractant. *J Exp Biol* 2002; **205**: 1439–1450.

[31] Zatylny C, Marvin L, Gagnon J, Henry J. Fertilization in Sepia officinalis: the first mollusk sperm-attracting peptide. *Biochem Biophys Res Comm* 2002; **296**: 1186–1193.

[32] Cosson J, Carré D, Cosson MP. Sperm chemotaxis in siphonophores: identification and biochemical properties of the attractant. *Cell Motil Cytoskeleton* 1986; **6**: 225–228.

[33] Coll JC, Bowden BF, Meehan GV *et al.* Chemical aspects of mass spawning in corals. I. Sperm-attractant molecules in the eggs of the scleractinian coral *Montipora digitata*. *Mar Biol* 1994; **118**: 177–182.

[34] Yanagimachi R, Cherr GN, Pillai MC, Baldwin JD. Factors controlling sperm entry into the micropyles of salmonid and herring eggs. *Dev Growth Differ* 1992; **34**: 447–461.

[35] Olson JH, Xiang X, Ziegert T *et al.* Allurin, a 21-kDa sperm chemoattractant from *Xenopus* egg jelly, is related to mammalian sperm-binding proteins. *Proc Natl Acad Sci USA* 2001; **98**: 11205–11210.

[36] Yoshida M, Murata M, Inaba K, Morisawa M. A chemoattractant for ascidian spermatozoa is a sulfated steroid. *Proc Natl Acad Sci USA* 2002; **99**: 14831–14836.

[37] Oishi T, Tuchikawa H, Murata M, Yoshida M, Morisawa M. Synthesis of endogenous sperm-activating and attracting factor isolated from ascidian *Ciona intestinalis*. *Tetrahedron Lett* 2003; **44**: 6387–6389.

[38] Oishi T, Tsuchikawa H, Murata M, Yoshida M, Morisawa M. Synthesis and identification of an endogenous sperm activating and attracting factor isolated from eggs of the ascidian *Ciona intestinalis*; an example of nanomolar-level structure elucidation of novel natural compound. *Tetrahedron* 2004; **60**: 6971–6980.

[39] Miller RL, Brokaw CJ. Chemotactic turning behaviour of *Tubularia* spermatozoa. *J Exp Biol* 1970; **52**: 699–706.

[40] Miller RL. Sperm chemotaxis in ascidians. *Am Zool* 1982; **22**: 827–840.

[41] Ishikawa M, Tsutsui H, Cosson J, Oka Y, Morisawa M. Strategies for sperm chemotaxis in the siphonopores and ascidians; A numerical stimulation study. *Biol Bull* 2004; **206**: 95–102.

[42] Miller RL. Chemotaxis of the spermatozoa of *Ciona intestinalis*. *Nature* 1975; **254**: 244–245.

[43] Singh S, Lowe DG, Thorpe DS *et al.* Membrane guanylate cyclase is a cell-surface receptor with homology to protein kinases. *Nature* 1988; **334**: 708–712.

[44] Cook SP, Brokaw CJ, Muller CH, Babcock DF. Sperm chemotaxis: Egg peptides control cytosolic calcium to regulate flagellar responses. *Dev Biol* 1994; **165**: 10–19.

[45] Quill TA, Ren D, Clapham DE, Garbers DL. A voltage-gated ion channel expressed specifically in spermatozoa. *Proc Natl Acad Sci USA* 2001; **98**: 12527–12531.

[46] Ren D, Navarro B, Perez G *et al.* A sperm ion channel required for sperm motility and male fertility. *Nature* 2001; **413**: 603–609.

[47] Kaupp UB, Solzin J, Hildebrand E *et al.* The signal flow and motor response controling chemotaxis of sea urchin sperm. *Nat Cell Biol* 2003; **5**: 109–117.

[48] Yoshida M, Ishikawa M, Izumi H, De Santis R, Morisawa M. Store-operated calcium channel regulates the chemotactic behavior of ascidian sperm. *Proc Natl Acad Sci USA* 2003; **100**: 149–154.

