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## A ram epididymal secretory protein shares common antigenic determinants with rat epididymal proteins and human seminal plasma proteins

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Mammalian spermatozoa leave the testis as immature cells unable to fertilize until they have traversed the epididymis. Sperm maturation is an orchestrated series of sequential events occurring at different levels of the epididymis: sperm-zona pellucida binding ability in the proximal epididymis, progressive motility and the ability to penetrate and fertilize the oocyte in the middle epididymis and the ability to initiate normal embryonic development in the distal epididymis (see reviews by Orgebin-Crist, 1986; Amann, 1987; Fournier-Delpech and Courot, 1987). In parallel, spermatozoa undergo a variety of surface changes (Hammerstedt and Parks, 1987). Although the functional significance of these changes has not been elucidated, it is believed that some could be related to the processes of sperm maturation. Sperm maturation itself is dependent upon androgen-controlled secretory products of the epididymal epithelium (see review by Orgebin-Crist, 1986). Since inhibitors of protein synthesis block the acquisition of fertilizing ability within the epididymis, particular attention has been focused on epididymal proteins which are secreted upon androgen stimulation and which interact with the maturing spermatozoa. Such proteins could mediate the androgenic regulation of fertilizing ability of the spermatozoa.

The rat epididymis synthesizes specific proteins under testosterone stimulation which have an affinity for immature spermatozoa from the testis or the proximal epididymis. One such protein migrates in the pre-albumin area on non-denaturing polyacrylamide gel electrophoresis and, therefore, has been called prealbumin epididymal specific (PES) (Fournier-Delpech, Bayard and Boulard, 1973). This rat PES protein has been studied under a number of other names: acidic epididymal glycoprotein (AEG) (Lea *et al.*, 1978, SEP (Kohane *et al.*, 1979), SP (Faye *et al.*, 1980), 32 kD protein (Wong and Tsang, 1982), proteins D and E (Garberi *et al.*, 1979). The PES protein, which apparently has two components (Garberi *et al.*, 1979; Brooks *et al.*, 1986), has been immunolocalized on the epididymal epithelium and has been shown to bind to a restricted region of the sperm head. Recently, from amino acid sequence data, Brooks *et al.* (1986) have shown that these proteins are homologous with carboxypeptidase Y, although they do not express this enzyme activity. In the ram there is a protein of

64 kD molecular weight which runs in the prealbumin region on non-denaturing gels. The protein, synthesized by the epididymis under testosterone stimulation, binds to the plasma membrane covering the acrosome of immature testicular ram spermatozoa and is still present after ejaculation. This protein could thus be involved in sperm-egg interaction at fertilization (Fournier-Delpech *et al.*, 1981 ; Jones, Fournier-Delpech and Willadsen, 1982 ; Fournier-Delpech *et al.*, 1988).

In this paper we present information on common antigenic determinants shared by androgen-dependent epididymal proteins in the rat (rat PES 32 kD) and the ram (ovine PES 64 kD) and also by human seminal plasma proteins.

## Materials and methods.

### *Materials.*

*Animals* : Epididymides were obtained from three adult Ile-de-France rams in breeding season and from 50 adult Wistar male rats (I.N.R.A. strain). Samples of human seminal plasma were provided by 10 donors of proven fertility.

*Products* were from Roussel-Uclaf (testosterone implants of 200 mg each), Vecta Laboratories (Vecta Stain, ABC kit) Sigma and Prolabo (other products).

### *Methods*

#### a. *Protein purification.*

1. *Ovine PES 64 kD.* — The rams were orchidectomized and 45 days later the left epididymis was removed. A sample of epididymal region 3 (Jones, Fournier-Delpech and Willadsen, 1982) was prepared for electron microscopy (see below). At the same time a testosterone implant (200 mg) was placed under the thorax skin. Three weeks later the right epididymis was removed and a piece of region 3 was also prepared for electron microscopy. After removal of the connective tissue envelope the remaining tissue was used for purification of Ovine PES 64 kD (Fournier-Delpech *et al.*, 1988). Briefly, the tissue was homogenized in saline and  $\text{SO}_4(\text{NH}_4)_2$  added to a final concentration of 25 % (w/v). After centrifugation, the supernatant was dialyzed against Tris-HCl buffer (10 mM, pH 8.8-9) and passed over a DEAE-Sephacel column. Proteins eluted by 0.3 M ClNa were submitted to preparative electrophoresis on non-denaturing polyacrylamide slab gels (7 % acrylamide, Tris-glycine buffer at pH 8.3). The PES was electroeluted, dialyzed against  $\text{CO}_3(\text{NH}_4)_2$  10 mM and lyophilized.

2. *Rat PES 32 kD.* — Forty-five (45) rats were orchidectomized and 45 days later a testosterone implant (10 mg) was placed under the skin of the thorax. Three weeks later, the epididymides were removed, homogenized in cold saline, centrifuged (30,000 g  $\times$  1 hour), and the supernatant passed through a column of Concanavalin A-agarose. Glycoproteins were eluted by  $\alpha$ -2-methyl-mannose (5 % in aceto-acetic buffer, 0.1 M, pH 5.6 containing 5 mM  $\text{CaCl}_2$  and  $\text{MgCl}_2$ ), dialyzed against Tris-HCl buffer (10 mM, pH 8.8-9) and were fractionated on

polyacrylamide slab gels under non-denaturing conditions (7 % acrylamide Tris-glycine buffer, pH 8.3). Protein D was separated from protein E, electroeluted, and lyophilized after extensive dialysis against  $\text{CO}_3(\text{NH}_4)_2$ .

3. *Human seminal plasma proteins*. — Samples of seminal plasma, after liquefaction, were treated with sodium azide (0.3 % final) and centrifuged (800 g × 30 min). The supernatants were pooled and centrifuged at 120,000 × g for 1 hour. The supernatant was dialyzed against Tris-HCl buffer (10 mM, pH 8.8-9) and submitted to preparative gel electrophoresis. The proteins were either transferred to nitrocellulose sheets or electroeluted, dialyzed, and lyophilized.

b. *Electron microscopy*.

Samples of ram epididymal region 3 were fixed in glutaraldehyde (2.5 % in cacodylate buffer 0.1 M, pH 7), embedded in Epon, stained with uranyl acetate and lead citrate, and viewed with a transmission electron microscope.

c. *Immunological procedures*.

