Site of the mammalian sperm physiological acrosome reaction

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In mammals and the many other animals in which fertilization involves the establishment of a penetration path through the egg coat, at the apex of the sperm head lies the acrosome—a membrane-limited organelle that houses a complex of enzymes. For fertilization to occur, spermatozoa must first undergo the acrosome reaction (AR), which involves point fusions between the outer acrosomal and overlying cell membrane. This reaction releases the acrosome's soluble lysins, which facilitate the creation of a path in the egg coat during penetration (although a variety of evidence now calls into question whether egg-coat lysis is the mechanism used by eutherian mammals). In PNAS, using in vitro fertilization, Jin et al. (1) report their tracking of the site and timing of the AR in spermatozoa from transgenic mice whose acrosome and sperm tail midpiece were tagged with different fluorescent markers. This approach, which used video imaging, allowed them to record that, compared with sperm on a naked zona surface, the AR was generally initiated more readily in the interstices of the cell mass of the cumulus oophorus that surrounds the newly ovulated mouse egg. Moreover, not only did the AR generally occur to a greater degree and often more rapidly in sperm penetrating the cumulus than on a naked zona surface, but this was also true for sperm interacting with naked eggs in the presence of cumulus-invested oocytes. Most important, Jin et al. (1) establish by retroactive examination of recorded images that some spermatozoa reacting within the cumulus were then able to go on to fertilize the egg. Given that the AR has been examined in different ways over the years and in a variety of mammals, including man, why then is this current study so important in the context of present efforts to understand the mechanisms of fertilization in eutherian mammals?

Initiation of the AR

It has long been clear for the nonmammalian models of fertilization in which spermatozoa must create a penetration path that, according to species, the AR is induced by the egg coat or often, by jelly that surrounds that coat. In eutherian mammals, however, establishing the site where the AR begins is made difficult by the fact that the egg coat—the zona pellucida—is surrounded for a species-variable number of hours after ovulation by the cumulus oophorus, a multilayered mass of cells often embedded in a hyaluronic acid-rich matrix. In early studies in the guinea pig and several rodents, Austin and Bishop (2) reported spermatozoa undergoing the AR within the cumulus and, mistakenly, they then suggested that the reaction was a morphological manifestation of sperm capacitation—a sperm membrane-related change undergone in the female tract, peculiar to therian mammals, that finally confers on spermatozoa the ability to fertilize. Later, as seen in the electron microscope, rabbit spermatozoa at the fringe of the cumulus oophorus generally had intact acrosomes, whereas most caught within

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the cumulus had undergone some phase of the AR by that time, as had most spermatozoa bound to the zona pellucida (3). Subsequently, similar observations of the AR within the cumulus were made for spermatozoa of the Syrian (4, 5) and Chinese hamster (6) and even man (7). Not long before that, however, inroads into the problem of defining fertilization in molecular terms had begun with the important finding that the (mouse) zona pellucida comprises three glycoproteins—ZP1, ZP2, and ZP3 (8). Because cumulus-free eggs of most mammals can be fertilized in vivo or in vitro, the equally exciting observation a few years later that the mouse AR can be induced by ZP3 (9) led to the view that the zona pellucida induces the AR. Although seeming to discount previous observations of the AR within the cumulus in other species and although, with occasional exceptions (10), most mouse studies were performed using cumulus-free eggs, this view of the zona pellucida as the physiological inducer of the mammalian AR has been a focus of other reports (11, 12), and is now widespread. Thus, the observations of Jin et al. (1) represent a return to biological reality in this regard. The particular merit of their study lies not only in the observation of events in living mouse spermatozoa. To reiterate, it shows that these spermatozoa frequently undergo the AR within the cumulus before reaching the zona pellucida and, using a fluorescent tail tag, it also shows the key fact that such spermatozoa can then go on to fertilize.

The observation that mouse spermatozoa with reacted acrosomes can then go on to adhere to and penetrate the zona pellucida has important implications for efforts to characterize the sperm head surface component(s) involved in sperm/egg binding at fertilization. However, in considering other implications of this work, it needs to be shown eventually that its findings represent the situation during fertilization in vivo. Moreover, despite the disappearance of the fluorescent acrosomal tag within the cumulus, it is presumed that the reacted acrosomal carapace is retained until and while the sperm head binds to the zona surface. Although this has not been stressed in the literature, a broad cross-species survey of the AR suggests that the reacted acrosome in eutherian mammals has an unusual stability and therefore, lifespan, possibly brought by an insoluble acrosomal matrix component. This stability seems to be an adaptation that then allows it to persist and tether the sperm head until penetration of the relatively formidable, dense zona matrix begins.

Significance of the Cumulus Oophorus?

Finally, the paper of Jin et al. (1) serves to focus two further issues. Although the identity of the AR induction agent in the mouse cumulus oophorus is not known, as a last point these authors note that in several mammals, including man, progesterone, which cumulus cells may produce, can induce the AR in capacitated spermatozoa (13). This brings an important question as to whether the stimulus for the AR in the cumulus oophorus and that on the zona surface, and the molecular steps involved, are the same or very different. Second, the study serves to highlight a broader question as to the adaptive significance of the cumulus oophorus, which appears to be unique to eutherian mammals, the follicular cells

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being shed before ovulation even in marsupial mammals that have comparably small eggs. In fact, there is no single or simple answer, because the cumulus oophorus displays a variety of radiations in regard to its post-ovulatory life, its form, and its function. For instance, in two very different species of shrew, the dense cumulus is stabilized by gap junctions and has no intercellular matrix, but does have an essential role in fertilization as the inducer of the AR, which the zona pellucida seems unable to stimulate (14). The acrosome is shed before spermatozoa reach the zona pellucida, and in one case, the reacted spermatozoa penetrate and kill a line of cumulus cells in reaching the zona surface (15). Because shrews are often considered to be akin to the eutherian progenitors, this essential role of the cumulus in fertilization may represent the evolutionary situation, and based on sperm head anatomy may apply at least to other insectivores. However, although fertilization can occur in the absence of a cumulus in most mammals studied, its form and the postovulatory life can vary widely. The cumulus oophorus may be compact and have little matrix in some species, but a hyaluronic acid-rich matrix expands the ovulated cumulus oophorus significantly where the eggs are fertilized in a spacious tubal ampulla, which is very common in eutherian mammals. Seen in a range of species from ungulates and primates to rodents and rabbits, this expanded state of the cumulus may act to provide a larger egg-associated target for and sequester the very few spermatozoa present in a voluminous ampulla (16), and consistent with the present study, it may foster early fertilization of the eggs (17).

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