[49] Morton B, Chang TSK. The effect of fluid from the cauda epididymis, serum components and caffein upon the survival of diluted epididymal hamster spermatozoa. *J Reprod Fertil* 1973; **35**: 255–263.

[50] Bavister BD, Roger BJ, Yanagimachi R. The effects of dauda epididymal plasma on the motility and acrosome reaction of hamster and guinea pig spermatozoa in vitro. *Biol Reprod* 1978; **19**: 358–368.

[51] Sheth AR, Gunjikar AN, Shank GV. The presence of progressive motility sustaining factor (PMSF) in human epididymis. *Andrologia* 1981; **13**: 142–146.

[52] Acott TS, Hoskins DD. Bovine sperm forward sperm motility protein: partial purification. *J Biol Chem* 1978; **253**: 6744–6750.

[53] Usselman MC, Cone RA. Rat sperm are mechanically immobilized in the cauda epididymis by immobilin, a high molecular weight glycoprotein. *Biol Reprod* 1983; **29**: 1241–1253.

[54] Carr DW, Usselman MC, Acott TS. Effects of pH, lactate, and viscoelastic drag on sperm motility: a species comparison. *Biol Reprod* 1985; **33**: 588–595.

[55] Lindholmer C. Survival of human spermatozoa in different functions of split ejaculate. *Fertil Steril* 1973; **24**: 521–526.

[56] Sanada S, Arai E, Ueda M, Ogura K, Yoshida O. Long term effects of human seminal plasma from whole semen and from different functions of split ejaculate on motility of human ejaculated spermatozoa. *Acta Urol Jpn* 1988; **34**: 165–1972. (In Japanese).

[57] Rozin S. Studies on seminal plasma I. The role of seminal plasma in motility of spermatozoa. *Fertil Steril* 1960; **11**: 278–285.

[58] Lindholmer C. The importance of seminal plasma for human sperm motility. *Biol Reprod* 1974; **10**: 533–542.

[59] Ranta T, Sillteri JE, Koistinen HT, Salem HT, Koskimies AI, Seppala M. Human seminal plasma contains a protein that shares physicochemical and immunochemical properties with placental protein 5 from the human placenta. *J Clin Endocrinol Metab* 1981; **53**: 1087–1089.

[60] Lee J-N, Lian J-D, Lee J-H, Chard T. Placental protein (human chorionic gonadotropin, human placental lactogen, pregnancy-specific beta 1-glycoprotein, and placental protein 5), in seminal plasma of normal men and patients with infertility. *Fertil Steril* 1983; **39**: 704–706.