1. *Preparation of antisera against ovine PES 64 kD*. — Pre-immune sera were obtained from 2 female adult rabbits, pooled, and frozen. The animals then received 50 µg of protein in 15 subcutaneous injection sites at days 0, 8, 15, and 22. The first injection was made with Complete Freund Adjuvant; the following injections were made with Incomplete Freund Adjuvant. At day 30, the rabbits were bled and the sera prepared. The preimmune and immune sera were diluted with 8 % BSA, incubated overnight at 4 °C, and centrifuged (120,000 × g × 1 hour) to discard the precipitated proteins, if any. The supernatants were sterilized by filtration, split into aliquots, and frozen. In some experiments, instead of preimmune sera non-immune sera were used. No positive reaction to the antigen was detected with either sera.

2. *Immunoblotting*. — Rat caudal fluid was diluted in saline, centrifuged (800 × g × 30 min) and the supernatant adjusted with 3 M Tris-HCl buffer at pH 8.8 to 10 mM final. Protein samples (100 µg/50 µl in Tris-HCl buffer) were run on non-denaturing slab gels (7 % acrylamide, Tris-glycine buffer at pH 8.3) with 2-h pre-electrophoresis. The proteins were transferred in potassium phosphate buffer (50 mM, pH 7.5) containing EDTA (2 mM) and ClNa (50 mM) to 2 nitrocellulose sheets by the sandwich method. The blots were treated in parallel with non-immune and immune sera diluted at 1/500. Goat anti-rabbit immunoglobulins labeled with the biotin-avidin horseradish peroxidase complex (Vecta Stain ABC kit) were visualized with diaminobenzidine HCl in presence of  $\text{Cl}_2\text{Ni}$ . Tween (0.2 %) was added to all steps until the second antibody reaction.

3. *Immunocytochemistry*.

*Spermatozoa*: Ram spermatozoa from the rete testis or the cauda were washed with medium 199 and incubated with non-immune or immune sera (1/200) in medium containing 1 % goat serum. They were in turn incubated for 1 hour with goat anti-rabbit IgG labeled with peroxidase, washed in medium 199, and stained with diaminobenzidine HCl in the presence of  $\text{Cl}_2\text{Ni}$ .

*Testicular and epididymal tissues* : Rat testis and epididymis were removed, fixed in paraformaldehyde (4 %)-glutaraldehyde (2 %) containing picric acid (0.2 %), and embedded in paraffin. Sections were treated with xylene to eliminate the paraffin, washed in absolute ethanol, and rehydrated with decreasing concentrations of ethanol in water. They were then treated with goat serum (1 %) in Tris-HCl containing 0.2 % Tween and 0.1 % SDS and non-immune or immune serum at 1/200 for 1 hour. The rabbit IgG were revealed with Vecta Stain ABC kit used according to the manufacturer's instructions.

d. *Analytical procedures.*

1. *SDS polyacrylamide gel electrophoresis.* — 1-D SDS electrophoresis was performed as previously described (Jones, Fournier-Delpech and Willadsen, 1982) on slab polyacrylamide gel containing 15 % acrylamide at pH 8.3.

2-Dimensional SDS electrophoresis : Protein samples (100 µg/15 µl) were denatured with 10 µl of 9 M urea, 1 % SDS, Tris-HCl 10 mM at pH 7.4, 5 % mercaptoethanol, subjected to isoelectrofocusing in tube gels using ampholine pH range of 3.5 to 10. The second dimension was run on 18 × 18 × 0.15 cm slab gels consisting of a linear gradient from 8 to 13 % acrylamide according to the method of O'Farrell. Gels were stained with Coomassie Brilliant Blue R 250 or ammoniacal silver.

2. *Amino acid analysis.* — Rat PES 32 kD and ovine PES 64 kD were electroeluted from the corresponding single spots after 2-D SDS polyacrylamide gel electrophoresis and staining with ammoniacal silver. Amino acid analysis was performed on a Waters Picotag Amino Acid analyzer. Amino acids per mole of protein were deduced from the molecular weights after subtraction of glycosidic residues : 7.5 % (data from Lea, Petrusz and French, 1978) for rat PES 32 kD and 4.5 % for the ovine PES 64 kD (unpublished data).

## Results.

a. *Morphology of the epididymis from which ovine PES 64 kD was purified.*

Regressive changes in the epididymal epithelium were noted 45 days after castration : reduction in the size of the stereocilia border and the cytoplasm and decrease in cell height. The nucleus occupied approximately half of the cell (fig. 1.1). The effect of testosterone supplementation is illustrated on figure 1.2. The supranuclear cytoplasm was more developed and the apical surface of the cells had long stereocilia.

b. *Electrophoretic characteristics of ovine PES 64 kD compared with rat PES 32 kD.*

The characteristics of the ovine PES 64 kD migrating in the prealbumin area are illustrated in figure 2. Purified ovine PES 64 kD (fig. 2A) migrated with molecular weight 64,000 kD on 1-D SDS-polyacrylamide slab gel (fig. 2B) ; after

