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Review
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Review

Sperm acrosome reaction: its site and role in fertilization†

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Abstract

Manner and roles of sperm acrosome reaction in a variety of animals were compared.

Summary Sentence

Key words: acrosome, acrosome reaction, egg, sperm, sperm–egg fusion.

Nearly two centuries after Antoine van Leeuwenhok (1677) first illustrated human spermatozoa using a simple microscope (magnifier) [1], a German zoologist, Oscar Hertwig (1876), and a Swiss zoologist, Herman Fol (1876), independently described the details of sperm entry into starfish and sea urchin eggs. Fol perhaps saw the acrosome process of starfish spermatozoa, but he misinterpreted it as a projection from the egg. We now know that spermatozoa of the starfish and many other species undergo a profound structural change, called the acrosome reaction (AR), before they fertilize. It was Jean Clark Dan (1952) who first documented the AR in the sea urchin [2]. Today, nearly 10 000 scientific papers are listed in PubMed database under keywords, "acrosome" or "acrosome reaction." The acrosome is seen in a diverse array of animal species from hydrozoans to humans [3]. It is now widely accepted that the AR is a regulated exocytotic event that occurs in response to certain stimuli. This led us to analyze the molecular basis of the AR in this review. Although several sperm receptors functioning as the AR-triggering substances have been proposed, none of them is unequivocal. It should be noted that the modes and consequences of the AR vary greatly among different species due to the uniqueness in the structure of egg investments as well as differences in the site of fertilization (e.g., open sea vs. within females' bodies).

The discovery of the acrosome and the acrosome reaction

The acrosome is a membrane-bound organelle of Golgi apparatus origin, commonly located at the tip of the head of mature spermatozoon. It was once called "apical body" because of its location, or "perforatorium" on the assumption that it might assist the spermatozoon boring into the egg [4]. Dan [2] was the first who clearly documented that sea urchin spermatozoa undergo a profound structural change in their acrosomes before fertilization. Using both phase-contrast and electron microscopies, she found that the sea urchin spermatozoon protrudes a filament-like structure (acrosomal process) from its tip upon contact with egg-water (seawater containing secretion of the egg's jelly coat). She stated: "*The spermatozoon actively swimming through the jelly coat responds to the chemical stimulation of the jelly substance by a breakdown of the membrane covering the front part of the acrosome, so that by the time the sperm reaches the egg surface, it carries at its tip a mass of freshly exposed lysin that facilitates penetration of the vitelline membrane as the first step in the fertilization process.*" This reaction, coined the AR by Dan, requires extracellular Ca^{2+} [5]. It is important to note that the AR of sea urchin spermatozoa can be triggered by alkaline seawater (pH 9) or direct contact with a solid surface [2], reminding us that

simple physiological or even physical conditions must be taken into consideration when we study this biological event.

Four roles of the sperm acrosome reaction in invertebrates

Acrosomes have been observed in many animal phyla including Arthropoda (shrimps, crabs, and horseshoe crabs), Mollusca (bivalves and sea snails), Annelida (polychaete worms), Echinoderma (feather stars, starfish, sea cucumbers, brittle stars, and sea urchins), Cephalochordata (amphioxus), Chordata (hagfish and lampreys), and Vertebrata (some fish, amphibians, reptiles, birds, mammals). In Cnidaria, a primitive form of the acrosome, small vesicles situated between the nucleus and the plasma membrane are found in marine hydrozoans such as *Tubularia*, *Hydractinia*, *Clava*, *Campanularia*, and *Pennaria* [3]. In some taxa such as sea anemones, nematodes, teleost fishes, the acrosome is absent. Obviously, acrosomes and the AR are not of absolute necessity for fertilization in some species. In ascidians, besides the AR [6], spermatozoa passing through the egg vitelline coat undergo a series of distinct structural changes such as swelling, translocation, and shedding of mitochondria, which is called "sperm reaction" [7, 8].

In teleost fish, the egg's envelope commonly called the chorion has a micropylar cannel, through which the fertilizing spermatozoon enters to reach the surface of egg plasma membrane [9]. It can be postulated that the evolution of a micropyle led teleost fish to abolish acrosomes. In other words, it could be perceived that the primary role of the AR is to assist a fertilizing spermatozoon in reaching the egg plasma membrane when the egg is surrounded and protected by a "tough" extracellular coat. In good agreement with this, nematodes (*C. elegans*) and sea anemones (*Actinia fragacea*) do not have any recognizable egg envelopes [10, 11] and coincidentally, their spermatozoa do not have well-defined acrosomes [12]. However, this cannot be generalized because in some species such as amphioxus, sturgeon and paddlefish, squids and insects, their spermatozoa and eggs possess acrosomes [13, 14] and micropyles, respectively, suggesting that the AR may have an alternative role(s) other than egg-coat penetration in these species. In *Drosophila*, an acrosome-intact sperm enters the egg followed by sperm plasma membrane breakdown, egg activation, sperm nuclear decondensation, and aster formation. Mutant males lacking the acrosomal membrane protein *snky* are sterile due to the inability of sperm plasma and acrosomal membranes to breakdown within egg cytoplasm [15].

The acrosomal vesicle contains substances that facilitate particular processes of fertilization. In abalones, for example, the sperm acrosomes contain *lysin*, a 16-kDa protein which nonenzymatically and species selectively creates a hole in the egg vitelline envelope through which the spermatozoon passes to reach the egg plasma membrane [16]. In sea urchins, an insoluble content of the acrosomal granule, known as a 30.5-kDa protein bindin, mediates species-specific binding of spermatozoa to the vitelline layer as well as sperm fusion with the egg plasma membrane [17, 18]. Fine electron microscopic studies as well as video microscopy mostly carried out during 1960s and 1970s using marine invertebrates such as sea urchins, starfish, horseshoe crabs (*Limulus*) and bivalves (*Mytilus*) identified an elongation of the acrosomal process as the result of actin polymerization at the apical tip of sperm head followed by discharge of acrosome contents (acrosome exocytosis) [19].

