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The role of the epididymis during maturation of mammalian spermatozoa *in vivo* and *in vitro*

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Introduction.

Fertilization in mammals involves a series of recognition processes between the spermatozoon and the oocyte. These interactions are often species and cell specific (Moore and Bedford, 1983; Bedford, 1981). For example, human spermatozoa will normally bind to oocytes and not to other somatic cells. They also display limited specificity towards foreign eggs and in fact will only attach to the eggs of Great Apes (Bedford, 1977). Such experiments suggest that mammalian spermatozoa have specific receptors on their surface which are able to recognize complementary binding sites on the zona pellucida. During evolution the deployment of these receptor mechanisms has become inextricably linked to the physiology and biochemistry of the whole animal. In order to understand how sperm/egg recognition processes may operate it is important to know when gamete surface molecules are first expressed and their nature.

Numerous studies have shown that mammalian spermatozoa acquire fertilizing capacity on passing through the proximal epididymis (Bedford, 1975; Moore, Hartman and Pryor, 1983). In the rabbit and hamster, studies using surgical manipulations of the excurrent ducts *in situ* (Orgebin-Crist *et al.*, 1975; Horan and Bedford, 1972) or using antibodies (Lea, Petrusz and French, 1978; Moore, 1981; Moore and Hartman, 1984) have established that there is a positive contribution by the epididymal epithelium to the development of this maturation. But the exact nature of the factors responsible for this maturation have yet to be identified. Unfortunately, the relationship between the epididymal epithelium and luminal spermatozoa has proved difficult to study *in vivo*. We have therefore investigated the possibility of carrying out *in vitro* sperm maturation and have used the hamster as an animal model (Moore, Hartman and Smith, 1986).

In vitro culture of hamster epididymal epithelium.

The microenvironment of the epididymal lumen is generated by the secretory and absorptive activity of the epithelium under the control of androgens, directly from the testis in rete testis fluid, or via the peripheral circulation. *In situ* this site

is not easily sampled, say by micropuncture technique, without altering epididymal function. Organ and tissue culture methods have therefore been attempted to mimic epididymal function. Principal cells cultured *in vitro* show normal morphological features for several days (Kierszebaum *et al.*, 1981 ; Klinefelter *et al.*, 1982 ; Joshi, 1985). Moreover, organ culture of epididymal tubules will induce limited sperm maturation although access to the lumen is still restricted (Orgebin-Crist and Jahad, 1979 ; Cuasnicú *et al.*, 1984).

To provide a simplified system to study epididymal function we have developed a cell culture preparation in which the ultrastructural epithelial architecture remains largely intact but spermatozoa can gain complete access to the apical surface of the epithelial cells (Moore, Hartman and Smith, 1986). Briefly, the method involves digesting lengths of tubule from hamster corpus or cauda epididymidis with collagenase in order to remove connective tissue. The tubules are then split along their longitudinal axis using fine needles. This procedure forms plaques of epithelium which in culture turn into spheres of tissue with epithelium facing outwards. The culture medium consists of RPMI 1640 supplemented with 10 % foetal calf serum, and containing 1 µM sodium pyruvate, 100 nM insulin, 200 nM hydrocortisone, 200 nM testosterone, 1 µM dihydrotestosterone, 5 µg transferrin/ml and 1 µg retinol/ml. The medium is filtered before use and supplemented with streptomycin and penicillin. Epithelial preparations are cultured in 5 % CO in air and within 24 hours many tissue spheres form. At high magnification epithelial cells (with microvilli present) can be observed around the edge of these balls of tissue (fig. 1). Electron microscopy revealed that spheres of epithelium consisted of an outer layer of epithelial cells, a basal lamina and an inner sub epithelial layer consisting of contractile myoid cells and fibroblasts. When tissue was prepared from adult males, spermatozoa also became entrapped within the balls as they formed. In general, epithelium cultured for 4-6 days displays a morphology consistent with secretory and absorptive function.

The secretory nature of the epithelial culture has been assessed using a monoclonal antibody (C5) against a 34 Kd protein (Smith, Hartman and Moore, 1986) first synthesised and secreted by principal cells in the proximal corpus epididymidis but not produced by cells in the caput or initial segment regions (fig. 2). On cultured epithelium this antigen is observed on the apical surface of principal cells for at least the first 4 days of culture and sometimes up to 7 days. In the absence of testosterone and dihydrotestosterone, antigen could not be detected after the second day of culture suggesting that its synthesis and secretion was under the control of androgens.

