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REVIEW

Loss of the Paternal Mitochondrion during Fertilization

CHARLES C. LAMBERT[1] and DAVID E. BATTAGLIA[2]

[1]*Department of Biology California State University Fullerton, CA 92634, and*
[2]*Department of Obstetrics and Gynecology SJ-70 University of Washington Medical School Seattle Washington 98195, U.S.A.*

INTRODUCTION

"If any apology is needed for presenting so strictly a morphological study of such an apparently threadbare subject as the fertilization of the ovum, I might say that the impulse to make it came from an experimental study ...." [22].

Spermatozoa are small cells specialized for the single function of delivering the haploid sperm nucleus to the egg and aiding in inserting this genome into the egg. As a specialized delivery vehicle, the sperm cell loses most of its original cytoplasm during spermatogenesis, leaving us with a basic means of locomotion (generally a tail with a functional axoneme), the nucleus and a means of generating ATP, the mitochondrion or several small mitochondria. Most sperm are divided into three functional regions: head with nucleus and acrosome, midpiece with mitochondria and tail with central axoneme [13, 15, for reviews].

Although the paternal mitochondrion generally makes no genetic or functional contribution to the zygote [5, 8], in most animals the sperm mitochondrion or multiple mitochondria enter the egg along with the nucleus, centriole and tail during fertilization [23 for review]. This occurs even though most of the spermatid mitochondria are shed during spermiogenesis. Here we review the apparently rare cases where the sperm mitochondrion is eliminated during fertilization. Hopefully this will foster additional interest in this area and additional cases will be uncovered. This process which often involves mitochondrial sliding along the sperm tail, occurs sporadically through-out the animal kingdom in such evolutionarily distant organisms as polychaetes and ascidians.

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Annelida

Lillie [22] appears to have been the first to call attention to loss of the paternal mitochondrion during the fertilization of animal eggs. He studied fertilization in the polychaete worm *Nereis limbata* and demonstrated that the sperm mitochondrion remains on the egg surface after sperm incorporation into the egg. The sequence of fertilization in this species begins with sperm binding to the egg vitelline coat (VC) *via* attachment of the tip of the acrosome. The egg reacts to sperm contact within 2–3 min by releasing egg jelly from its cortical granules and forcing supernumerary sperm from the VC surface. Subsequently a fertilization cone forms at the site of contact with the fertilizing sperm. The fertilization cone disappears leaving the sperm on the the egg surface until 40–50 min after insemination when it is rapidly internalized by the egg. The midpiece with its mitochondrion remains on the egg surface (Fig. 1)

A similar process occurs in another polychaete, *Platynereis megalops* [14]. Here fertilization is internal following copulation and the eggs are extruded with sperm attached and the jelly forming. Penetration, as in *Nereis* requires over 30 minutes and results in the midpiece being left on the egg surface after the completion of sperm penetration. However, the large fertilization cone seen during *Nereis* fertilization is not formed in this