In general, AR-associated morphological changes are much more prominent in marine invertebrates than in mammals. An extreme example is the sea cucumber, *Thyone briareus*, where the spermatozoon protrudes a 90-μm-long actin filament within 10 s after its contact with egg's jelly coat [20]. The fertilizing spermatozoon makes contact with the egg plasma membrane using the tip of acrosomal process. In starfish, a ball-shaped sperm head that adheres to the periphery of a thick gelatinous coat called egg jelly (EJ) protrudes a very long process, by which the head of fertilizing spermatozoon is rapidly "dragged" into the egg cortex [21]. Sperm head is then pulled into the egg without significant flagellar movement. In the sea urchin, *Strongylocentrotus purpuratus*, EJ contains sulfated fucose homopolymers (FSPs) [22] that induces the AR. EJs of closely related species also contain structurally very similar but not identical FSPs, which explains species preferentiality of the AR [23, 24]. In starfish, *Asterias amurensis*, AR-inducing substance (ARIS) also consists of sulfated polysaccharides with various sugar compositions [25]. Hence, spermatozoa must have receptors for these sulfated polysaccharides. In sea urchins, the best available evidence supports a model in which spermatozoa bind FSP via a protein called receptor for EJ (REJ-1), and this binding triggers the AR [26].

When species from multiple taxa, mostly marine broadcast spawners, are compared, we are reminded that the AR has at least four different roles. First, the acrosome contents assist sperm adherence to and/or penetration through the egg coat such as a proteinaceous envelope, a glycan-rich matrix, and in some cases a cellular (follicle cell) layer. Second, the newly exposed inner acrosomal membrane is a fusogenic membrane that may contain specific molecules that may enable the spermatozoon to fuse with egg plasma membrane. Third, the perforatorium (or acrosomal process) exposed or formed after the AR facilitates disruption of a physical barrier surrounding the egg with or without assistance from sperm's flagellar propulsion or enzymes contained in the acrosome. Fourth, the acrosomal content(s) may be involved egg activation [15].

It is conceivable that the AR is a process that makes the spermatozoon fusible with the egg proper. Since 1952, marine invertebrates (e.g., sea urchin) have contributed greatly to our understanding of animal sperm AR. In sea urchins and perhaps most other marine invertebrates, acrosome-reacted spermatozoa after treatment with EJ lose their fertilization competence within a few minutes [27]. This led to the belief that the AR must occur very rapidly, like "lighting a match," at the right place and right time. A large number of molecules which were believed to be key players in the AR and sperm–egg interactions [28] must now be re-examined by using genome-editing technologies, e.g., TALEN and CRISPR/Cas9, which recently have become available [29, 30].

In contrast to marine invertebrates with broadcast spawning and external fertilization, fertilization in many terrestrial and semi-terrestrial animals (insects, mollusks, newts, salamanders, reptiles, birds, and mammals) takes place within female's body. Before we discuss the mammalian sperm AR, we will first consider the AR in nonmammalian vertebrates with external and internal fertilization.

The sperm acrosome reaction in fish

Spermatozoa of common fish (teleosts) like trout, flounder, and zebrafish do not have acrosomes. The egg's thick envelope (chorion) has a thin tapered canal (micropyle) through which a fertilizing spermatozoon reaches the surface of egg (plasma membrane). The spermatozoon that enters the micropyle first fuses with the egg. The second and succeeding spermatozoa are all blocked or pushed out of the micropyle following egg activation. Non-teleost fish, such as lamprey and sturgeon, are different from higher bony fishes.

Lamprey spermatozoa have acrosomes but there is not a micropyle. The fertilizing spermatozoon seems to undergo the AR after its contact with the chorion in the animal pole of the egg. Interestingly, the chorion of this region of the egg has a gelatinous tuft through which many spermatozoa reach the chorion surface perpendicularly. It was found that the tuft is not essential, but its presence facilitates fertilization [31]. Hagfish also possess sperm with large acrosomes that contain actin, and undergo filament extension during the AR-like invertebrate sperm [32]. Sturgeon spermatozoa also have acrosomes that produce a long filament. Eggs have numerous micropyles (up to 25) at the animal pole [33]. The AR, which is similar to invertebrate sperm with the long acrosomal process (10 μm) containing actin filaments, seems to occur at the upper 1/3 of the micropylar canal where a glycoconjugate with AR-inducing activity is localized [34]. It must be the tip of the inner acrosomal membrane [34] that fuses with the egg plasma membrane. It has been suggested that numerous micropyles exist due to the broadcast spawning that occurs in sturgeon and they facilitate fertilization. It is unknown why sperm possess acrosomes however. Perhaps because sturgeon is ancient, their sperm have yet to lose the acrosome [35].

The sperm acrosome reaction in amphibians

In all gymnophiona (caecillians), most urodela and a few anura, fertilization is internal. In others, fertilization is external. Herein, fertilizations in two well-studied species, red-bellied newt, and African clawed frog are introduced.

The newt, *Cynops pyrrhogaster*, has long been studied as a model animal with internal fertilization. This newt, like several other urodeles, displays a unique courting behavior. Male releases a female-attracting pheromone, a decapeptide called "sodefrin" [36], in front of the female's snout. A male then moves forward and a female chases him. Spermatophore deposition by the male is triggered by a tail-touching behavior of the female. A female then picks up spermatophores using her cloaca. The spermatozoa released from the spermatophore are stored in cloacal glands called spermathecae. In *C. pyrrhogaster*, females pick up spermatophores in fall and use spermatozoa for insemination in spring [37]. In salamandrids, the longest period of sperm storage ever reported is 2 years [38]. The spermatozoa stored in spermathecae are quiescent. During oviposition, spermatozoa are released onto eggs passing down the oviduct. Upon contact with the outermost layer of EJ, spermatozoa undergo the AR before initiating flagellar movement, which is quite unusual. It is known that both ARIS and sperm motility-initiating substance (SMIS) are in the outer rim of EJ layer [39]. These substances were identified as 120/90-kDa proteins with AR-inducing activity [40] and a 34-kDa protein with sperm motility imitation activity [41]. Immunofluorescence microscopy revealed that granules containing SMIF are covered with ARIS, which is in agreement with the order of processes that occur in spermatozoa during fertilization [41].

