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Commentary

Mammalian Sperm Acrosome Reaction: Where Does It Begin Before Fertilization?

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Spermatozoa of many animal species, including humans, must undergo a Ca^{2+}-dependent exocytotic process known as the acrosome reaction (AR) before fertilizing oocytes. The AR, first described in the sea urchin and starfish by Dan [1, 2], involves multiple fusions of the outer acrosomal membrane with the overlying sperm plasma membrane. This allows release of the contents of the acrosome to the outside of the sperm cell. Lytic acrosomal materials digest or dissociate the oocyte's glycoprotein coat, making a hole through which the sperm head advances to reach the surface of the oocyte proper before fusing with it. In the sea urchin, an acrosomal protein called bindin adheres to the extended inner acrosomal membrane; this is what mediates sperm head attachment to the vitelline envelope as well as fusion with the egg's plasma membrane [3, 4].

In some species of sea urchins (e.g., *Pseudocentrotus depressus*), the AR may occur on a very thin vitelline envelope covering the egg proper [5] (Fig. 1). However, in many other species, the AR begins while spermatozoa pass through a jelly coat overlying the vitelline envelope. In the starfish, the AR occurs at the outer surface of a thick jelly coat [6]. In some other invertebrate species (e.g., the annelid *Hydroides hexagonus*), the AR begins at the outer border of the vitelline envelope [7].

In mammals, fully mature oocytes that are ready for fertilization are each surrounded by a thick vitelline envelope called the zona pellucida that in turn is surrounded by numerous follicular cells embedded in an acellular matrix (hyaluronic acid polymers). Collectively, these are known as the cumulus-oocyte complex. Although we are certain that spermatozoa undergo the AR by the time they enter the zona pellucida with the aid of active flagellar propulsion, the site where fertilizing spermatozoa begin their AR has been the subject of controversy. Early investigators, who examined cumulus-oocyte complexes collected from the oviducts of naturally mated or inseminated females, found spermatozoa with intact, modified, or no acrosomes within the cumulus at about the time of fertilization [8–14]. Yanagimachi and Phillips [14] inferred that most fertilizing hamster spermatozoa in vivo initiate their AR while advancing though the cumulus. Other investigators, in particular those who studied mouse fertilization in vitro, opposed this idea and maintained that the site of the physiologically relevant AR is the zona pellucida [15–17]. This hypothesis was based on two observations: Acrosome-intact spermatozoa bind to the zona in vitro and then undergo the AR [18–20], and solubilized zona pellucida, in particular solubilized ZP3, binds specifically to the acrosomal region of sperm head and induces the AR as effectively as Ca^{2+} ionophore in vitro [21–23]. It should be noted that no one has ever examined a single spermatozoon continuously from the beginning of the AR until the end of fertilization (syngamy). The spermatozoa that begin their AR on the zona pellucida might not be those that actually fertilize. Gahlay et al. [24] recently cast doubt on the ability of the zona pellucida to induce the AR. According to these investigators, zonae of transgenic mice ($ZP2^{Mut}$, $ZP3^{Mut}$) are unable to induce the AR, yet oocytes are still fertilized in these strains, suggesting that fertilizing spermatozoa undergo the AR either before their contact with the zona or during their passage through it.

Jin et al. [25] approached the problem of sperm AR and fertility by video recording mouse spermatozoa after inseminating cumulus-enclosed oocytes in vitro. They placed a single cumulus-oocyte complex under a miniature coverslip, then slightly compressed and inseminated it with capacitated spermatozoa before undertaking continuous video recording. They used transgenic male mice whose spermatozoa express green fluorescent protein in their acrosomes. Sperm heads show green fluorescence when acrosomes are intact. This disappears upon initiation of the AR [26]. The strength of the study by Jin et al. [25] was that investigators could distinguish fertilizing spermatozoa from their nonfertilizing counterparts by examination of recorded images; this is something that no one has ever done before. The results of their exploration were startling. Most fertilizing mouse spermatozoa had already undergone the AR when first seen in the cumulus. Although a few fertilizing spermatozoa did undergo the AR on the zona, most acrosome-intact spermatozoa swam away from the zona without entering it. In other words, the initiation of sperm AR on the zona was the exception rather than the rule. This is consistent with what has been observed for guinea pig spermatozoa that exclusively bind to and penetrate the zona pellucida after the AR [27]. One wonders why previous researchers believed that acrosome-reacted mouse spermatozoa are infertile because of their inability to bind the zona [21]. The report by Jin et al. [25] reminds us that we must reinvestigate the process and mechanism of sperm-oocyte interactions by paying more attention to the spermatozoa that actually participate in fertilization. In many species, fertilization in vitro is certainly possible without an intact cumulus oophorus. Undoubtedly, the zona pellucida has the ability to induce or accelerate the AR, but the zona cannot be considered the sole innate substance that induces the physiological AR.

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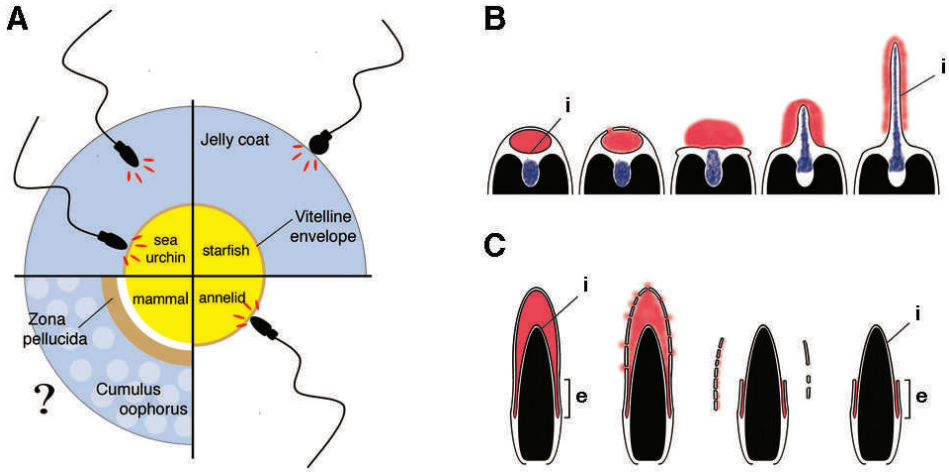

FIG. 1. The AR. **A**) Sites where the AR begins for the sea urchin (in the jelly coat or on the vitelline envelope), the starfish (on the outer periphery of jelly coat), the annelid (on the vitelline envelope), and the mammal (the subject of the study by Jin et al. [25]). **B**) Diagrams of longitudinal sections of the anterior ends of sea urchin sperm heads showing successive stages of the AR; note that the inner acrosomal membrane (i) extends to form a process that fuses with the egg plasma membrane. **C**) Diagrams of successive stages of the AR in mammal; the inner acrosome membrane (i) remains structurally unchanged during and after the AR. The plasma membrane of the equatorial segment region (e) of sperm head changes its characteristics upon the AR to fuse with the egg plasma membrane.

Jin et al. [25] did not clarify the site where fertilizing mouse spermatozoa began their AR. Because the cumulus oophorus is large, investigators used a low-magnification objective to search for spermatozoa within the cumulus. This made it difficult to determine when and where the AR began in the spermatozoa being viewed. However, what is clear from their study is that mouse spermatozoa that have begun the AR before reaching the zona were able to fertilize. The site where fertilizing spermatozoa begin their AR and what triggers the AR remain to be determined.

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