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Ultrastructural Features of Mammalian Sperm: Applications of Cold Field-Emission Scanning Electron Microscopy

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The ultrastructure of mammalian spermatozoa is typically studied using TEM thin sectioning [1] or TEM surface replicas [2]. Recently, we have applied cold field-emission scanning electron microscopy (FE-SEM) to answer diverse questions about sperm morphology and ultrastructure. The specialized electron gun technology of FE-SEM allows for imaging at resolutions that rival TEM, at least for biological samples [3]. Here, we describe studies of the sperm tail annulus, perinuclear theca, and other sperm structures. We present examples of how FE-SEM provides data not easily obtained by more conventional imaging methods.

The mammalian sperm annulus is a ring-like structure composed mainly of septin proteins and is found in the sperm tail between the mitochondrial and fibrous sheaths. Recent knockouts of the septin 4 gene in mouse showed that the annulus does not form in the absence of septin 4 protein [4, 5]. We are currently using FE-SEM to characterize the sperm tail and annulus in septin 4 wild-type, heterozygous and null mutant mice (Figs. 1A-1C). Initial results indicate that the annulus might act as an anchor for both the mitochondrial sheath and the fibrous sheath. An advantage of FE-SEM for this work lies in the ability to rapidly acquire statistically significant data about the thickness of the annulus in heterozygous mice.

The acrosome of guinea pig sperm is unusually large and therefore this species is often used as a model to study the acrosome reaction in mammals. We are using FE-SEM to characterize changes in the proteinaceous layer (perinuclear theca) that underlies the acrosome and plasma membrane in mammal sperm using the guinea pig as a model. We have found a novel feature in the equatorial segment region of acrosome-reacted guinea pig sperm: a band of pores in the perinuclear theca, the function of which is currently unknown. Figs. 2A and 2B compare intact and acrosome-reacted guinea pig sperm.

Fig. 3 shows further examples of the utility of FE-SEM for characterizing sperm ultrastructural features. Figs. 3A and 3B are surface views of intact mouse and hamster sperm heads. In both these species, we have detected circular and tube-like structures in the acrosomal region of the sperm head. These structures have only been previously reported once in the literature [2]. They may represent the presence of membrane or underlying cytoskeletal microdomains in the sperm head. Fig. 3C demonstrates the presence of a recently reported structure in the mammalian sperm head termed the equatorial subsegment [6, 7]. The function of the equatorial subsegment is unknown, but it may be involved in sperm-egg interactions as this region of the sperm head has been reported to be the first to fuse with the egg membrane during the initial stages of fertilization [8].

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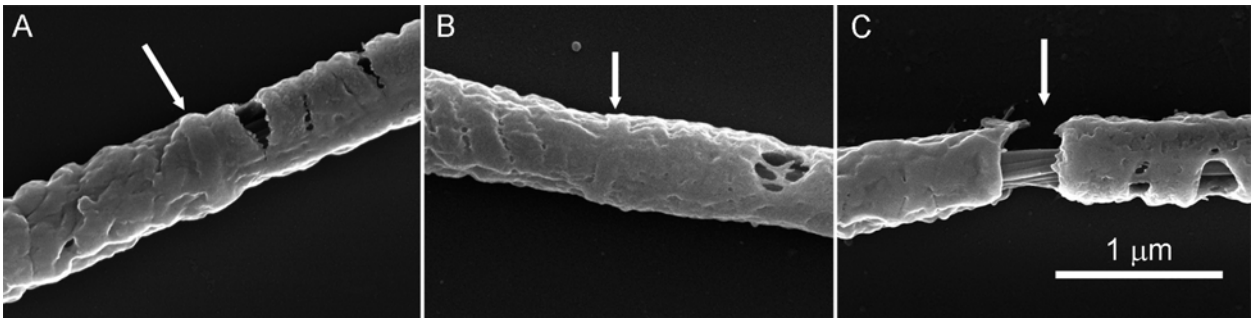

Fig. 1. Epididymal sperm from mice that are (A) wild-type, (B) heterozygous, and (C) null for the septin 4 gene. In (A) and (B) arrows indicate the annulus; in (C) the arrow indicates where the mitochondrial and fibrous sheaths have pulled apart due to the missing annulus. Bar = 1 micron for all panels.