[61] Bolton AE, Pinto-Furtado LG, Andrew CE, Chapman MG. Measurement of the pregnancy-associated placental protein 14 and pregnancy associated plasma protein A in human seminal plasma. *Clin Reprod Fertil* 1986; **4**: 233–240.
[62] Cohen MS, Colin MJ, Golimbu M, Hotchkiss RS. The effect of prostaglandins on sperm motility. *Fertil Steril* 1977; **28**: 78–85.
[63] Peitz B, Old-clarke P. Effects of seminal vesicle removal on fertility and uterine sperm motility in the house mouse. *Biol Reprod* 1986; **35**: 608–617.
[64] Peitz B. Effect of seminal vesicle fluid components on sperm motility in the mouse. *J Reprod Fert* 1988; **83**: 169–176.
[65] Ashizawa K, Okauchi K. Stimulation of sperm motility and oxygen consumption of fowl spermatozoa by low molecular weight fraction of seminal plasma. *J Reprod Fert* 1984; **71**: 593–598.
[66] Dott HM. The effects of bovine seminal plasma on the impedance change frequency and glycolysis of bovine epididymal spermatozoa. *J Reprod Fert* 1974; **38**: 147–156.
[67] Dott HM, Harrison RAP, Foster GCA. The maintenance of motility and the surface properties of spididymal spermatozoa from bull, rabbit and ram in homologous seminal and epididymal plasma. *J Reprod Fertil* 1979; **55**: 113–124.
[68] Bass JW, Molan PC, Shannon P. Factors in seminal plasma of bull that affect the viability and motility of spermatozoa. *J Reprod Fert* 1983; **68**: 275–280.
[69] Stoltenberg M, Sorensen MB, Danscher G. Histochemical demonstration of zinc ions in ejaculated human semen. *Int J Androl* 1997; **20**: 229–236.
[70] Stoltenberg M, Sorensen MB, Danscher G, Juhl S, Andreasen A, Ernst E. Autometallographic demonstration of zinc ions in rat sperm cells. *Mol Human Reprod* 1997; **3**: 763–767.
[71] Sorensen MB, Bergdahl IA, Hjollund NHI, Bonde JPE, Stoltenberg M, Ernst E. Zinc, magnesium and calcium in human seminal fluid. relations to other semen parameter and fertility. *Mol Human Reprod* 1999; **5**: 331–337.
[72] Andrew JC, Nolan JP, Hammerstedt RH, Bavister BD. Role of zinc during hamster sperm capacitation. *Biol Reprod* 1994; **51**: 1238–1247.
[73] Henkel R, Bitter J, Weber R, Hunter F, Miska W. Relevance of zinc in human sperm flagella and its relation to motility. *Fertil Steril* 1999; **71**: 1138–1143.
[74] Lindholmer C. Toxicity of zinc ions to human spermatoatozoa and the influence of albumin. *Andrologia* 1974; **6**: 7–15.
[75] Lindholmer C, Eliasson R. The effects of albumin, magnesium and zinc on human sperm survival in different fractions of split ejaculates. *Fertil Steril* 1974; **25**: 424–431.
[76] Sheth AR, Mugatuwala PP, Shah GV, Rao SS. Occurrence of prolactin in human semen. *Fertil Steril* 1975; **26**: 905–907.
[77] Smith M, Lugman WA, Rakoff JS. Correlation between seminal radioimmunoreactive prolactin, sperm count, and sperm motility in pre-vasectomy and infertility clinic patients. *Fertil Steril* 1979; **32**: 312–315.
[78] Biswas S, Fergson KM, Stedronska J, Baffoe G, Mansfield MD, Kosbab MH. Fructose and hormone levels in semen: their correlation with sperm counts and motility. *Fertil Steril* 1978; **30**: 200–204.
[79] Sueldo CE, Berger T, Kletzky O, Marrs RP. Seminal prolactin concentration and sperm reproductive capacity. *Fertil Steril* 1985; **43**: 632–635.
[80] Kucukkomurcu S, Delogne-Desnoeck J, Robyn C. Prolactin and fructose in human seminal fluid. *Int J Fertil* 1980; **25**: 117–121.
[81] Shah GV, Desai RB, Sheth AR. Effect of prolaction in metabolism of human spermatozoa. *Fertil Steril* 1976; **27**: 1292–1294.
[82] Shah GV, Sheth AR. Is prolactin involved in sperm capacitation? *Med Hypothesis* 1979; **5**: 909–914.
[83] Garbers DL, Lust WD, First NL, Lardy HA. Effect of phosphodiesterase inhibitors and cyclic nucleotides on sperm respiration and motility. *Biochemistry* 1971; **10**: 1825–1831.
[84] Lindemann CB. A cAMP-induced increase in the motility of demembranated bull sperm models. *Cell* 1978; **13**: 9–18.
[85] Chaistitvanich N, Boonsaeng V. Molecular structure of human seminal coagulum: the role of disulfide bonds. *Andrologia* 1983; **15**: 446–451.
[86] Lilja H, Abrahamsson PA, Lundwall A. Semenogelin, the predominant protein in human semen. Primary structure and identification of closely related protein in the male accessory sex glands and on the spermatozoa. *J Biol Chem* 1989; **264**: 1894–1900.
[87] Robert M, Gibbs BF, Jacobson E, Gagnon C. Characterization of prostate-specific antigen proteolytic activity on itrs major physiological substrate, the sperm motility inhibitor precursor/semenogelin I. *Biochemistry* 1997; **36**: 3811–3819.
[88] Lilja H, Oldbring J, Rannevik G, Laurell CB. Seminal vesicle-secreted proteins and their reactions during gelation and liquefaction of human semen. *J Clin Invest* 1987; **80**: 281–285.
[89] De Lamirande E, Bardin CW, Gagnon C. Aprotinin and a seminal plasma factor inhibit the motility of demembranated reactivated rabbit spermatozoa. *Biol Reprod* 1983; **28**: 788–796.
[90] Iwamoto T, Gagnon C. Purification and characterization of a sperm motility inhibitor in human seminal plasma. *J Androl* 1988; **9**: 377–383.
[91] Iwamoto T, Gagnon C. A human seminal plasma protein blocks the mtility of human spermatozoa. *J Urol* 1988; **140**: 1045–1048.
[92] Robert M, Gagnon C. Purification and characterization of the active precursor of a human sperm motility inhibitor secreted by the seminal vesicle: identity with semenogelin. *Biol Reprod* 1996; **55**: 813–821.
[93] Frenette G, Tremblay RR, Dube JY. Zinc binding to major human seminal plasma coagulum protein. *Arch Androl* 1989; **23**: 155–163.
[94] Kise H, Nishioka J, Kawamura J, Suzuki K. Characterization of semenogelin II and its molecular interaction with prostate-specific antigen and protein C inhibitor. *Eur J Biochem* 1996; **238**: 88–96.