Development of the fertilizing capacity of hamster spermatozoa *in vitro.*

In the hamster, spermatozoa are fully fertile when recovered from the proximal cauda epididymidis but display low fertilizing capacity (5-15 %) when recovered from the distal corpus region. This lack of fertility is associated with a reduced

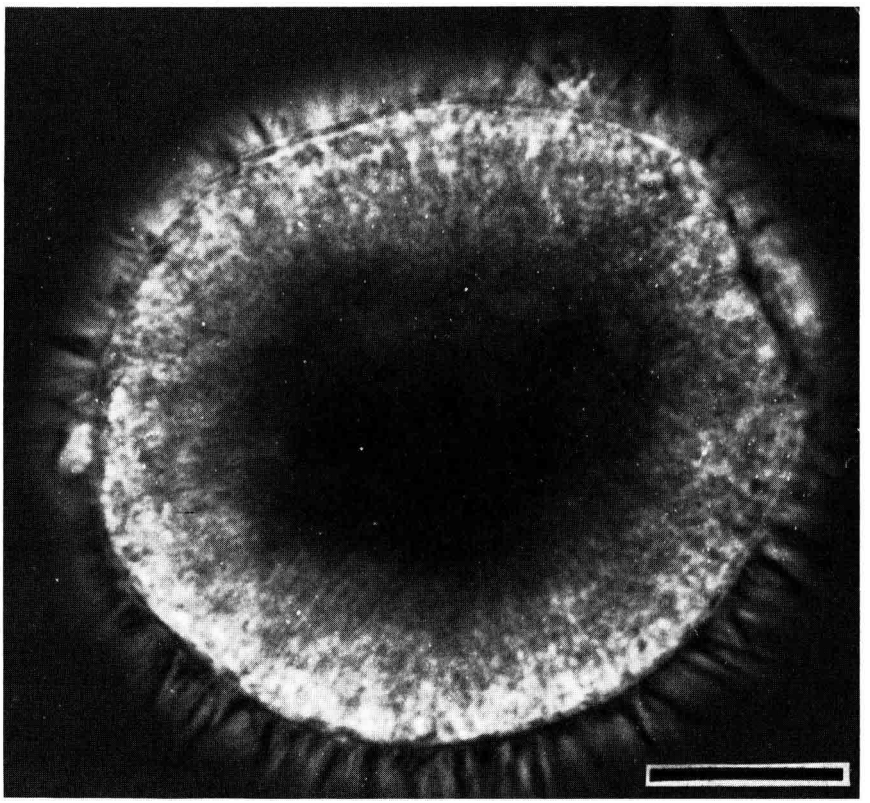

FIG. 1. — *A micrograph of an epithelial ball after 24 hours of culture.* Microvilli are clearly visible on the surface. Phase contrast. Bar = 10 µm.

ability to bind to the zona pellucida and slow progressive motility. To examine whether *in vitro* maturation of immature spermatozoa could be achieved, spermatozoa were recovered from the corpus region and incubated with 3 day-old epithelial cultures for 6 hours in 50 µl microdrops under oil. Sperm fertility was then assessed by *in vitro* fertilization or *in vivo* insemination of females 2-3 hours before the estimated time of ovulation (Moore and Hartman, 1986). Although a pronounced increase in the binding of spermatozoa to the zona pellucida was evident following incubation with epithelial culture there was no significant increase in fertilizing ability compared with control spermatozoa. This acquisition of zona-binding receptors on spermatozoa after co-culture was in agreement with previous work by (Cuasnicú *et al.*, 1984) but failure to promote fertility was contrary to the report again by Blaquier's group (Gonzales-Echieverría *et al.*, 1984) where maturation was successful with epididymal protein extract.

Since spermatozoa from the distal corpus region do not exhibit the same motility characteristics as those from the cauda epididymidis it was surmised that this could be the reason for the lack of fertility. By ligating the hamster epididymis