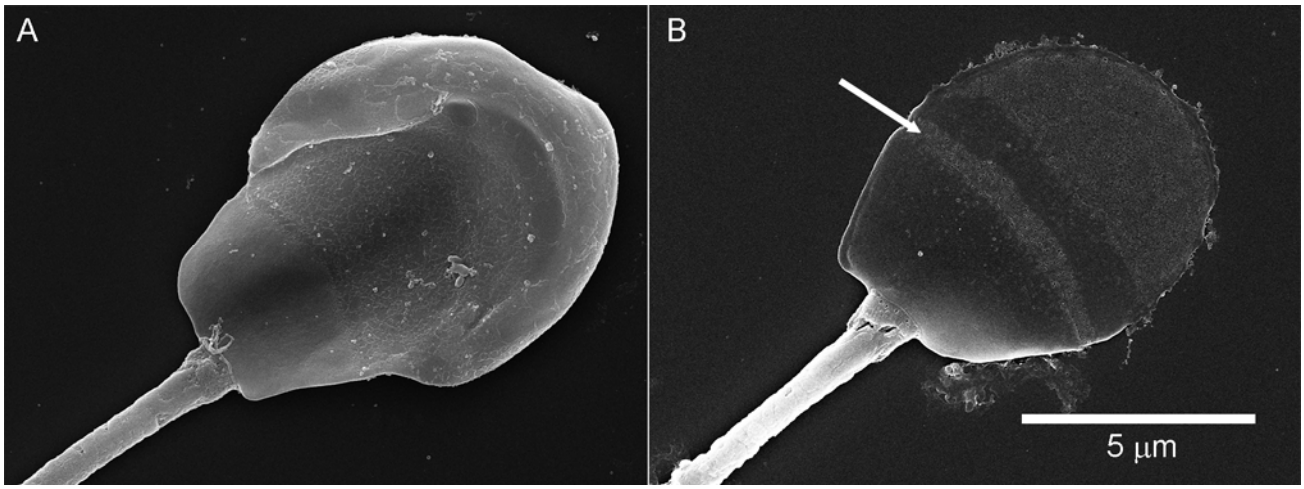

Fig. 2. Epididymal guinea pig sperm. (A) Acrosome intact. (B) Acrosome-reacted. Arrow indicates region where band of pores is located. Bar = 5 microns for both panels.

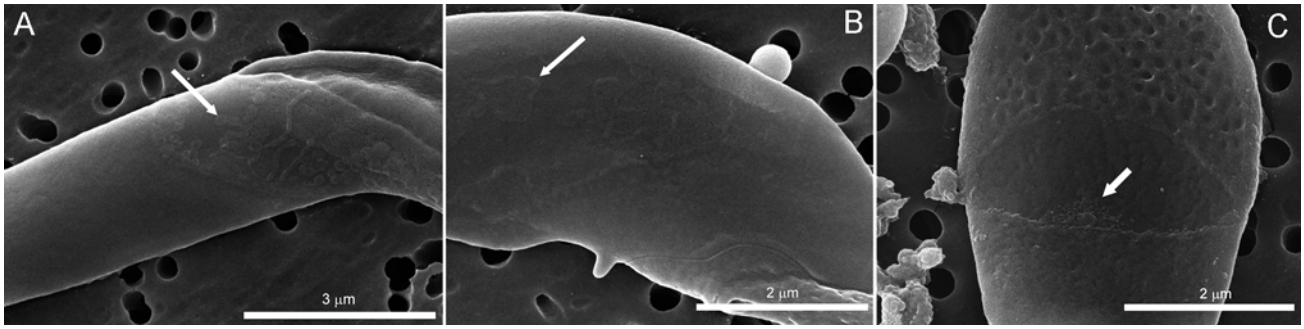

Fig. 3. (A and B) Epididymal hamster and mouse sperm, respectively, showing circular and tube-like structures (arrows) in acrosomal region. (C) Equatorial subsegment (arrow) within the equatorial segment of collard peccary sperm.

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