[95] Sherwood OD. Relaxin. In: Knobil E, Neill JD, eds. *The Physiology of Reproduction*, Vol. 3, 2nd edn. Raven Press, New York, 1994; 861–1009.

[96] De Cooman S, Gilliaux P, Thomas K. Immunoreactive relaxin-like substance in split ejaculates. *Fertil Steril* 1983; **39**: 111–113.

[97] Schieferstein G, Voelter W, Seeger H, Lippert TH. Immunoreactive relaxin in seminal plasma of men. *Int J Fert* 1989; **34**: 215–218.

[98] Kohsaka T, Kobayashi M, Muraki T, Sato T, Sasada H, Masaki J. Male relaxin. Evidence for relaxin immunoreactivity in the reproductive organs of boar. In: *11th International Congress of Animal Reproduction and Artificial Insemination (Proceedings 375–377)*, June 1988, Dublin, Ireland. Congress Committee, Dublin, 1988.

[99] Juang HH, Musah AI, Schwabe C, Anderson LL. Immunoreactive relaxin in boar seminal plasma and its correlation with sperm motility. *Anim Reprod Sci* 1990; **22**: 47–53.

[100] Sasaki Y, Kohsaka T, Kawarasaki T *et al.* Immunoreactive relaxin in seminal plasma of fertile boars and its correlation with sperm motility characteristics determined by computer-assisted digital image analysis. *Inter J Androl* 2001; **24**: 24–30.

[101] Kohsaka T, Hamano K, Sasada H *et al.* Seminal immunoreactive relaxin in domestic animals and its relationship to sperm motility as possible index for predicting the fertilizing ability of sires. *Int J Androl* 2003; **26**: 115–120.

[102] Kohsaka T, Takahara H, Sasada H *et al.* Evidence for immunoreactive relaxin in boar seminal vesicle using combined light and electron microscope immunocytochemistry. *J Reprod Fertil* 1992; **95**: 397–408.

[103] Carrell DT, Peterson CM, Urry RL. The binding of recombinant human relaxin to human spermatozoa. *Endcrine Res* 1995; **21**: 697–707.

[104] Kohsaka T, Sasasda H, Takahara H, Sato E, Baba K, Sherwood OD. The presence of specific sites on boar spermatozoa for porcine relaxin and its action on their motility characteristics. *J Reprod Dev* 2001; **47**: 197–204.