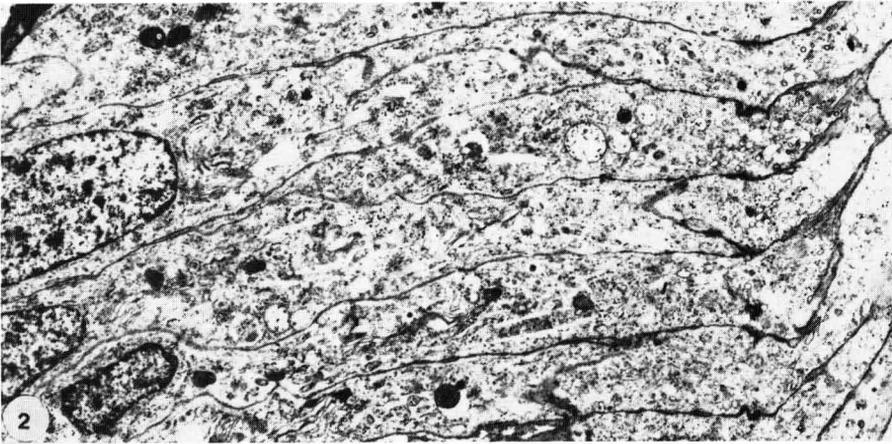
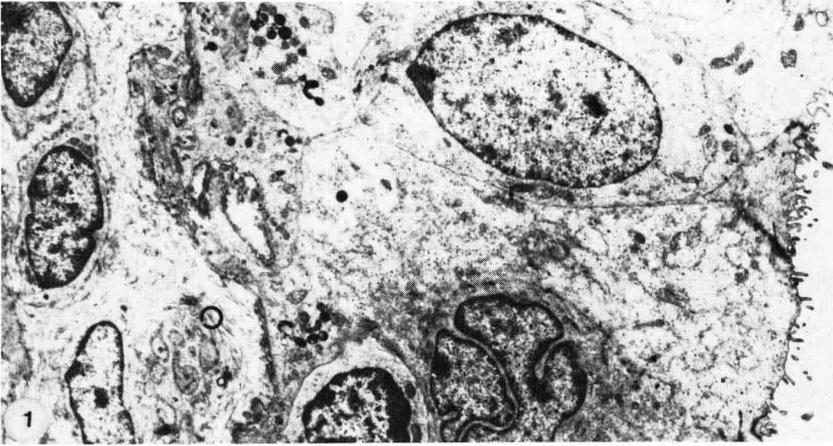
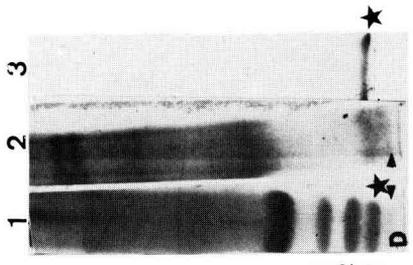
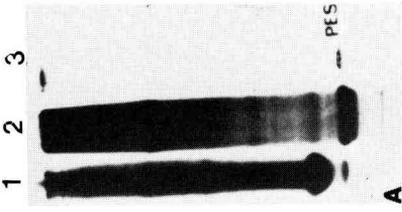
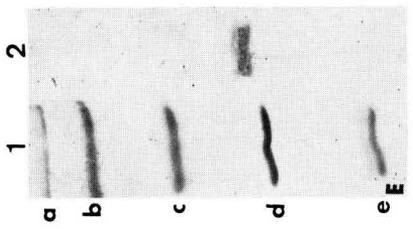
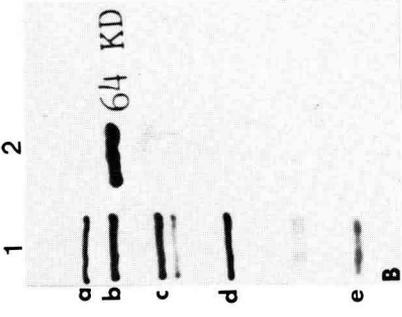
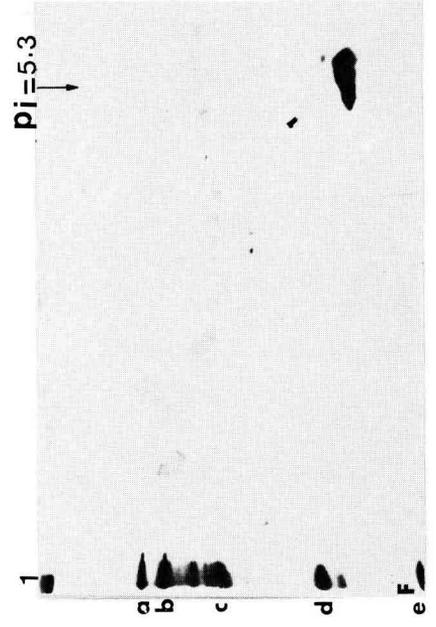
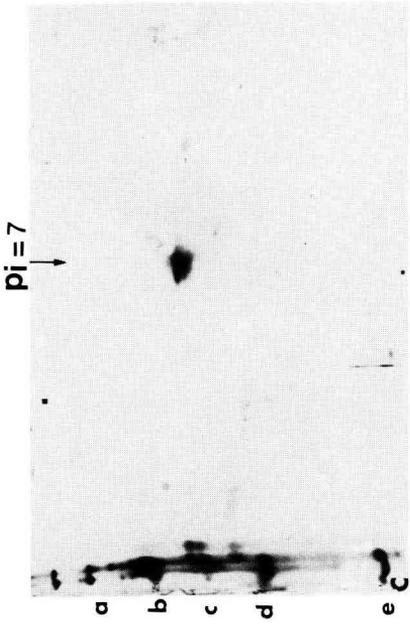


FIG. 1. — *Androgendependency of the epididymis in the ram : effects of the testosterone on the epithelium in the castrate.*

FIG. 1.1 : Epithelium lining the duct of the left epididymis in ram castrated along 45 days ( $\times 1\,400$ ).

FIG. 1.2 : Epithelium lining the duct of the right epididymis in the same ram 3 weeks after the deposit of the testosterone implant : note the development of the supranuclear cytoplasm and the stereocilia ( $\times 1\,400$ ).

denaturation with urea, the apparent molecular weight was 57,000 kD after 2-D SDS electrophoresis and the isoelectric point was 7 (fig. 2C). The rat PES 32 kD migrated in a similar position to protein D (fig. 2D), that is, as a single band of molecular weight 32,000 kD on 1-D SDS polyacrylamide slab gel (fig. 2E). After urea denaturation and electrofocusing, it migrated on 2-D SDS polyacrylamide gel with a molecular weight of 27,000 kD and an isoelectric point 5.3 (fig. 2F).



c. *Immunocytochemical localization of ovine PES 64 kD antigenic determinants.*

The anti-ovine PES 64 kD serum (fig. 3.II.1) cross-reacted with the rat PES electroeluted from 2-D gels or the proteins D and E present in the epididymal fluid. Slight staining of the protein C was also detected (fig. 3.II.2 and 3.II.4). Furthermore, the ovine antibody reacted with three human seminal plasma proteins identified by their relative coefficient of mobility in relationship to albumin : 0.46, 0.63, 0.80 (fig. 3.I and 3.III). Reaction with rat protein C and human protein 0.46 was nonspecific, since the non-immune serum also reacted with them (fig. 3.I.2 and 3.I.3). Therefore, rat proteins D and E and human proteins 0.63 and 0.80, that reacted with the antibody against ovine PES 64 kD and not with the non-immune serum, shared common antigenic determinants with ovine PES 64 kD.