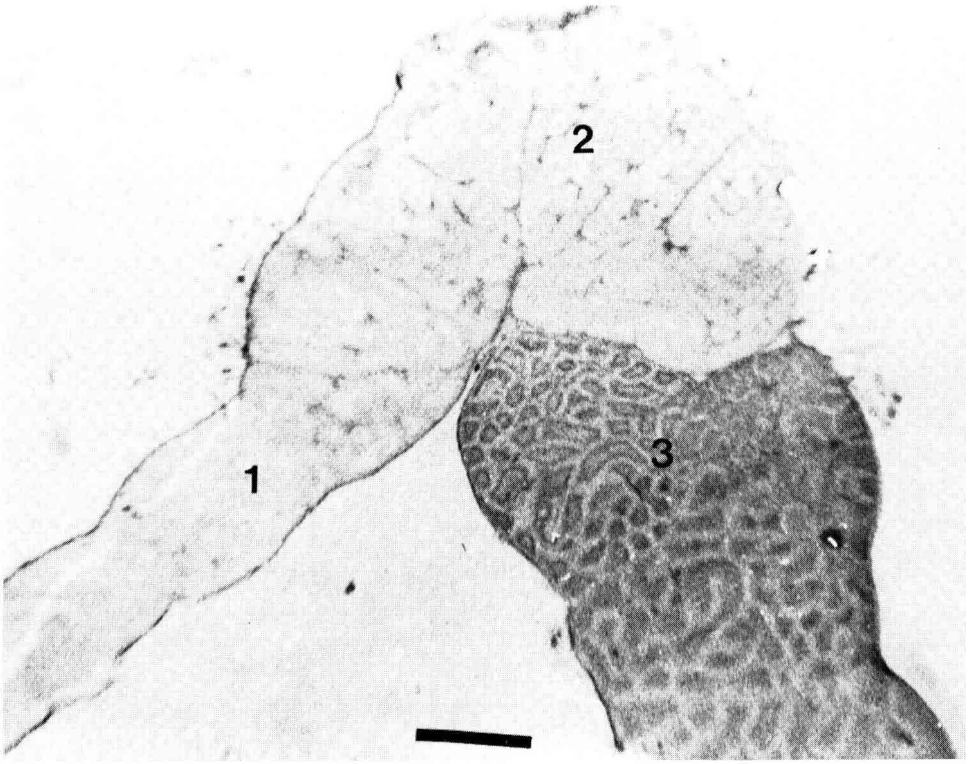

FIG. 2. — *A low power micrograph of a section of proximal epididymis from an adult hamster following immunocytochemical localisation with C5 antibody.* Staining was limited to the proximal corpus, first on the epithelium and more distally on spermatozoa. Bar = 1 mm.

at the junction of the distal corpus and proximal cauda regions for 3 days, spermatozoa recovered from the corpus region attain increased progressive motility and head to head autoagglutination but still have low fertility. However, the incubation of these spermatozoa with epididymal epithelium for 6 hours resulted in a significant increase in fertilising capacity *in vitro* or *in vivo* to 30-40 %. The fertilising ability of distal corpus spermatozoa incubated with 3 day old cultures without androgens, or with 8-12 days old epithelial cells with fibroblast overgrowth, or without epithelial cells remained low (5 %). Since all the sperm samples maintained their motility and underwent similar capacitation and fertilization conditions it can be concluded that factors present in the culture of proximal cauda epithelium promoted the final stages of hamster sperm maturation (fig. 3).

One of the processes of maturation probably involves the acquisition of the receptor for recognizing the oocyte although it remains to be determined how this receptor is formed. Studies with C5 monoclonal antibody indicate that epitopes can be specifically transferred from the epididymal epithelium to spermatozoa *in vitro*. If spermatozoa from the caput region are incubated with corpus epithelial

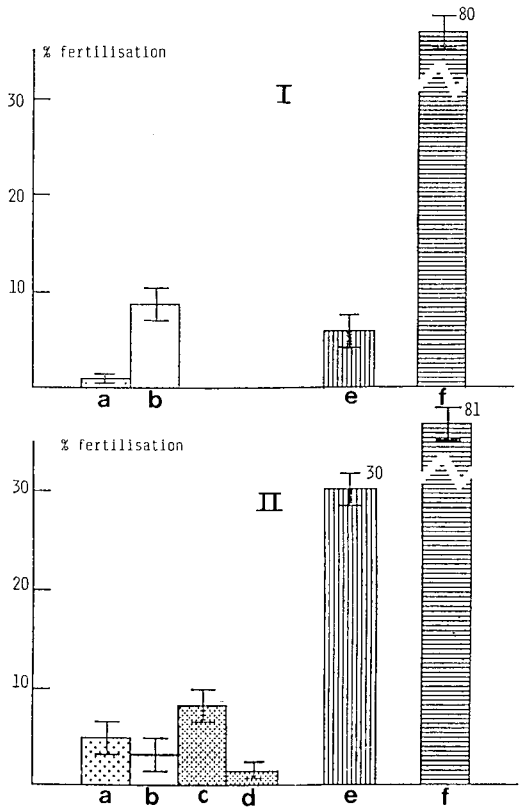

FIG. 3. — *Histograms of the fertilising ability* in vitro *of spermatozoa from the distal corpus epididymidis of intact* (I) *and ligated* (II) *adult hamsters following pre-incubation treatments.* (a) culture without androgen ; (b) without incubation ; (c) old epithelial culture ; (d) without epithelial culture ; (e) culture with androgen ; (f) cauda sperm positive control.