In most anurans, electron microscopic studies have identified a tiny acrosome at the anterior tip of sperm head. Because of its small size, the AR in living anuran spermatozoa remained unknown for a long time. In 2002, in virtue of confocal microscopy combined with the use of acidotropic dye, LysoSensor Green DND-189, the AR was first visualized in living *Xenopus* spermatozoa [42]. These "green" spermatozoa that were bound to isolated vitelline envelopes underwent the AR. Heat-solubilized vitelline envelops and overlying EJ had much less AR-inducing activity. It is known that the vitelline envelope of coelomic eggs is modified by the secretion from the uppermost region of oviduct known as *pars recta*, while eggs are descending through this part of oviduct. The extracts of *pars recta* have AR-inducing activity [42]. Monoclonal antibodies were generated against *pars recta* extracts and screened by a neutralizing effect of ARIS in *Xenopus*. Using this approach, a 300-kDa VE glycoprotein was identified as a possible ARIS [43]. Deglycosylation of this protein renders this glycoprotein inactive, again suggesting that glycan mediates anuran AR [44].

In amphibians, sperm may undergo the AR as they make contact with EJ (*Cynops*) or the vitelline envelope (*Xenopus*). In both cases, glycoproteins are prime candidates for the ARIS and their sugar chains are likely essential for AR-inducing activity [40, 44]. Although the sites of the AR and manner of fertilization are different between amphibians and marine invertebrates, the mechanisms of the AR in these distant animals seem to be fundamentally the same.

The sperm acrosome reaction in birds

To our knowledge, fertilization in all birds is internal. A male bird deposits his ejaculate in female's vagina and spermatozoa are transported to the utero-vaginal junction where the sperm storage tubes (SSTs) are present. SSTs serve as the primary sperm reservoirs [45].

The duration of sperm life in the SSTs varies from 2 weeks (domestic fowl) to 15 weeks (turkey). Fertilization occurs in the uppermost part of the oviduct, infundibulum, such that spermatozoa must travel through a long female reproductive tract, including the uterus, isthmus, and magnum, with the maximum length between 87.57 ± 37 cm (turkey) [46] and 133.18 ± 9.45 cm (emu) [47]. How spermatozoa pass through such a long oviduct is unknown. It is postulated that less than 0.1% of spermatozoa deposited in the vagina reach the site of fertilization (reviewed by [48]). At least in some birds, additional SSTs are present in the infundibulum and a significant number of spermatozoa can be recovered from both infundibulum and proximal magnum. A physiological relevance of infundibular SSTs remains elusive. Secondary sperm reservoir may ensure high fertility of spermatozoa within female tract [49]. Avian spermatozoa, in general, do not seem to require capacitation and are ready to undergo the AR upon leaving the testis [50]. Nevertheless, avian spermatozoa can be stored for a considerable length of time in the SSTs. This indicates that the sperm AR is somehow prohibited for a long time until the time of fertilization.

At ovulation, each egg is covered by the perivitelline membrane (PVM) that is analogous to mammalian egg's zona pellucida (ZP). Avian egg is very large. Its cytoplasm is accumulated in a small area in the animal pole, called germinal disk. Although several or more spermatozoa enter the germinal disk, only one units with egg nucleus, which is called physiological polyspermy. The reason why multiple spermatozoa enter an egg is likely that a single spermatozoon does not carry enough amount of oocyte-activating factor, phospholipase Cζ [51, 52]. In the quail, the AR can be induced by purified ZP1, one of two major glycoproteins in the PVM, but not by ZP3. Contrary to this, chicken spermatozoa interact with ZP3 [53]. Removal of N-linked oligosaccharides (N-glycans) from quail ZP1 abolished its AR-inducing activity. In the chicken, N-glycans released from the PVM have the AR-inducing activity [54]. In both cases, sugar chains are likely to play an important role in the induction of sperm AR. Quail spermatozoa treated with pertussis toxin failed to induce ZP1-induced AR. They remained attached to PVM surface without entering PVM. These observations in two representative birds suggest that avian spermatozoa undergo the AR on the PVM before dissolving it to create holes.

PVM consists of two major (ZP1 and ZP3) and three minor (ZP2, ZP4, and ZPD) glycoproteins. Gene expression profile shows that ZP2 and ZP4 are expressed during the earlier folliculogenesis, whereas ZP3 and ZPD are highly expressed during later stages of folliculogensis. ZP1 is synthesized in the liver [55], transported by blood circulation and incorporated in the PVM [56]. Of all avian ZP proteins, chicken ZP2 is the only protein that exhibits specific localization, albeit at low abundance, in the germinal disc region. Given that the germinal disc region is the only place where spermatozoa penetrate the PVM [57], at least in chicken, ZP2 seems to be the likely sperm receptor of the PVM [58].

The acrosome reaction of human and primate spermatozoa

Acrosome and the AR of human spermatozoa have been studied extensively in various aspects including (a) acrosome biogenesis, (b) chemical components of the acrosome contents, (c) membrane dynamics before and during the AR, (d) relationship between capacitation and AR, (e) relationship between the AR and development of sperm's ability to fuse with the egg, (f) relationship between acrosomal dysfunction and male infertility, and (g) acrosome and AR as targets for contraceptive measures. Because of ethical tissue and technical problems, we are unable to study where human spermatozoa undergo the AR and what trigger it in vivo [59]. Presumptive ARIS include follicular fluid of ovarian follicle [60, 61], secretions from the cumulus-oocyte-complex [62–64], and oocyte's ZP [65–67]. It is known that human spermatozoa in vitro undergo the AR in response to progesterone [68, 69] and neurotransmitters [70].