Human protein 0.63 characterized on 2-D SDS polyacrylamide gel electrophoresis had 3 components (16,000 kD, 35,000 kD, and 53,000 kD at isoelectric point 5.5 and one component (16,000 kD) at isoelectric point 5. Human protein 0.80 under the same conditions had 2 components of approximately 40,000 kD and isoelectric point 5.5.

1. *Localization on ram spermatozoa.* — The antibody against ovine PES 64 kD reacted positively with the midpiece of ram spermatozoa from the testis, epididymis, or ejaculate and with the periacrosomal area of spermatozoa from the epididymis or the ejaculate. The pre-immune serum reacted positively with the sperm mid-piece. Therefore, only the periacrosomal surface ; had antigenic determinants common to the secreted ovine PES 64 kD protein. This antigen appeared on the spermatozoa in the epididymis and remained on ejaculated spermatozoa (fig. 4: 1 to 4). It was localized on the plasma membrane (fig. 4-6).

FIG. 2. — *Electrophoretic pattern of the ovine PES 64 kD and rat PES 32 kD proteins.*

— A and D : Non denaturing electrophoresis (7 % acrylamide, pH 8.3) of :

A : Ram proteins : 1 = Proteins of the epididymal fluid ; 2 = DEAE-Sephacel fraction 0.3 M ClNa ; 3 = Purified ovine PES.

D : Rat proteins : 1 = Proteins of the epididymal fluid : prealbumin 1(p1), 2(p2), 3(p3) ; 2 = Con A-Agarose fraction eluted by  $\alpha$ 2-D-methylmannose : protein D (★) and E (▲) ; 3 = Purified rat PES 32 kD.

— B and E : 1-D SDS polyacrylamide gel electrophoresis (15 % acrylamide, pH 8.3) of purified PES proteins having molecular weight 64 kD in the ram ( $E_2$ ) and 32 kD on the rat ( $E_2$ ) referring to the standardized molecular weights under number 1.

— C and F : 2-D SDS polyacrylamide gel electrophoresis (8 to 13 % acrylamide, pH 3 to 10) of the purified PES having molecular weight 57 kD at isoelectric point 7 in the ram (C), and 27 kD at isoelectric point 5.3 in the rat (F) referring to standardized molecular weights under number 1. Standardized molecular weights were : 94 kD (a), 67 kD (b), 45 kD (c), 30 kD (d), 20 kD (e). Gels A, B, D and E were stained with Coomassie R 250 brillant blue, gel C and F with ammoniacal silver. Note that the purified rat PES and ovine PES present under number 3 on panels A and D, migrated as a single spot on 2-D SDS polyacrylamide gel electrophoresis.

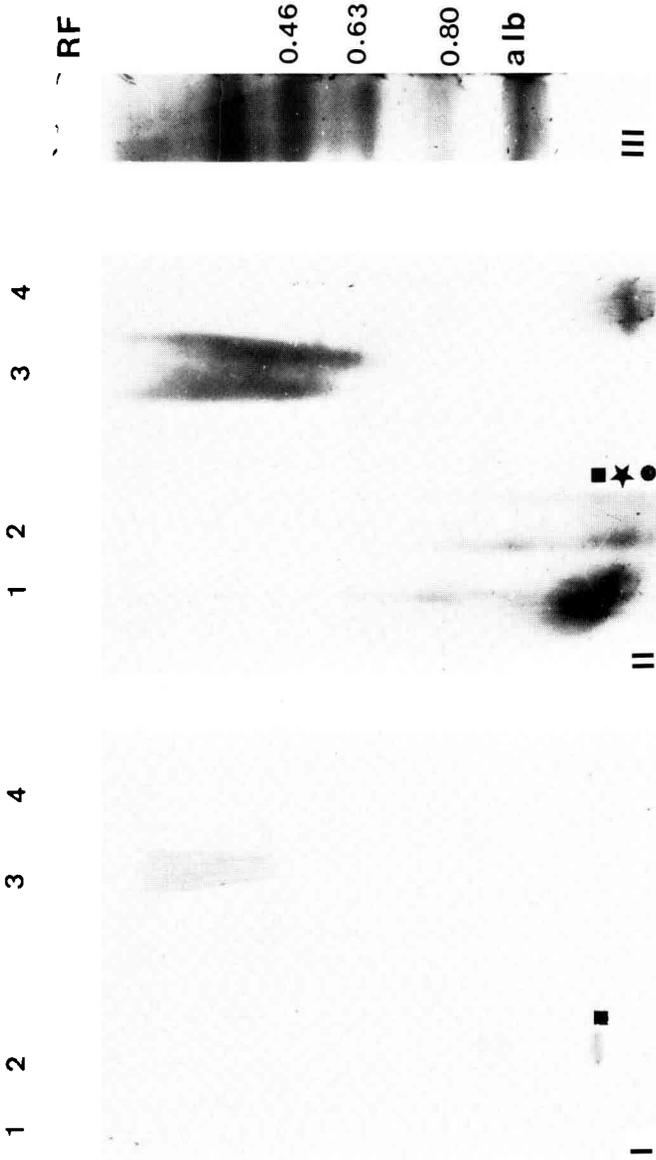


FIG. 3.—Immunostaining of ovine PES 64 kD, rat PES 32 kD and human seminal plasma proteins with anti-ovine PES serum.  
 — I and II: Nitrocellulose sheet treated with preimmune serum and immune serum respectively after transfer of proteins ;  
 1 = Ovine PES 64 kD ; 2 = Proteins of the rat epididymal fluid ; C ■ : D ★ ; E ● ; 3 = Human seminal plasma proteins ;  
 4 = Rat PES 32 kD.  
 Note on the panel A, unspecific positive staining of the rat protein C and unidentified human proteins in this sheet treated with preimmune serum.  
 — III : Preparative electrophoresis of the human seminal plasma proteins ; The proteins cross-reacting with the anti-ovine PES serum have Rf of 0.46, 0.63 and 0.80 in relationship with the albumin (alb.)

2. *Localization on rat testis and epididymis.* — Rat testis did not exhibit significant staining with the ovine PES 64 kD antibody. By contrast the intralumenal content of the proximal epididymis and the packed spermatozoa were heavily stained ; the stereocilia and the cytoplasm of the cells lining the duct were also stained ; the staining decreased in the corpus and the cauda epididymidis (fig. 5.1A, 5.2A, 5.3A, 5.4A). The staining was specific as assessed by the absence of staining of controls treated with non-immune serum (fig. 5.2B, 5.3B, 5.4B).