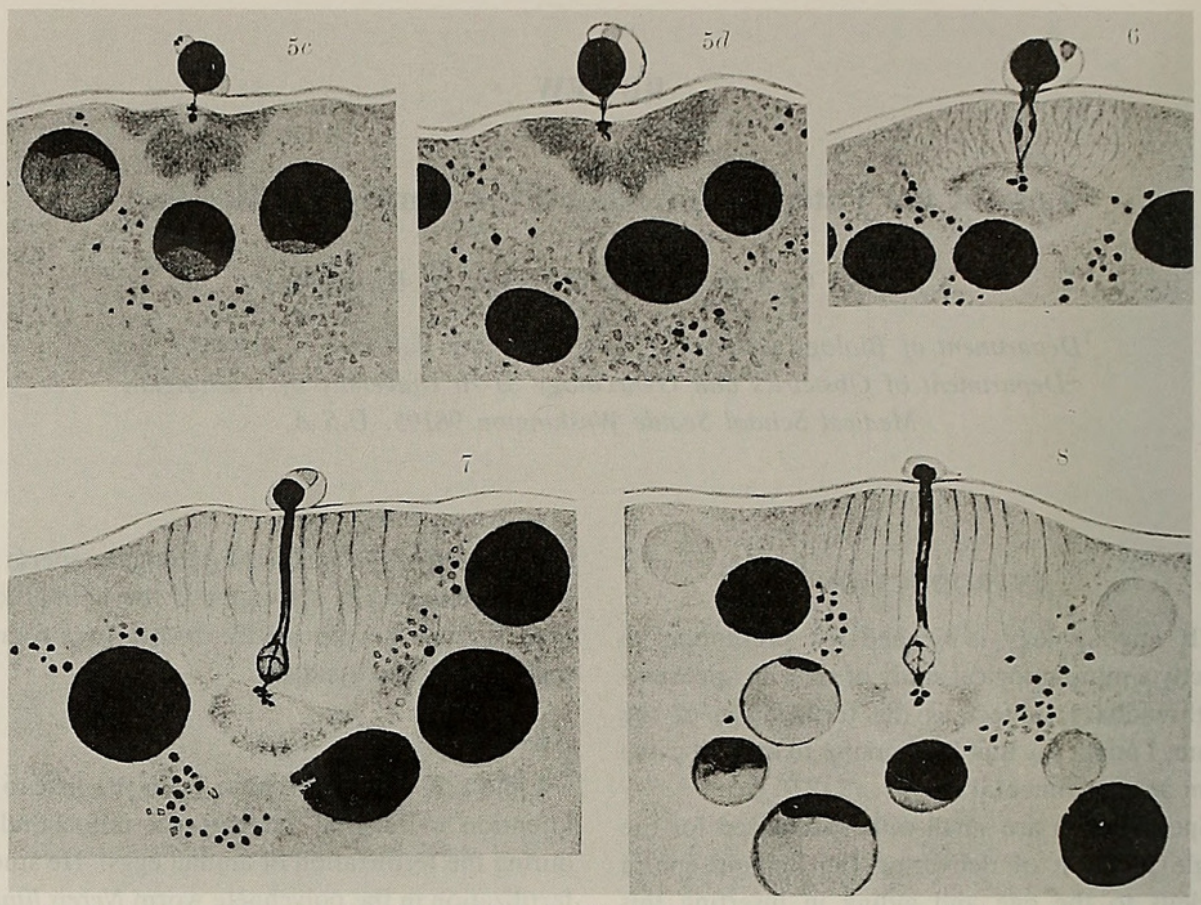

FIG. 1. Fertilization in the polychaete *Nereis limbata* (22) showing the sperm head and tail entering the egg and leaving the mitochondrion on the surface of the vitelline coat.

species.

In all other known cases of fertilization the sperm mitochondrion enters the fertilization cone and is taken into the zygote cytoplasm [23].

Mollusca

During the fertilization of most molluscs the sperm mitochondrion enters the egg and may even persist in the egg cytoplasm [24]. The sperm mitochondrial genome seems to be inherited to a high degree in the bivalve *Mytilus edulis* [10, 35] but this is clearly not the general case in bivalves.

Chitons with their heavily sculptured egg hulls have evolved a unique way to fertilize the egg without forcing the wide midpiece through the VC [1]. Chiton sperm are typical primitive sperm except for the locations of the mitochondria where one lies lateral to the nucleus as in the ascidian sperm and the other encircles the base of the nucleus [1]. In *Tonicella lineata* the sperm passes into cupules in the hull which give the sperm access to the underlying VC. Sperm-egg fusion occurs between the acrosomal membrane and an egg microvillus which penetrates into a pore in the VC. This composite tube formed from the fusion of the two membrane bound cylinders then serves as the connection through which the sperm chromatin mass is injected into the egg without even a nuclear envelope. This leaves the mitochondria and tail behind on the VC surface (Fig. 2).

Laternula limicola is a brackish water bivalve in which a golgi derived "temporary acrosome" moves from the sperm tip to the midpiece during spermatogenesis, finally taking up a position posterior to the mitochondrion in the midpiece [17]. During fertilization, the sperm first encounters a 24 μm thick jelly coat, then penetrates a 7 μm thick metachromatic layer before reaching the VC. The sperm then passes through the VC to the egg interior leaving the midpiece with its mitochondrion and temporary acrosome on the outside (Fig. 3). The temporary acrosome remains unchanged

throughout the entire process [17]. Another bivalve, *Lyonsia ventricosa* also has a temporary acrosome similar to that of *Laternula* [16] but we know of no studies of fertilization in this species.