In a nonhuman primate macaque, spermatozoa in vitro first attach to the ZP loosely, followed by a tight binding to ZP before undergoing the AR [71]. According to Chiu [66], human ZP components ZP3 and ZP4 have the AR-inducing activity. Gupta [72] who generated baculo-virus recombinant human ZP proteins maintain that ZP1, ZP3, and ZP4, but not ZP2, induce human sperm AR. Baibakov et al. [73] produced transgenic mice whose ZP proteins were replaced by human ZP proteins. They found that only the zonae with human ZP2 protein allowed human spermatozoa to bind and penetrate the ZP, suggesting that ZP2 is likely the zona component inducing human sperm AR.

Mouse sperm acrosome reaction

Mouse sperm AR has been studied almost extensively under in vitro fertilization (IVF) conditions. Mouse IVF is routinely successful after coincubation of epididymal spermatozoa with eggs with or without cumulus oophorus in appropriate media. It has been thought that capacitated, acrosome-intact spermatozoa adhere to the ZP surface before undergoing the AR. Of three major components of mouse zona (ZP1, ZP2, and ZP3), ZP3 is considered the AR-inducing component of the zona [74]. AR-inducing activity of the purified ZP3 has been reported in many other mammalian species including humans [75]. ZP2, another zona glycoprotein of the mouse, is responsible for the binding of acrosome-reacted spermatozoa to ZP. Upon fertilization, the egg releases ovastain, a cortical granule protease, that cleaves ZP2 to prevent further penetration of spermatozoa [76], which has been called the "zona reaction" [73, 77].

Until recently, not much effort had been directed to see how the AR takes place in individual mouse spermatozoa during the course of fertilization. The mouse acrosome is thin and flat. Distinguishing acrosome-intact spermatozoa from acrosome-reacted ones using the ordinary light microscope is difficult. Therefore, the acrosomal status of mouse spermatozoa has been evaluated after staining of spermatozoa with a fluorescent dye, chlortetracycline which stains sperm head differently according to the status of acrosome [78]. Studies using this technique led investigators to infer that mouse spermatozoa undergo the AR after attachment to ZP [79].

In 1999, transgenic mice with green fluorescent protein (GFP) in the acrosome were generated [80]. When spermatozoa of these mice were exposed to calcium ionophore, green fluorescence of the acrosome disappeared in ~3 s, indicating that the AR is a quick event. Nakanishi et al. [80] found that during IVF mouse spermatozoa remained attached to ZP without AR for a long time. Other researchers who used GFP spermatozoa also found that spermatozoa bound to the ZP retained their acrosomes intact for a long time [81], while those exposed to solubilized ZP quickly lost acrosomal GFP [82]. This discrepancy could be due to differences in the state (e.g., density, conformation or configuration) of AR-inducing agents or medium conditions. Alternatively, spermatozoa may initiate the AR in response to some stimuli other than ZP or in addition to ZP [81]. Jin et al. [83] modified mouse IVF systems in such a way that the acrosome status of individual spermatozoa could be followed continuously by video microscopy. They found that most fertilizing spermatozoa were acrosome-reacted before contacting the ZP. Obviously, fertilizing mouse spermatozoa do not need to have intact acrosomes when they reach the ZP surface, as it was once thought [84].

Studies with electron microscopy have clearly demonstrated that initial sperm–egg fusion event occurs between the plasma membranes overlaying the equatorial segment of sperm head and egg plasma membrane; thus, it has been puzzling for many years why acrosome-intact spermatozoa are unable to fuse with eggs even when they are brought directly in contact with the egg's plasma membrane [85]. Satouh et al. [86] discovered that the intra-acrosomal protein Izumo-1 migrates, by an as yet ill-defined mechanism, from the outer acrosomal membrane to the plasma membrane in the equatorial region of sperm head during the AR. This explains clearly why acrosome-intact spermatozoa are fusion-incompetent.

The acrosome reaction of mammalian spermatozoa under physiological circumstances

In vitro studies are certainly important for understanding the process and mechanism of sperm–egg interactions in mammals, but we must understand what is really going on within the oviduct where fertilization takes place under natural conditions. Austin and Bishop [87], who first reported mammalian sperm AR, found acrosome-reacted, motile spermatozoa in the oviduct fluid of guinea pig and in the cumulus oophorus of the guinea pig and Libyan jird eggs. They found acrosome-reacted spermatozoa within the ZP and perivitelline space of guinea-pig, golden hamster, Chinese hamster, and Libyan jird eggs. They inferred that the acrosomes are modified while spermatozoa are passing though the female genital tract and detach before spermatozoa penetrate the ZP. One should note that acrosomes of these rodent species are large and their structural changes can be detected readily under phase-contrast microscopes without fixation and staining of spermatozoa. Cummins and Yanagimachi [88] reported that golden hamster spermatozoa collected from oviduct ampulla appear to be ready to undergo the AR and complete the AR, while they are passing through the cumulus or shortly before contacting

the surface of the ZP. According to Suarez et al. [89], 89% of rabbit spermatozoa collected from oviduct's ampulla 11 h after mating (about the beginning of fertilization) were acrosome-intact. While ZPs of various species are certainly able to induce or accelerate AR in the mouse [90], hamster [91], guinea pig [92], rabbit [93], bovine [94], monkey [71], and human [95, 96], this does not mean that ZP is the sole ARIS.

As stated earlier, acrosome-reacted mouse spermatozoa are able to attach to and penetrate in ZP. In fact, mouse spermatozoa within the oviduct seem to undergo the AR before ascending from the isthmus to the ampulla [97–99]. One should note that the mouse has been the most commonly used model animal for the study of mammalian fertilization. The concept that egg's ZP is the physiological ARIS must be reconsidered.

