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Golden hamster perivitelline spermatozoa do not show proacrosin/acrosin at the inner acrosomal membrane

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It has been suggested that acrosin may function in penetration of the zona pellucida and of the highly structured extracellular matrix of the perivitelline space. In this study we investigated whether golden hamster perivitelline spermatozoa contain proacrosin/acrosin, as evidenced by the silver enhanced immunogold technique using the monoclonal antibody antiacrosin C2E5. None of the 197 spermatozoa recovered from the perivitelline space showed proacrosin/acrosin associated with the acrosomal region, suggesting that acrosin would not play a role in the penetration of the perivitelline extracellular matrix.

INTRODUCTION

Acrosin –a trypsin-like serine proteinase present at the outer surface of inner acrosomal membrane (Barros *et al.*, 1992)– is believed to play a central role in mammalian fertilization (Tesarik *et al.*, 1988; Barros *et al.*, 1990; Töpfer-Petersen *et al.*, 1990, 1991; Capote *et al.*, 1991). Its proteolytic activity and carbohydrate-affinity could be important in binding to and penetrating into the zona pellucida. Moreover, the presence of a highly structured extracellular matrix in the perivitelline space of several mammalian species (Talbot and DiCarlantonio, 1984a, 1984b; Dandekar and Talbot, 1992), suggests that acrosin could also play a role in penetration of this extracellular matrix (Planchenault *et al.*, 1991). For this to occur it would be necessary that perivitelline spermatozoa contain active proacrosin/acrosin at the outer surface of the inner acrosomal membrane. To test this hypothesis the presence of acrosin was studied in golden hamster perivitelline spermatozoa by the silver enhanced immunogold technique.

MATERIALS AND METHODS

Caudal epididymal spermatozoa were incubated for 2 hours at 37° C and 5% of CO_2 in Tyrode albumin pyruvate lactate culture medium (TAPL-10K culture medium; Yanagimachi, 1982; Barros *et al.*, 1984). For this purpose, 100 µl drops of medium containing 2 x 10^6 spermatozoa/ml were placed in sterile Petri dishes covered with mineral oil. Perivitelline spermatozoa were obtained from aged hamster oocytes in which spermatozoa cross the zona pellucida but gamete membrane fusion does not take place. Cumulus-free aged oocytes, recovered by flushing the oviducts of superovulated females, were stored at 4° C for 24 hours. Oocytes were inseminated for 10 min with capacitated spermatozoa and then transferred to fresh culture medium without spermatozoa for 2-3 additional hours. Eggs were thoroughly washed by pipetting and, at the end of the incubation, spermatozoa bound to the outer surface of the zona pellucida were removed. Perivitelline spermatozoa were isolated by breaking the zona pellucida with tweezers (Nº 7, Dumont) on slides previously treated with 0.1% poly-l-lysine (Sigma). Spermatozoa were processed by the silver enhanced immunogold technique (Auro Probe One GAM and Intense EM, Janssen; Leunissen *et al.*, 1989), using the monoclonal antibody to proacrosin/acrosin C2E5 (Elce *et al.*, 1986) as described elsewhere (Barros *et al.*, 1992). Control sperma-

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tozoa obtained from the incubation drops at the start of incubation, were permeabilized with cold methanol, and then treated as described above.

RESULTS AND DISCUSSION

Aged oocytes (Fig. 1a) do not undergo the cortical reaction and thus may bind capacitated spermatozoa (Barros and Yanagimachi, 1972). Moreover, the zona pellucida of freshly ovulated and aged oocytes does not differ in its ability to induce the acrosome reaction and in its susceptibility to be digested by trypsin (unpublished results). These data support the view that the zona pellucida of aged oocytes maintains many of its biological properties. That is why, in this study, all aged oocytes were penetrated by many spermatozoa (Fig. 1b). The zona pellucida of 23 eggs was ruptured and 197 perivitelline spermatozoa were recovered ($x = 8.6$ spermatozoa/egg). Unlike the controls (Fig. 1c), none of these