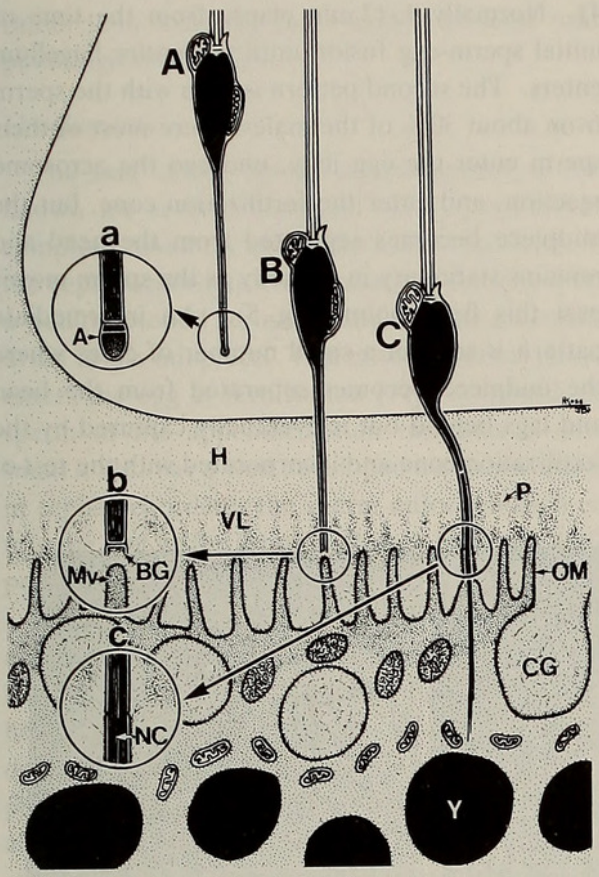

FIG. 2. Fertilization in the chiton *Tonicella lineata* (24). In this mollusc the sperm acrosome fuses with an egg surface microvillus and the nucleus is injected through the composite tube leaving the tail and 3 mitochondria on the egg surface.

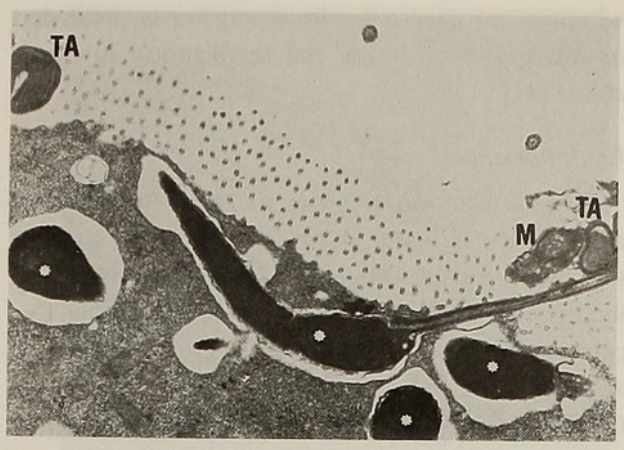

FIG. 3. Fertilization in the bivalve mollusc *Laternula limicola* (10). Here the mitochondrion (M) remains embedded in the vitelline coat with the "temporary acrosome" (TA) as the remainder of the sperm enters the egg cytoplasm.

Bryozoa

Both external and internal fertilization can be found in the bryozoans. In several brooded species fertilization occurs within the ovary during oogenesis. Markus [25] reported that only the nucleus enters the egg in several species, a finding confirmed by Matawari [26]. In a more detailed study, Temkin [31] found that sperm of *Dendrobeania lichenoides*, *Hippodiplosia insculpta* and *Tricellaria gracilis* leave both the midpiece and tail on the VC surface at fertilization; only the sperm head actually enters the oocyte. In another study [6], both the sperm nucleus and midpiece can be seen within the oocyte of *Chartella papyracea*, a species with precocious intra-ovarian fertilization. Thus there may be interspecific variation of