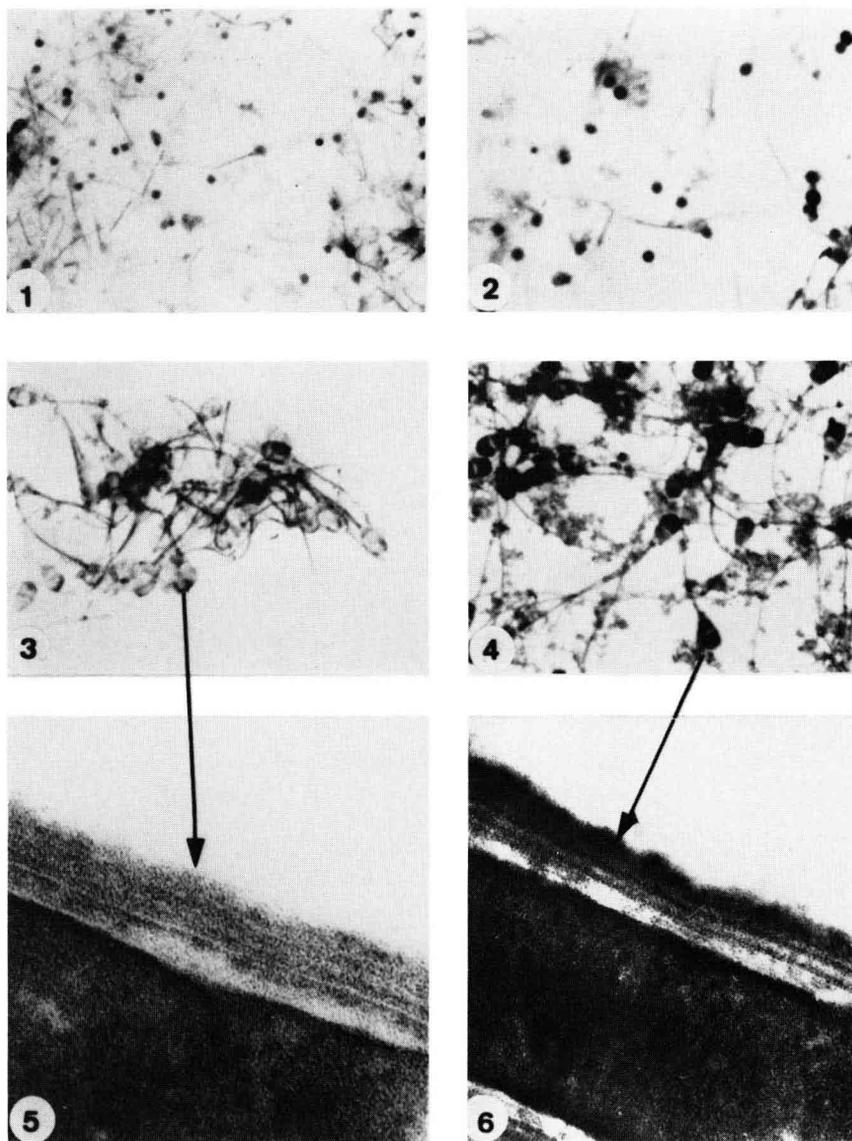


FIG. 4. — Immunocytological localization of ovine PES 64 kD antigenic determinants on ram spermatozoa. FIG. 4.1 and 4.2: Unspecific labeling of the midpiece of testicular spermatozoa treated with preimmune (1) or immune (2) serum. FIG. 4.3 and 4.4: Specific labeling of periacrosomal area of ejaculated spermatozoa treated with preimmune serum (3) or immune serum (4). FIG. 4.5 and 4.6: Ultrastructural localization of the labeling of the plasma membrane from an aliquot of the sample presented on the picture 4.4; the control from an aliquot of the sample presented on the picture 4.3 is negative 4.5.