Conclusions and perspectives

The AR is a widespread phenomenon among animals that use various fertilization tactics. Most commonly, the AR is required for sperm's passage through the "wall" (egg envelope) surrounding the egg proper. While fertilization process can be readily examined in species with external fertilization (such as sea urchin and as starfish), it is next to impossible to observe the AR of mammalian spermatozoa within the oviduct where normal fertilization takes place. Thus, studies in marine invertebrates have offered much basic information on this biological event. In mammals, it has been a long-standing question where and what to trigger the AR in vivo. To address these questions, studies have been conducted using two different approaches, one being oriented to search and identification of substances with specific biological activities for various steps of fertilization in vitro, and the other being oriented to the identification of sites in the female tract where the AR and fertilization really occur. The latter approach was resumed rather recently by using transgenic mice with green fluorescent acrosomes. Although the results obtained from these two approaches have many contradictions, it is hoped that two approaches eventually become complementary to each other, rather than contradictory.

In nonmammalian vertebrates such as newts and birds, egg's coats carry ARIS. Although it is tempting to speculate that a specific ARIS exists universally throughout vertebrates, one must be aware that spermatozoa of nonmammalian vertebrates may not need "capacitation" prior to fertilization. At the end of capacitation process, mammalian spermatozoa may undergo the AR spontaneously, and this may be enough to render spermatozoa fertilization competent. In the mouse, spontaneous AR has been considered nonphysiological, because it renders spermatozoa fertilization incompetent [84]. We now know that fertilizing mouse spermatozoa within oviducts are acrosome-reacted before meeting eggs [83, 97–99]. What trigger the AR and what were found in the mouse is true for other species remain to be determined. As far as the "initiator" of mammalian sperm AR is concerned, we are now back to the starting point.

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References

1. Howards SS. Antoine van Leeuwenhoek and the discovery of sperm. *Fertil Steril* 1997; **67**:16–17.
2. Dan JC. Studies on the acrosome. I. Repose to egg water and other stimuli. *Biol Bull* 1952; **103**:54–66.
3. Hinsch GW, Clark WH, Jr. Comparative Fine structure of Cnidaria spermatozoa. *Biol Reprod* 1973; **8**:62–73.
4. Wilson EB. *The Cell in Development and Heredity*. New York: MacMillan. 1925; 1232 pp.
5. Dan JC. Studies on the acrosome. III. Effect of calcium deficiency. *Biol Bull* 1954 **107**:335–349.
6. Fukumoto M. Acrosome reaction in ascidians induced by a calcium ionophore. *J Struct Biol* 1993; **111**:77–83.
7. Lambert CC. The ascidian sperm reaction: evidence for Cl- and HCO_3-involvement in acid release. *Dev Biol* 1982; **91**:257–262.
8. Nakazawa S, Shirae-Kurabayashi M, Otsuka K, Sawada H. Proteomics of ionomycin-induced ascidian sperm reaction: Released and exposed sperm proteins in the ascidian *Ciona intestinalis*. *Proteomics* 2015; **15**:4064–4079.
9. Yanagimachi R, Harumi T, Matsubara H, Yan W, Yuan S, Hirohashi N, Iida T, Yamaha E, Arai K, Matsubara T, Andoh T, Vines C et al. Chemical and physical guidance of fish spermatozoa into the egg through the micropyle. *Biol Reprod* 2017; **96**:780–799.
10. Xu XZ, Sternberg PW. A *C. elegans* sperm TRP protein required for sperm-egg interactions during fertilization. *Cell* 2003; **114**:285–297.
11. Larkman AU, Carter MA. The apparent absence of a cortical reaction after fertilization in a sea anemone. *Tissue Cell* 1984; **16**:125–130.
12. Nishimura H, L'Hernault SW. Spermatogenesis-defective (*spe*) mutants of the nematode *Caenorhabditis elegans* provide clues to solve the puzzle of male germline functions during reproduction. *Dev Dyn* 2010; **239**:1502–1514.
13. Su CX, Chen J, Shi FM, Guo MS, Chang YL. Formation of the acrosome complex in the bush cricket *Gampsocleis gratiosa* (Orthoptera: Tettigoniidae). *Arthropod Struct Dev* 2017; **46**:579–587.
14. Morisawa S, Mizuta T, Kubokawa K, Tanaka H, Morisawa M. Acrosome reaction in spermatozoa from the amphioxus *Branchiostoma belcheri* (Cephalochordata, Chordata). *Zoolog Sci* 2004; **21**:1079–1084.
15. Wilson KL, Fitch KR, Bafus BT, Wakimoto BT. Sperm plasma membrane breakdown during *Drosophila* fertilization requires sneaky, an acrosomal membrane protein. *Development* 2006; **133**:4871–4879.
16. Vacquier VD, Carner KR, Stout CD. Species-specific sequences of abalone lysin, the sperm protein that creates a hole in the egg envelope. *Proc Natl Acad Sci USA* 1990; **87**:5792–5796.
17. Vacquier VD, Moy GW. Isolation of bindin: the protein responsible for adhesion of sperm to sea urchin eggs. *Proc Natl Acad Sci USA* 1977; **74**:2456–2460.
18. Glabe CG. Interaction of the sperm adhesive protein, bindin, with phospholipid vesicles. II. Bindin induces the fusion of mixed-phase vesicles that contain phosphatidylcholine and phosphatidylserine in vitro. *J Cell Biol* 1985; **100**:800–806.
19. Tilney LG, Fukui Y, DeRosier DJ. Movement of the actin filament bundle in *Mytilus* sperm: a new mechanism is proposed. *J Cell Biol* 1987; **104**:981–993.
20. Tilney LG, Inoue S. Acrosomal reaction of the Thyone sperm. III. The relationship between actin assembly and water influx during the extension of the acrosomal process. *J Cell Biol* 1985; **100**:1273–1283.
21. Kyozuka K, Osanai K. Fertilization cone formation in starfish oocytes: the role of the egg cortex actin microfilaments in sperm incorporation. *Gamete Res* 1988; **20**:275–285.
22. Alves AP, Mulloy B, Moy GW, Vacquier VD, Mourao PA. Females of the sea urchin *Strongylocentrotus purpuratus* differ in the structures of their egg jelly sulfated fucans. *Glycobiology* 1998; **8**:939–946.
23. Alves AP, Mulloy B, Diniz JA, Mourao PA. Sulfated polysaccharides from the egg jelly layer are species-specific inducers of acrosomal reaction in sperms of sea urchins. *J Biol Chem* 1997; **272**:6965–6971.
24. Hirohashi N, Vilela-Silva AC, Mourao PA, Vacquier VD. Structural requirements for species-specific induction of the sperm acrosome reaction by sea urchin egg sulfated fucan. *Biochem Bioph Res Commun* 2002; **298**:403–407.