cultures they acquire antigen to C5 on their post-acrosomal region and on their annulus (fig. 4). This limited binding of determinant suggests a specific coupling to the sperm surface. The membrane changes were concomitant with the induction of forward motility in a proportion of spermatozoa. While the molecular weight of 34 000 for the C5 determinant would indicate that it is not forward motility protein, the specific binding to the annulus might well induce changes in the flagellum movement.

Conclusion.

These *in vitro* studies support previous *in situ* investigations and extend our understanding of the role of the epididymal epithelium during mammalian sperm maturation. The use of immunological techniques with polyclonal and monoclo-

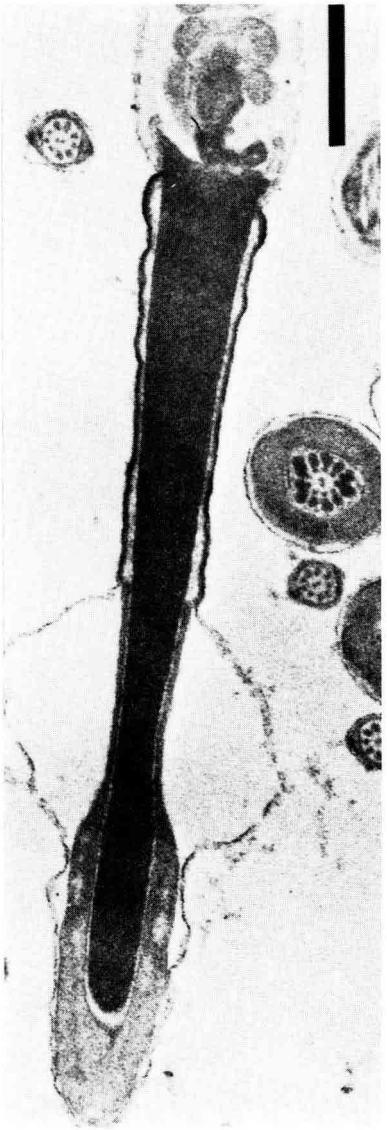

FIG. 4. — *Electron micrograph of a section of hamster spermatozoa from the corpus epididymidis after immunolocalisation for C5 determinant.* Reaction product is present on the post-acrosomal region. Bar = 1 μm.

nal antibodies have shown conclusively that mammalian sperm surface proteins and glycoproteins undergo extensive but specific reorganization during epididymal maturation due to (a) inherent membrane modifications related to morphological reorganization and membrane fluidity changes and (b) the addition of components (protein or sugars) secreted by the epididymal epithelium under the control of androgens. Recent investigations indicate that the foundation for these changes is first laid down during spermatogenesis.

In our laboratory we are currently devising methods to culture human epididymal epithelium from patients undergoing epididymovasectomy. These techniques may allow us to directly observe human sperm maturation processes *in vitro.*

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Résumé. *Le rôle de l'épididyme au cours de la maturation* in vivo *et* in vitro *des spermatozoïdes chez les mammifères.*

Chez les mammifères les spermatozoïdes ne sont pas fécondants à la sortie du testicule et n'acquièrent leur capacité à féconder qu'après leur transit à travers les régions proximales de l'épididyme. Chez l'homme la maturation finale des spermatozoïdes s'effectuerait dans le corps de l'épididyme, ce qui serait en accord avec les résultats obtenus par des techniques chirurgicales telles que l'épididymovasostomie.

Afin d'explorer les processus de la maturation des spermatozoïdes des méthodes de culture *in vitro* ont été mises au point. Chez le hamster l'activité fonctionnelle de l'épithélium épididymaire a été maintenue *in vitro* pendant 5 jours. Des spermatozoïdes immatures incubés avec ces cultures acquièrent leur pouvoir fécondant. L'usage d'anticorps monoclonaux a permis de montrer que des produits sécrétoires spécifiques sont transférés de la surface des cellules principales épididymaires en culture sur les spermatozoïdes. Des travaux similaires sur l'épithélium épididymaire humain sont maintenant en cours de développement. Les résultats indiquent que l'activité fonctionnelle des cellules épithéliales peut être maintenue en culture et devraient permettre une exploration plus poussée de la maturation des spermatozoïdes humains.

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