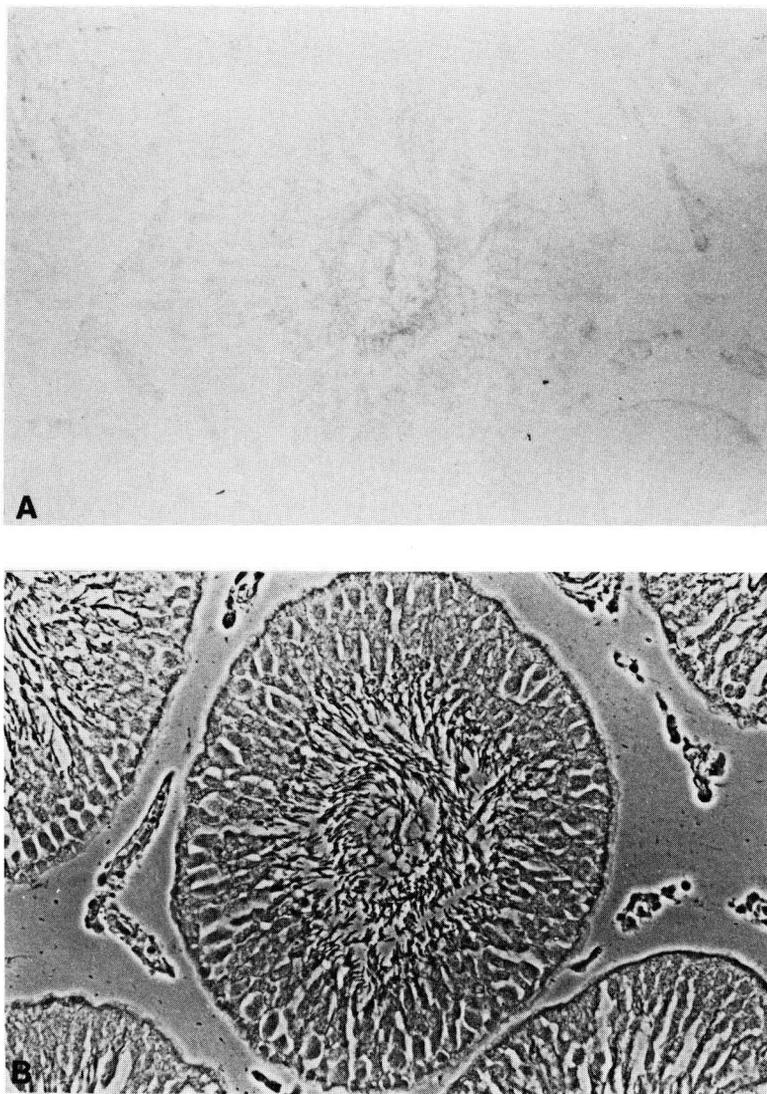
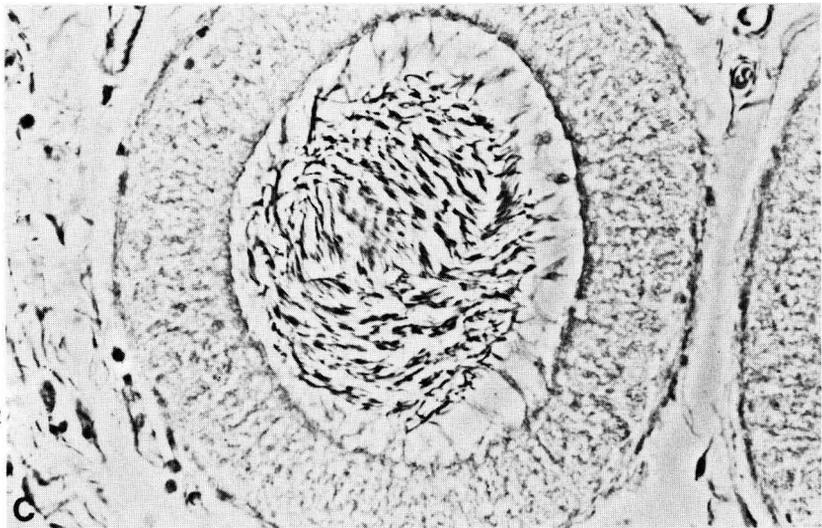
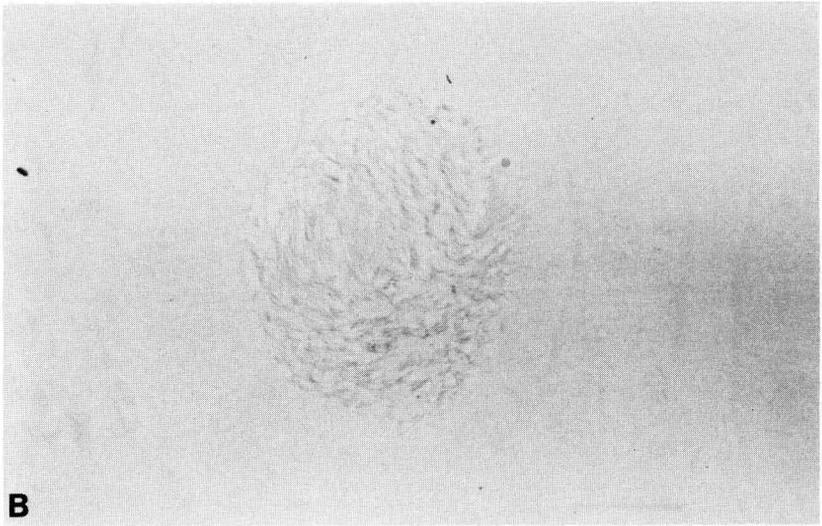
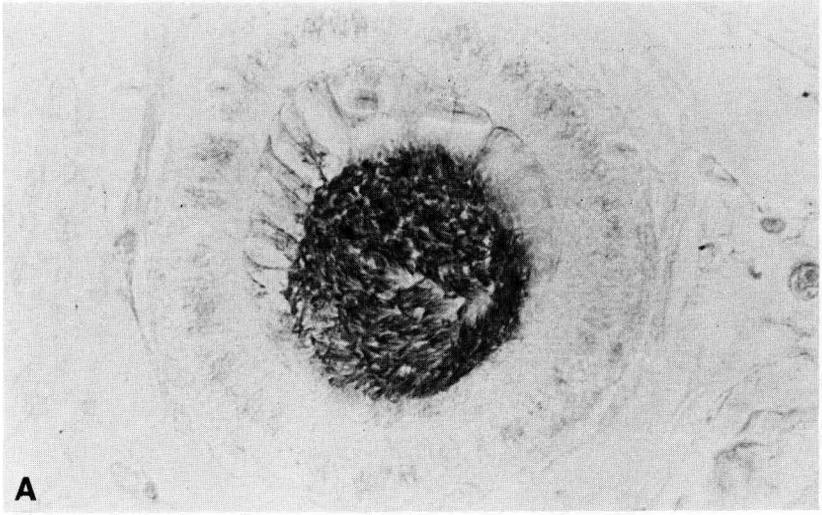


FIG. 5.1.

FIG. 5. — *Immunocytochemical localization of ovine PES 64 kD epitopes on the testis and epididymis of the rat.* FIG. 5.1 : Testicular tissues treated with anti-ovine PES 64 kD serum (A) do not exhibit significant staining on seminiferous tubules; (B) phase contrast. FIG. 5.2, 5.3, 5.4 : Are representative of tissues of the proximal, middle or distal epididymis respectively. They were treated with anti-ovine PES 64 kD serum (A), non immune serum (B) and observed under phase contrast (C).