[105] Park JM, Ewing K, Miller F, Fredman CI, Kim MH. Effects of relaxin on the fertilization capacity of human spermatozoa. *Am J Obstet Gynecol* 1988; **158**: 974–979.

[106] Yanagimachi R. Mammalian fertilization. In: Knobil E, Neill JD, eds. *The Physiology of Reproduction*: Raven Press, New York, 1994; 189–317.

[107] Yanagimachi R. In vitro capacitation of hamster spermatozoa by folicular fluid. *J Reprod Fert* 1969; **18**: 275–286.

[108] Visconti PE, Galantino-Homer H, Ning X *et al.* Cholesterol efflux-mediated signal transduction in mammalian sperm. Beta-cyclodextrins initiate transmembrane signaling leading to an increase in protein tyrosine phosphorylation and capacitation. *J Biol Chem* 1999; **274**: 3235–3242.

[109] Arnoult C, Kazam IG, Visconti PE, Kopf GS, Villaz M, Floman HM. Control of the low voltage-activated calcium channel of mouse sperm by egg ZP3 and by membrane hyperpolarization during capacitation. *Proc Natl Acad Sci USA* 1999; **96**: 6757–6762.

[110] Baldi E, Luconi M, Bonaccorsi L, Muratori M, Forti G. Intracellular events and signaling pathways involved in sperm acquisition of fertilizing capacity and acrosome reaction. *Front Biosci* 2000; **5**: E110–E123.

[111] Flesch FM, Gadella BM. Dynamics of the mammalian sperm plsma membrane in the process of fertilization. *Biochim Biophys Acta* 2000; **1469**: 197–235.

[112] Okamura N, Tajima Y, Soejima A, Sugita Y. Sodium bicarbonate in seminal plasma stimulates the motility of mammalian spermatozoa through direct activation of adenylate cyclase. *J Biol Chem* 1985; **260**: 9699–9705.

[113] Lee S-L, Kuo Y-M, Kao C-C, Hong C-Y, Wei Y-H. Purification of a sperm motility stimulator from porcine follicular fluid. *Comp Biochem Physiol* 1992; **101**: 591–594.

[114] Jeng H, Lin K-M, Chang W-C. Purification and characterization of reversible sperm motility inhibitor from porcine seminal plasma. *Biochem Biophys Res Commun* 1993; **191**: 435–440.

[115] Fritz H, Schleuning WD, Schiessler H, Schill WB, Wendt V, Winkler G. Boar, bull and human sperm acrosin-isolation, properties and biological aspects. In: Reich E, Ritkin DB, Shaw E, eds. *Proteases Bio Control*. Cold Spring Harbor, New York 1975; 715–735.

[116] Morisawa M, Tanimoto S, Ohtake H. Characterization and partial purification of sperm-activating substance from eggs of the herring, *Clupea pallasi*. *J Exp Zool* 1992; **264**: 225–230.

[117] Oda S, Igarashi Y, Manaka K *et al.* Sperm-activating proteins obtained from the herring egg are homologous to trypsin inhibitors and synthesized in follicle cells. *Dev Biol* 1998; **204**: 55–63.

[118] Yoshida K, Inaba K, Ohtake H, Morisawa M. Purification and characterization of prolyl endopeptidase from the Pacific herring, *Clupea pallasi*, and its role in the activation of sperm motility. *Devlop Growth Differ* 1999; **41**: 217–225.

[119] Moricard R, Bossu J. Arrival of fertilyzing sperm at the follicular cell of the secondary oocyte. *Fert Steril* 1951; **2**: 260–266.

[120] Schwartz R, Brooks W, Zinsser HH. Evidence of chemotaxis as a factor in sperm motility. *Fertil Steril* 1957; **9**: 300–308.

[121] Bronson R, Hamada Y. Gamete interactions *in vitro*. *Fertil Steril* 1977; **28**: 570–576.