25. Koyota S, Wimalasiri KM, Hoshi M. Structure of the main saccharide chain in the acrosome reaction-inducing substance of the starfish, Asterias amurensis. *J Biol Chem* 1997; **272**:10372–10376.
26. Vacquier VD, Moy GW. The fucose sulfate polymer of egg jelly binds to sperm REJ and is the inducer of the sea urchin sperm acrosome reaction. *Dev Biol* 1997; **192**:125–135.
27. Christen R, Schackmann RW, Shapiro BM. Interactions between sperm and sea urchin egg jelly. *Dev Biol* 1983; **98**:1–14.
28. Hirohashi N, Kamei N, Kubo H, Sawada H, Matsumoto M, Hoshi M. Egg and sperm recognition systems during fertilization. *Dev Growth Differ* 2008; **50**:S221–S238.
29. Yoshida K, Treen N, Hozumi A, Sakuma T, Yamamoto T, Sasakura Y. Germ cell mutations of the ascidian *Ciona intestinalis* with TALE nucleases. *Genesis* 2014; **52**:431–439.
30. Lin CY, Su YH. Genome editing in sea urchin embryos by using a CRISPR/Cas9 system. *Dev Biol* 2016; **409**:420–428.
31. Kille RA. Fertilization of the lamprey egg. *Exp Cell Res* 1960; **20**:12–27.
32. Morisawa S, Cherr GN. Acrosome reaction in spermatozoa from hagfish (Agnatha) *Eptatretus burgeri* and *Eptatretus stouti*: Acrosomal exocytosis and identification of filamentous actin. *Dev Growth Differ* 2002; **44**:337–344.
33. Cherr GN, Clark WH, Jr. Fine structure of the envelope and micropyles in the eggs of the white sturgeon, Acipenser transmontanus Richardson. (micropyle/chorion/egg envelopes/sturgeon/egg jelly). *Dev Growth Differ* 1982; **24**:341–352.
34. Cherr GN, Clark WH, Jr. An egg envelope component induces the acrosome reaction in sturgeon sperm. *J Exp Zool* 1985; **234**:75–85.
35. Cherr GN, Clark WH, Jr. Gamete interaction in the white sturgeon *Acipenser transmontanus*: a morphological and physiological review. *Environ Biol Fish* 1985; **14**:11–22.
36. Kikuyama S, Toyoda F, Ohmiya Y, Matsuda K, Tanaka S, Hayashi H. Sodefrin: a female-attracting peptide pheromone in newt cloacal glands. *Science* 1995; **267**:1643–1645.
37. Akiyama S, Iwao Y, Miura I. Evidence for true fall-mating in Japanese newt *Cynops pyrrhogaster*. *Zoolog Sci* 2011; **28**:758–763.
38. Boisseau C, Joly J. Transport and survival of spermatozoa in female amphibia. In: Hafez ESE, Thibault CG (eds.), *The Biology of Spermatozoa*. Basel: Karger; 1975:94–104.
39. Takahashi S, Nakazawa H, Watanabe A, Onitake K. The outermost layer of egg-jelly is crucial to successful fertilization in the newt, Cynops pyrrhogaster. *J Exp Zool* 2006; **305**:1010–1017.
40. Watanabe A, Fukutomi K, Kubo H, Ohta M, Takayama-Watanabe E, Onitake K. Identification of egg-jelly substances triggering sperm acrosome reaction in the newt, *Cynops pyrrhogaster*. *Mol Reprod Dev* 2009; **76**:399–406.
41. Watanabe T, Kubo H, Takeshima S, Nakagawa M, Ohta M, Kamimura S, Takayama-Watanabe E, Watanabe A, Onitake K. Identification of the sperm motility-initiating substance in the newt, *Cynops pyrrhogaster*, and its possible relationship with the acrosome reaction during internal fertilization. *Int J Dev Biol* 2010; **54**:591–597.
42. Ueda Y, Yoshizaki N, Iwao Y. Acrosome reaction in sperm of the frog, *Xenopus laevis*: its detection and induction by oviductal pars recta secretion. *Dev Biol* 2002; **243**:55–64.
43. Ueda Y, Kubo H, Iwao Y. Characterization of the acrosome reaction-inducing substance in *Xenopus* (ARISX) secreted from the oviductal pars recta onto the vitelline envelope. *Dev Biol* 2003; **264**:289–298.
44. Ueda Y, Imaizumi C, Kubo H, Sato K, Fukami Y, Iwao Y. Analysis of terminal sugar moieties and species-specificities of acrosome reaction-inducing substance in *Xenopus* (ARISX). *Dev Growth Differ* 2007; **49**:591–601.
45. Sasanami T, Matsuzaki M, Mizushima S, Hiyama G. Sperm storage in the female reproductive tract in birds. *J Reprod Dev* 2013; **59**:334–338.
46. Dalrymple JR, Macpherson JW, Friars GW. The reproductive tract of the turkey hen (a biometrical study). *Can J Comp Med* 1968; **32**:435–436.
47. Vijayakumar K, Balasundaram K, Paramasivan S, Kumaravel A, Madhu N. Macro anatomy of female reproductive tract during laying and non-laying period in adult emu birds (*Dromaius novaehollandiae*). *Asian J Sci Technol* 2014; **5**:793–795.