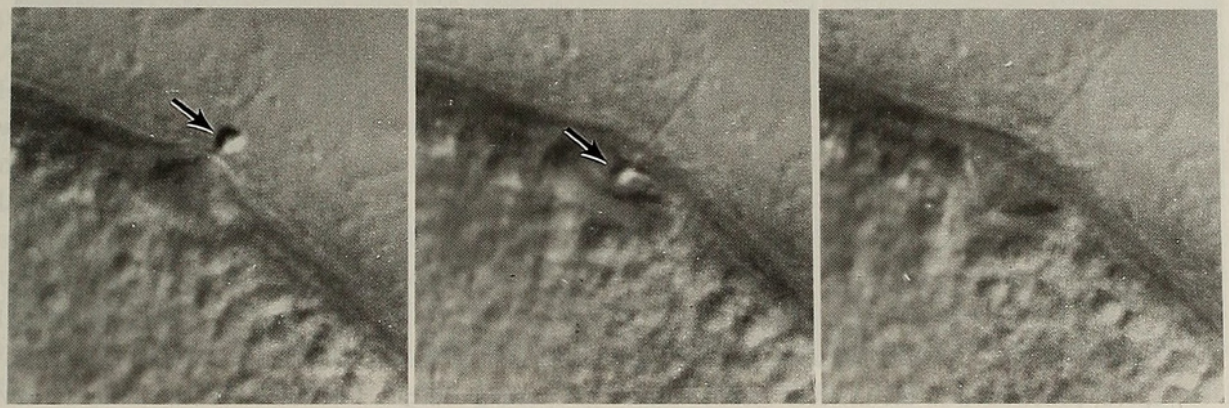

FIG. 4. Sperm penetration in the asteroid *Pisaster ochraceus* showing the usual pattern with the mitochondrion (arrow) entering the fertilization cone and egg.

whether the paternal mitochondrion is discarded or incorporated during the fertilization of bryozoans [31].

Echinodermata

In most echinoderms the sperm mitochondrion experiences a change in orientation at the time of the acrosome reaction [2, 29] but is generally incorporated into the egg during fertilization. Exceptions to this have been seen in a holothurian and an asteroid which seem to demonstrate facultative rather than obligatory sperm mitochondrial loss during fertilization. Colwin and Colwin [4] report that while the sperm mitochondrion usually enters the egg of the holothurian *Thyone briareus,* occasionally the midpiece remains outside as the sperm is incorporated.

In the asteroid *Pisaster ochraceus* careful observation reveals that there are three patterns of mitochondrial behavior during fertilization to be found among different males in the populations around San Juan Island, Washington [Battaglia, unpublished observations). In the most common pattern the head and midpiece remain intact and pass through the fertilization cone together (Fig. 4). Normally 9–12 min elapse from the time of initial sperm-egg fusion until the entire flagellum enters. The second pattern is seen with the sperm from about 30% of the males where most of their sperm enter the egg jelly, undergo the acrosome reaction, and enter the fertilization cone, but the midpiece becomes separated from the head and remains stationary in the jelly as the sperm moves past this fixed point (Fig. 5). An intermediate pattern is seen in a small number of cases where the midpiece becomes separated from the head and lags behind but is eventually captured by the fertilization cone and incorporated with the rest of

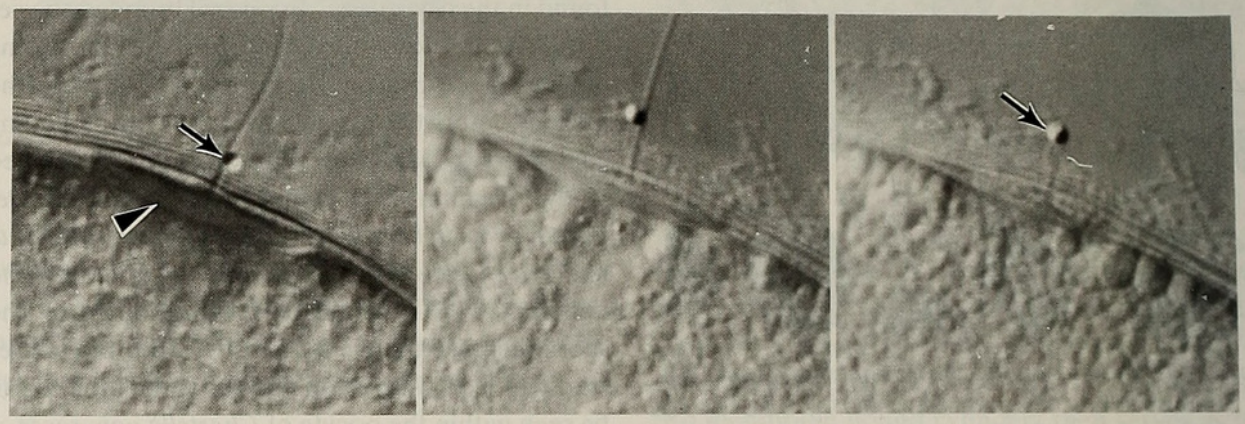

Fig. 5. Sperm penetration in the asteroid *Pisaster ochraceus* showing the case where the mitochondrion (arrow) remains on the surface of the vitelline coat. This pattern was seen in 30% of the males.