[122] Oliveira RG, Tomasi L, Rovasio RA, Giojalas NLC. Increased velocity and induction of chemotactic response in mouse spermatozoa by follicular and oviductal fluids. *J Reprod Fertil* 1999; **115**: 23–27.

[123] Vijayasarathy S, Shivaji S, Iqbal M, Balaram P. Formyl-Met-Lau-Phe induces chemotaxis and acrosomal enzyme release in bull sperm. *FEBS Lett* 1980; **115**: 178–180.

[124] Gnessi L, Ruff MR, Fraioli F, Pert CB. Demonstration of receptor-mediated chemotaxis by human spermatozoa a novel quantitative bioassay. *Exp Cell Res* 1985; **161**: 219–230.

[125] Sliwa L. Effect of heparin on human spermatozoa migration in vitro. *Arch Androl* 1993; **30**: 177–181.

[126] Villanueva-Diaz C, Arias-Martinez J, Bermejo-Martinez L,

Vadillo-Ortega F. Progesteron induces human sperm chemotaxis. *Fertil Steril* 1995; **64**: 1183–1188.

[127] Anderson RA, Feathergili KA, Rawlins RG, Mack SR, Zaneveld LJD. Atrial natriuretic peptide: a chemoattractant of human spermatozoa by a guanylate cyclase-dependent pathway. *Mol Reprod Dev* 1995; **40**: 371–378.

[128] Jaiswal BS, Tur-Kaspa I, Dor J, Mashiach S, Eisenbach M. Human sperm chemotaxis: is progesterone a chemoattractant? *Biol Reprod* 1999; **60**: 1314–1319.

[129] Ralt D, Goldenberg M, Fetterolf P *et al.* Sperm attraction of follicular factor(s) correlates with human egg fertilizability. *Poc Natl Acad Sci USA* 1991; **88**: 2840–2844.

[130] Makler A, Reichler A, Stoller J, Feigin PD. A new model for investigating in real-time the existence of chemotaxis in human spermatozoa. *Fert Steril* 1992; **57**: 1066–1074.

[131] Villanueva-Diaz C, Diaz-Perez MDIA, Vadillo-Ortega F, Krivitzky SK, Kably-Ambe A. Evidence that human follicular fluid contains a chemoattractant for spermatozoa. *Fertil Steril* 1990; **54**: 1180–1182.

[132] Cohen-Dayag A, Ralt D, Tur-Kaspa I *et al.* Sequential acquisition of chemotactic responsiveness by human spermatozoa. *Biol Reprod* 1994; **50**: 786–790.

[133] Eisenbach M, Tur-Kaspa I. Do human eggs attract spermatozoa? *Bioessays* 1999; **21**: 203–210.

[134] Spehr M, Gisselmann G, Poplawski A *et al.* Identification of a testicular odorant receptor mediating human sperm chemotaxis. *Science* 2003; **299**: 2054–2058.

[135] Fukuda N, Yomogida K, Okabe M, Touhara K. Functional characterization of a mouse testicular olfactory receptor and its role in chemosensing and in regulation of sperm motility. *J Cell Sci* 2004; **117**: 5835–5845.

[136] Inoda T, Morisawa T. Effect of osmolality on the initiation of sperm motility in *Xenopus laevis*. *Comp Biochem Physiol* 1987; **88A**: 539–542.

[137] Ukita M, Itoh T, Watanabe T, Watanabe A, Onitake K. Substances for the initiation of sperm motility in egg-jelly of the Japanese newt, *Cynopus pyrrhogaster*. *Zool Sci* 1999; **16**: 793–802.

[138] Ashizawa K, Maeda S, Okauchi K. The mechanisms of reversible immobilization of fowl spermatozoa at body temperature. *J Reprod Fert* 1989; **86**: 271–276.

[139] Vines CA, Yoshida K, Griffin FJ *et al.* Motility initiation in herring sperm is regulated by reverse sodium-calcium exchange. *Proc Natl Acad Sci USA* 2002; **99**: 2026–2031.