48. Blesbois E, Brillard JP. Specific features of in vivo and in vitro sperm storage in birds. *Animal* 2007; **1**:1472–1481.
49. Fujii S, Tamura T. Location of Sperms in the Oviduct of the Domestic Fowl with. Special Reference to Storage of Sperm in the Vaginal Gland. *J Fac Fish Anim Husb Hiroshima Univ* 1963; **5**:145–163.
50. Nixon B, Ewen KA, Krivanek KM, Clulow J, Kidd G, Ecroyd H, Jones RC. Post-testicular sperm maturation and identification of an epididymal protein in the Japanese quail (*Coturnix coturnix japonica*). *Reproduction* 2014; **147**:265–277.
51. Mizushima S, Takagi S, Ono T, Atsumi Y, Tsukada A, Saito N, Shimada K. Phospholipase Czeta mRNA expression and its potency during spermatogenesis for activation of quail oocyte as a sperm factor. *Mol Reprod Dev* 2009; **76**:1200–1207.
52. Mizushima S, Hiyama G, Shiba K, Inaba K, Dohra H, Ono T, Shimada K, Sasanami T. The birth of quail chicks after intracytoplasmic sperm injection. *Development* 2014; **141**:3799–3806.
53. Han L, Monne M, Okumura H, Schwend T, Cherry AL, Flot D, Matsuda T, Jovine L. Insights into egg coat assembly and egg-sperm interaction from the X-ray structure of full-length ZP3. *Cell* 2010; **143**:404–415.
54. Horrocks AJ, Stewart S, Jackson L, Wishart GJ. Induction of acrosomal exocytosis in chicken spermatozoa by inner perivitelline-derived N-linked glycans. *Biochem Biophys Res Commun* 2000; **278**:84–89.
55. Sasanami T, Pan J, Mori M. Expression of perivitelline membrane glycoprotein ZP1 in the liver of Japanese quail (*Coturnix japonica*) after in vivo treatment with diethylstilbestrol. *J Steroid Biochem Mol Biol* 2003; **84**:109–116.
56. Kinoshita M, Mizui K, Ishiguro T, Ohtsuki M, Kansaku N, Ogawa H, Tsukada A, Sato T, Sasanami T. Incorporation of ZP1 into perivitelline membrane after in vivo treatment with exogenous ZP1 in Japanese quail (*Coturnix japonica*). *FEBS J* 2008; **275**:3580–3589.
57. Wishart GJ. Quantitative aspects of sperm:egg interaction in chickens and turkeys. *Anim Reprod Sci* 1997; **48**:81–92.
58. Nishio S, Kohno Y, Iwata Y, Arai M, Okumura H, Oshima K, Nadano D, Matsuda T. Glycosylated chicken ZP2 accumulates in the egg coat of immature oocytes and remains localized to the germinal disc region of mature eggs. *Biol Reprod* 2014; **91**:107.
59. De Jonge C. Biological basis for human capacitation-revisited. *Hum Reprod Update* 2017; **23**:289–299.
60. Siiteri JE, Dandekar P, Meizel S. Human sperm acrosome reaction-initiating activity associated with the human cumulus oophorus and mural granulosa cells. *J Exp Zool* 1988; **246**:71–80.
61. Yudin AI, Gottlieb W, Meizel S. Ultrastructural studies of the early events of the human sperm acrosome reaction as initiated by human follicular fluid. *Gamete Res* 1988; **20**:11–24.
62. Kirkman-Brown JC, Bray C, Stewart PM, Barratt CL, Publicover SJ. Biphasic elevation of $[Ca^{2+}]_i$ in individual human spermatozoa exposed to progesterone. *Dev Biol* 2000; **222**:326–335.
63. Osman RA, Andria ML, Jones AD, Meizel S. Steroid induced exocytosis: the human sperm acrosome reaction. *Biochem Bioph Res Commun* 1989; **160**:828–833.
64. Harper CV, Barratt CL, Publicover SJ, Kirkman-Brown JC. Kinetics of the progesterone-induced acrosome reaction and its relation to intracellular calcium responses in individual human spermatozoa. *Biol Reprod* 2006; **75**:933–939.
65. Hoshi K, Sugano T, Endo C, Yoshimatsu N, Yanagida K, Sato A. Induction of the acrosome reaction in human spermatozoa by human zona pellucida and effect of cervical mucus on zona-induced acrosome reaction. *Fertil Steril* 1993; **60**:149–153.
66. Chiu PC, Wong BS, Chung MK, Lam KK, Pang RT, Lee KF, Sumitro SB, Gupta SK, Yeung WS. Effects of native human zona pellucida glycoproteins 3 and 4 on acrosome reaction and zona pellucida binding of human spermatozoa. *Biol Reprod* 2008; **79**:869–877.
67. Ganguly A, Bukovsky A, Sharma RK, Bansal P, Bhandari B, Gupta SK. In humans, zona pellucida glycoprotein-1 binds to spermatozoa and induces acrosomal exocytosis. *Hum Reprod* 2010; **25**:1643–1656.