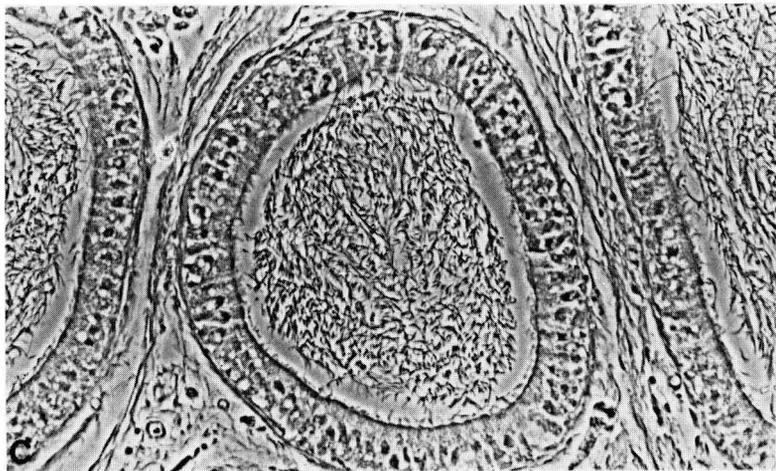
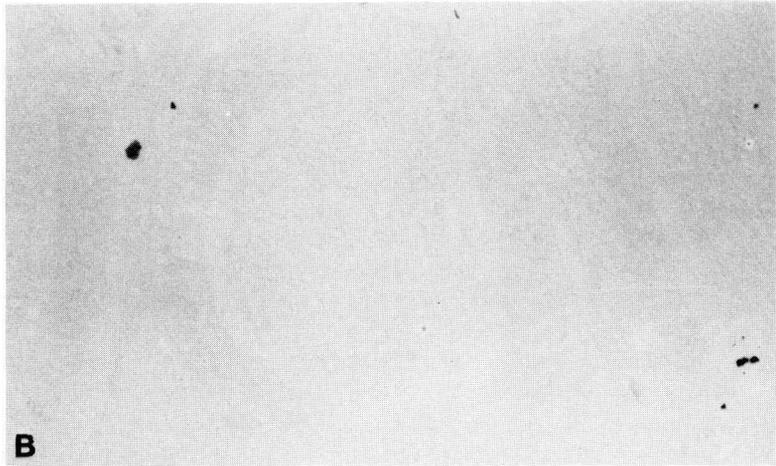
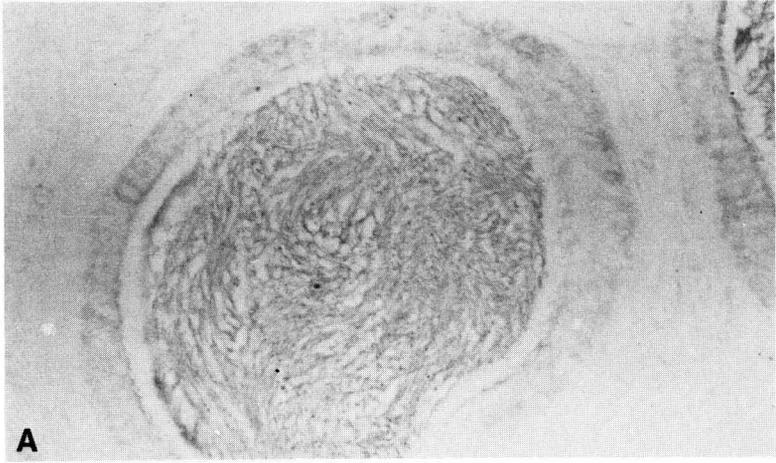


FIG. 5.3 — See legend p. 129.

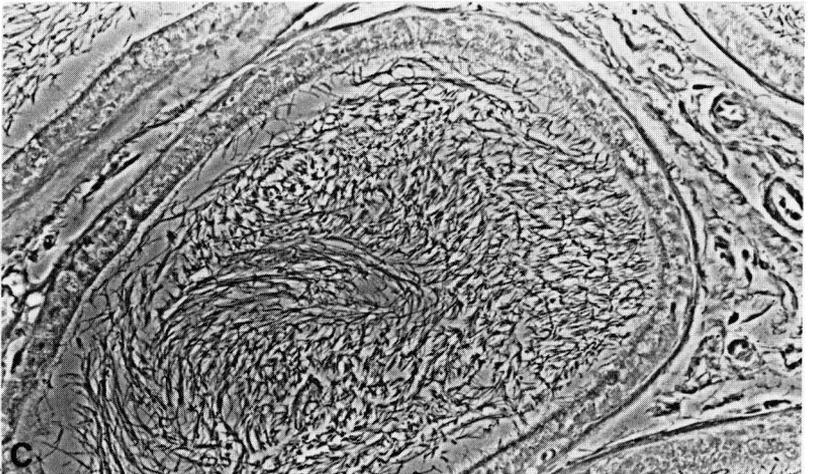
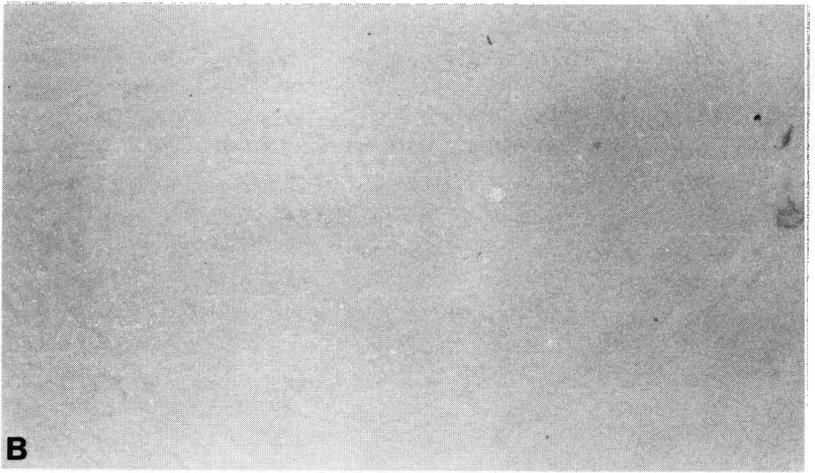
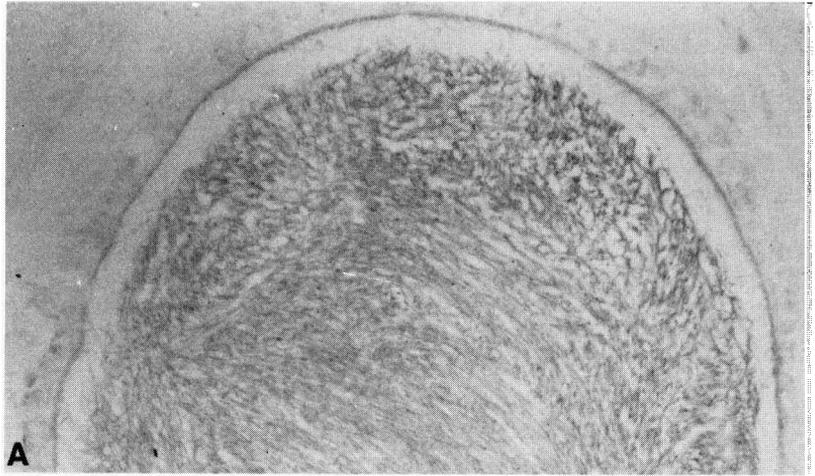


FIG. 5.4 — See legend p. 1292.

d. *Preliminary amino acid analysis of rat PES, ovine PES, and human proteins having common antigenic determinants as revealed by the ovine PES antibody.*

Amino acid analysis showed 244 amino acids per mole for rat PES 32 kD and 509 per mole for ovine PES 64 kD (table 1). The most prominent amino acid in both the rat and the ram protein was glycine. It was likely a contaminant from the preparative buffer still present despite extensive dialysis. The major amino acids in rat protein were serine, asparagine, alanine, leucine, threonine, valine, and proline. In ram protein, they were serine, alanine, glutamine, leucine, and threonine.