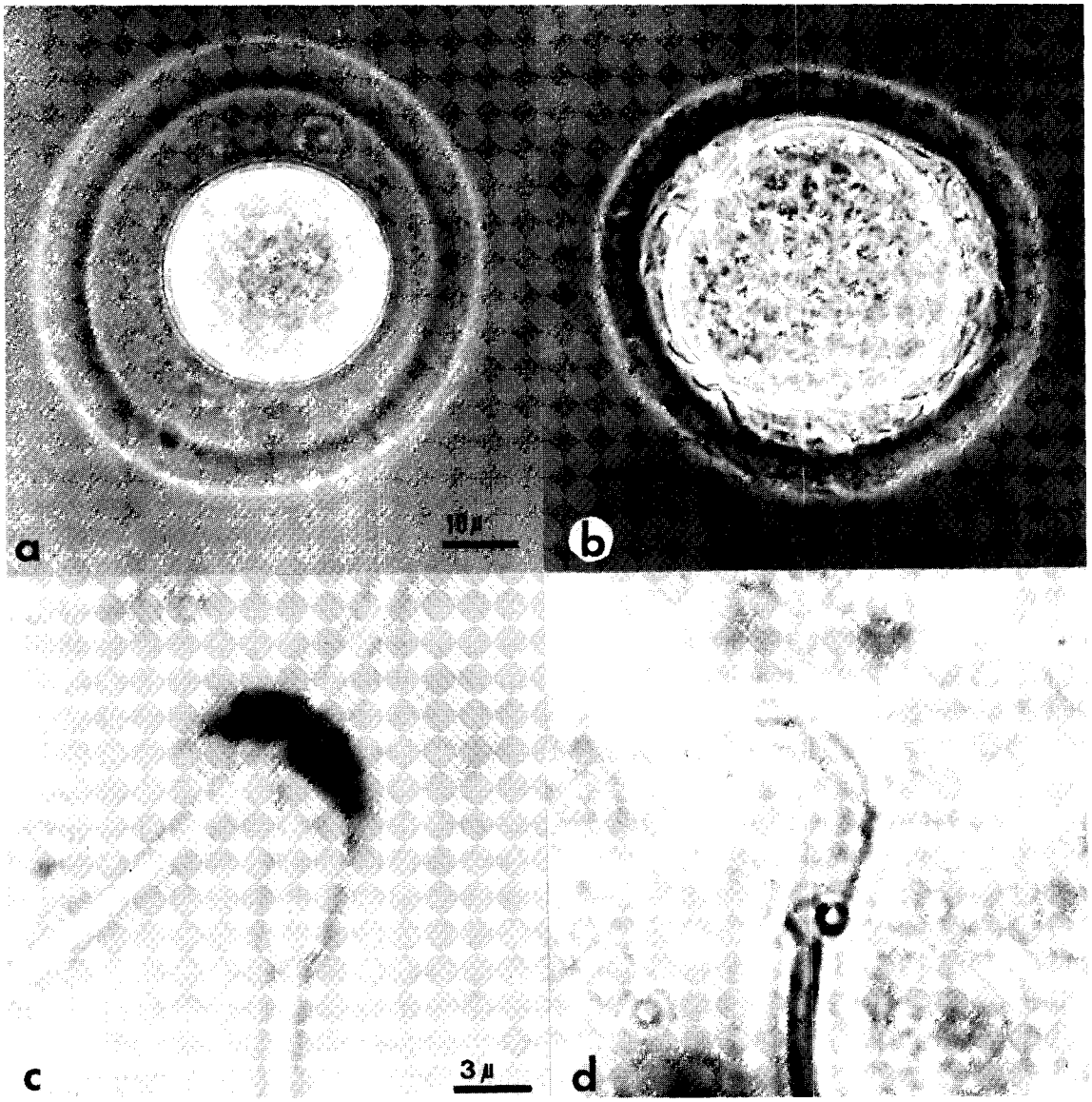

Fig. 1: (a) Hamster aged oocyte. Notice the broad perivitelline space (phase contrast microscopy, 1060x). (b) Oocyte showing many spermatozoa located in the perivitelline space after elimination of spermatozoa bound to the outer surface of the zona pellucida (phase contrast microscopy, 1060x). (c) Control spermatozoon showing intense acrosin labeling (bright field microscopy, 3400x). (d) Perivitelline spermatozoon showing no labeling (bright field microscopy, 3400x).

spermatozoa showed labeling associated to the acrosomal region (Fig. 1d).

These results are consistent with previous findings that shortly after detaching from the acrosomal cap, hamster spermatozoa lack acrosin (Barros *et al.*, 1992). In contrast, rabbit spermatozoa that have penetrated the zona pellucida showed acrosin associated with its acrosomal region (Meléndez *et al.*, 1992). It seems posible that in golden hamster, acrosin is used up during the penetration of the zona pellucida and therefore no enzyme would be present in perivitelline spermatozoa, as it has been suggested by Planchenault *et al.* (1991).

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