68. Meizel S, Turner KO. Progesterone acts at the plasma membrane of human sperm. *Mol Cell Endocrinol* 1991; **77**:R1–R5.
69. Sagare-Patil V, Bhilawadikar R, Galvankar M, Zaveri K, Hinduja I, Modi D. Progesterone requires heat shock protein 90 (HSP90) in human sperm to regulate motility and acrosome reaction. *J Assist Reprod Genet* 2017; **34**:495–503.
70. Meizel S. The sperm, a neuron with a tail: 'neuronal' receptors in mammalian sperm. *Biol Rev* 2004; **79**:713–732.
71. Tollner TL, Yudin AI, Cherr GN, Overstreet JW. Real-time observations of individual macaque sperm undergoing tight binding and the acrosome reaction on the zona pellucida. *Biol Reprod* 2003; **68**:664–672.
72. Gupta SK. Role of zona pellucida glycoproteins during fertilization in humans. *J Reprod Immunol* 2015; **108**:90–97.
73. Baibakov B, Boggs NA, Yauger B, Baibakov G, Dean J. Human sperm bind to the N-terminal domain of ZP2 in humanized zonae pellucidae in transgenic mice. *J Cell Biol* 2012; **197**:897–905.
74. Bleil JD, Wassarman PM. Mammalian sperm-egg interaction: identification of a glycoprotein in mouse egg zonae pellucidae possessing receptor activity for sperm. *Cell* 1980; **20**:873–882.
75. van Duin M, Polman JE, De Breet IT, van Ginneken K, Bunschoten H, Grootenhuis A, Brindle J, Aitken RJ. Recombinant human zona pellucida protein ZP3 produced by chinese hamster ovary cells induces the human sperm acrosome reaction and promotes sperm-egg fusion. *Biol Reprod* 1994; **51**:607–617.
76. Burkart AD, Xiong B, Baibakov B, Jimenez-Movilla M, Dean J. Ovastacin, a cortical granule protease, cleaves ZP2 in the zona pellucida to prevent polyspermy. *J Cell Biol* 2012; **197**:37–44.
77. Bleil JD, Beall CF, Wassarman PM. Mammalian sperm-egg interaction: fertilization of mouse eggs triggers modification of the major zona pellucida glycoprotein, ZP2. *Dev Biol* 1981; **86**:189–197.
78. Saling PM, Storey BT. Mouse gamete interactions during fertilization in vitro. Chlortetracycline as a fluorescent probe for the mouse sperm acrosome reaction. *J Cell Biol* 1979; **83**:544–555.
79. Storey BT, Lee MA, Muller C, Ward CR, Wirtshafter DG. Binding of mouse spermatozoa to the Zónae Pellucidae of mouse eggs in cumulus: evidence that the acrosomes remain substantially intact. *Biol Reprod* 1984; **31**:1119–1128.
80. Nakanishi T, Ikawa M, Yamada S, Parvinen M, Baba T, Nishimune Y, Okabe M. Real-time observation of acrosomal dispersal from mouse sperm using GFP as a marker protein. *FEBS Lett* 1999; **449**:277–283.
81. Baibakov B, Gauthier L, Talbot P, Rankin TL, Dean J. Sperm binding to the zona pellucida is not sufficient to induce acrosome exocytosis. *Development* 2007; **134**:933–943.
82. Buffone MG, Rodriguez-Miranda E, Storey BT, Gerton GL. Acrosomal exocytosis of mouse sperm progresses in a consistent direction in response to zona pellucida. *J Cell Physiol* 2009; **220**:611–620.
83. Jin M, Fujiwara E, Kakiuchi Y, Okabe M, Satouh Y, Baba SA, Chiba K, Hirohashi N. Most fertilizing mouse spermatozoa begin their acrosome reaction before contact with the zona pellucida during in vitro fertilization. *Proc Natl Acad Sci USA* 2011; **108**:4892–4896.
84. Florman HM, Storey BT. Mouse gamete interactions: the zona pellucida is the site of the acrosome reaction leading to fertilization in vitro. *Dev Biol* 1982; **91**:121–130.
85. Yanagimachi R. Mammalian fertilization. In: Knobil E, Neill JD (eds.), *The Physiology of Reproduction*. New York: Raven Press; 1994:189–317.
86. Satouh Y, Inoue N, Ikawa M, Okabe M. Visualization of the moment of mouse sperm-egg fusion and dynamic localization of IZUMO1. *J Cell Sci* 2012; **125**:4985–4990.
87. Austin CR, Bishop MW. Role of the rodent acrosome and perforatorium in fertilization. *Proc R Soc Lond B Biol Sci* 1958; **149**:241–248.
88. Cummins JM, Yanagimachi R. Sperm-egg ratios and the site of the acrosome reaction during in vivo fertilization in the hamster. *Gamete Res* 1982; **5**:239–256.
89. Suarez SS, Katz DF, Overstreet JW. Movement characteristics and acrosomal status of rabbit spermatozoa recovered at the site and time of fertilization. *Biol Reprod* 1983; **29**:1277–1287.
90. Bleil JD, Wassarman PM. Sperm-egg interactions in the mouse: sequence of events and induction of the acrosome reaction by a zona pellucida glycoprotein. *Dev Biol* 1983; **95**:317–324.
91. Uto N, Yoshimatsu N, Lopata A, Yanagimachi R. Zona-induced acrosome reaction of hamster spermatozoa. *J Exp Zool* 1988; **248**:113–120.
92. Schroer SC, Yudin AI, Myles DG, Overstreet JW. Acrosomal status and motility of guinea pig spermatozoa during in vitro penetration of the cumulus oophorus. *Zygote* 2000; **8**:107–117.
93. O'Rand MG, Fisher SJ. Localization of zona pellucida binding sites on rabbit spermatozoa and induction of the acrosome reaction by solubilized zonae. *Dev Biol* 1987; **119**:551–559.
94. Florman HM, First NL. The regulation of acrosomal exocytosis. *Dev Biol* 1988; **128**:453–463.
95. Cross NL, Morales P, Overstreet JW, Hanson FW. Induction of acrosome reactions by the human zona pellucida. *Biol Reprod* 1988; **38**:235–244.
96. Gupta SK, Bhandari B, Shrestha A, Biswal BK, Palaniappan C, Malhotra SS, Gupta N. Mammalian zona pellucida glycoproteins: structure and function during fertilization. *Cell Tissue Res* 2012; **349**:665–678.
97. La Spina FA, Puga Molina LC, Romarowski A, Vitale AM, Falzone TL, Krapf D, Hirohashi N, Buffone MG. Mouse sperm begin to undergo acrosomal exocytosis in the upper isthmus of the oviduct. *Dev Biol* 2016; **411**:172–182.
98. Muro Y, Hasuwa H, Isotani A, Miyata H, Yamagata K, Ikawa M, Yanagimachi R, Okabe M. Behavior of mouse spermatozoa in the female reproductive tract from soon after mating to the beginning of fertilization. *Biol Reprod* 2016; **94**:80.
99. Hino T, Muro Y, Tamura-Nakano M, Okabe M, Tateno H, Yanagimachi R. The behavior and acrosomal status of mouse spermatozoa in vitro, and within the oviduct during fertilization after natural mating. *Biol Reprod* 2016; **95**:50–50.