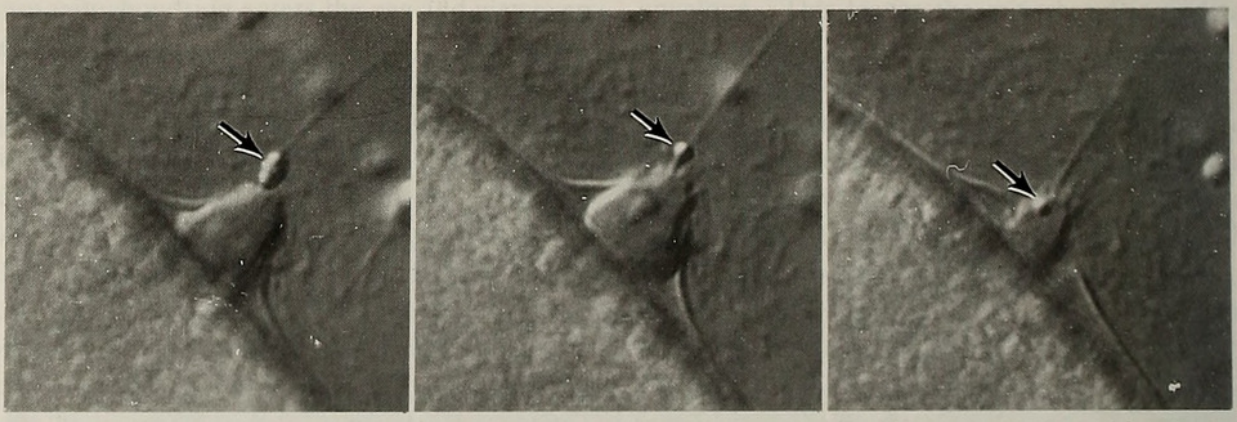

Fig. 6. Sperm penetration in the asteroid *Pisaster ochraceus* showing the case where the mitochondrion (arrow) separates from the nucleus but is eventually incorporated in the fertilization cone and taken into the egg cytoplasm.

the sperm (Fig. 6). These patterns were found in the sperm from different males and independent of the source of oocytes. Since these observations were made at the peak of the breeding season and led to perfectly normal development we conclude that they reflect normal modes of fertilization rather than testicular degeneration or some other pathological condition.

Urochordata

The fate of the sperm mitochondrion is now known for several members of the invertebrate complex commonly called the lower chordates. In the cephalochordate *Branchiostoma* the sperm has a well developed acrosome and a single mitochondrion in the midpiece. This mitochondrion clearly enters the egg at fertilization [11]. Although the sperm of the appendicularian tunicates is quite small it also has a well developed acrosome and as in cephalochordates the sperm mitochondrion enters the egg at fertilization (Linda Holland, 1992, personal communication).

Ascidian sperm lack a conspicuous acrosome and midpiece. Instead of a midpiece these sperm have the mitochondrion next to the nucleus in the head [20, for review of sperm structure]. The first detailed account of fertilization in ascidians reported that the mitochondrion entered the egg along with the nucleus and tail [27] but subsequent studies [7, 32, 33] report that sperm between the VC surface and egg surface lack the mitochondrion. Richard Cloney (personal communication, 1975 and 3) first observed the mitochondrion of *Molgula occidentalis* to remain on the VC surface as the sperm moved past this fixed point. This pattern of fertilization was subsequently seen in *Ascidia ceratodes* and other ascidians [9, 18, 19]. Figure 7 shows an *Ascidia ceratodes* sperm head within the inner layer of the VC surface while the mitochondrion remains on the outer surface of this layer between the follicle cells. Mitochondrial sliding can be induced in vitro by a number of treatments including alkaline SW and low Na^+ SW [18, 19]. Mitochondrial migration down the tail results in the sperm moving past this point when the sperm mitochondrion sticks to the cover slip *in vitro*. This sliding occurs at the rate of 6.7 μm/sec *in vitro* but 3.1 μm/sec *in vivo* into the egg and

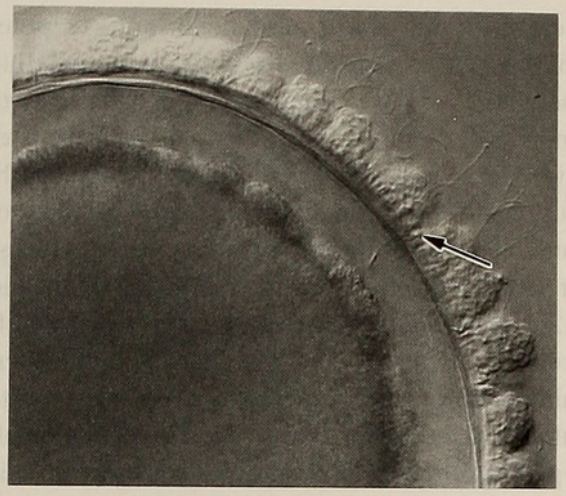

FIG. 7. Sperm penetration in the ascidian *Ascidia ceratodes*. Here the mitochondrion (arrow) remains on the vitelline coat surface between the follicle cells as the remainder of the sperm penetrates through the egg envelopes to reach the egg surface.

appears to be a rate limiting step in fertilization (Yim, Abadi and Lambert, unpublished data). Mitochondrial sliding is thought to drive the sperm through the VC and perivitelline space and into the egg [15, 20 for reviews]. In addition to the mechanical forces generated by mitochondrial sliding, sperm surface proteases are also involved [28 and Koch, Norton and Lambert, unpublished data].

Conclusions

The interplay between the physical and biochemical capabilities of the sperm and egg is instructive as to how the remarkable process of fertilization is accomplished. The management of sperm mitochondria during fertilization is rarely considered in studies of sperm-egg interaction. Generally it may be assumed that all of the mitochondria that survive in the zygote are of maternal origin. Thus, the prevention of paternal mitochondrial replication after fertilization may be a universal feature of all species. The importance of disposing of paternal mitochondria during fertilization is speculative; however, the mechanisms underlying the success of this phenomenon are interesting. Recent evidence suggests that mitochondrial DNA has an extremely high rate of mutation which may be exacerbated by free radical formation [34]. It is

clear that the sperm mitochondrion is a potent source of free radicals due to its extremely high metabolic activity. Thus, oxidative damage to the unprotected mitochondrial genome may be extensive in mature, active sperm. It is conceivable that this could result in inefficient/abnormal zygote mitochondria if the replication of these damaged organelles were allowed to occur after fertilization [12]. Most animal eggs appear to deal with this scenario by inactivating and destroying the sperm mitochondria after they have been incorporated into the egg cytosol. Fertilization in the mammal is a good example of a class of animals where active destruction of sperm mitochondria is mediated by the egg [30]. The complexity of the mammalian midpiece precludes its expulsion during sperm incorporation. It contains many mitochondria which rapidly swell and are destroyed upon entry into the egg cytosol. Thus, all mitochondria in the mammalian embryo are considered to be of maternal origin (although recent evidence suggests that 0.1% of human sperm mitochondria are replicated in the fertilized egg) [34].

However, the mechanisms attendant to the inactivation or destruction of mitochondria within the egg may not exist in all species, particularly in animals where mitochondrial expulsion is observed as described in this review. In these species it is the physical elimination of this organelle that prevents its incorporation into the egg, thereby avoiding any replication of the paternal mitochondrial genome. The results are the same, but the mechanisms that accomplish these phenomena may be different. For example, the ascidian sperm appear to have a complex mechanism that utilizes cytoskeletal elements in the sperm to actively expel the mitochondrial compartment [21]. In contrast, the expulsion of sperm mitochondria in some starfish and polychaetes utilizes a more passive approach; the physical constraints imposed by the egg extracellular matrix aid in holding the mitochondrial compartment in place while the rest of the sperm slides past. One unifying principle to all of these species is that so-called "primitive" sperm are involved, thereby suggesting that mitochondrial expulsion can only be accomplished in species possessing a simple sperm architecture.

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