The human proteins 0.63 and 0.80 had similar amino acid compositions. The major amino acids were glutamine, serine, asparagine, lysine, leucine, and threonine.

TABLE 1

*Relative amino-acid composition of rat PES 32 kD, ram PES 64 kD and human seminal plasma protein 40 kD (H5).*

|                                      | Rat    | Ram    | Human  |
|--------------------------------------|--------|--------|--------|
| Molecular weight                     | 27 000 | 55 000 | 40 000 |
| mol amino acid/<br>100 mol recovered |        |        |        |
| — Asparagine                         | 14     | 3.6    | 13     |
| — Glutamine                          | 6      | 12     | 17     |
| — Serine                             | 19     | 28     | 13     |
| — Histidine                          | 2.4    | 3      | 2.9    |
| — Arginine                           | 3.5    | 3      | 7.9    |
| — Threonine                          | 7.5    | 6      | 4      |
| — Alanine                            | 8.5    | 13     | 5      |
| — Proline                            | 7.2    | 5      | 6      |
| — Tyrosine                           | 4.5    | 1      | 2.3    |
| — Valine                             | 7.6    | 5      | 5.6    |
| — Methionine                         | 0.4    | 1      | 0      |
| — Cysteine                           | 1.5    | 2      | 3.4    |
| — Isoleucine                         | 2      | 2      | 4.2    |
| — Leucine                            | 8.4    | 11     | 6.6    |
| — Phenylalanine                      | 2.7    | 2      | 2.3    |
| — Lysine                             | 5      | 3      | 7.2    |

Molecular weights estimated from 2D gel electrophoresis.

## Discussion.

This work demonstrates that androgen-dependent proteins synthesized by the epididymis have interspecific epitopes which are both expressed in the cells of the epididymal epithelium (ram, rat), on the periacrosomal area of the plasma membrane of spermatozoa after entering into the epididymis (ram), and on specific proteins of rat epididymal plasma and human seminal plasma.

The two proteins studied in the rat and in the ram have slightly lower molecular weight on 2-D SDS gel electrophoresis than on 1-D SDS electrophoresis: 57 kD *versus* 64 kD for ovine PES 64 kD protein and 27 kD *versus* 32 kD for the rat PES 32 kD protein. This difference in molecular weight of rat protein has been reported previously (Brooks, 1982) and is likely an artifact of the electrophoresis conditions employed. The ovine and rat proteins had different isoelectric points: 7 for the ovine PES 64 kD and 5.3 for the rat PES 32 kD. Brooks (1983) gave a similar isoelectric point for rat protein D. The number of amino acids (244) identified from the analysis of the rat PES 32 kD protein in this study is in agreement with the number (246) obtained from the sequencing of the nucleic acids encoding for the synthesis of protein D (Brooks *et al.*, 1986). The number of amino acids of the ovine PES 64 kD is approximately double.

The antibody was specific for ovine PES 64 kD since the preimmune or non-immune serum did not react with the protein on Western blots (fig. 3.I and 3.II). This antibody cross-reacted with rat PES 32 kD and with proteins D and E that represent the rat acid glycoprotein in the epididymal fluid; the latter is in agreement with Brooks' finding showing that proteins D and E shared common antigenic determinants (Brooks, 1982). Moreover, the antibody cross-reacted with two human seminal plasma proteins which migrated in the postalbumin area under non-denaturing electrophoresis; they could be related to epididymal proteins with same relative electrophoretic mobility which have been shown to associate with spermatozoa in the epididymis (Tezón *et al.*, 1985). This implies that the ovine PES 64 kD protein has epitopes common to analogous proteins in other species. This deserves further investigation, since hybridization data with cDNA to proteins D and E shows little cross species hybridization (Brooks *et al.*, 1986). Amino acid analysis revealed a relative abundance of asparagine and serine/threonine which could support N-glycosylation and O-glycosylation, respectively. Therefore, the interspecific epitopes of ovine PES 64 kD protein may be glycoconjugates.

There is evidence of the expression of ovine PES 64 kD antigenic determinants on the periacrosomal plasma membrane of ram epididymal spermatozoa. In this regard, the ovine PES 64 kD protein is analogous to the rat PES 32 kD, since the latter has identical characteristics: secretion by the epididymis under androgenic stimulation and localization on the acrosomal surface on the concave area of the spermatozoa in the epididymis (Kohane *et al.*, 1979). Moreover, the anti-ovine PES serum did not stain testicular tissue but reacted heavily with the luminal content of the rat epididymis. The localization of the ovine PES 64 kD antigenic determinants on rat epididymal spermatozoa, on human spermatozoa, and in human epididymal fluid remains to be determined. If the antigenic determinant present in human seminal plasma originates in the epididymis, it could become a useful marker of human epididymal function.

In *conclusion*, it appears that the ovine epididymal protein PES 64 kD shares antigenic determinants with both rat epididymal protein PES 32 kD and human

seminal plasma proteins. The biological function of these proteins remains to be determined.

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**Résumé.** *Une protéine sécrétée par l'épididyme chez le bélier porte des déterminants antigéniques communs avec des protéines de l'épididyme du rat et du plasma séminal humain.*

L'androgénodépendance de l'épithélium épидидymaire de bélier a été établie par des observations ultrastructurales chez des animaux, 45 jours après la castration suivie ou non par un traitement avec des implants de testostérone durant 3 semaines. Une protéine épидидymaire androgénodépendante caractérisée par sa migration dans la région des préalbumines en électrophorèse non dénaturante et dont le poids moléculaire de 64 kD a été déterminé en gel SDS-PAGE (acrylamide 15 %) a été purifiée chez les animaux traités par l'hormone. Un antisérum monospécifique de cette protéine a présenté une réaction croisée avec la préalbumine 3 de rat (protéine D-E) et des protéines non identifiées du plasma séminal humain. L'analyse de la composition en acides aminés des protéines du rat, de l'homme et du bélier a montré des taux élevés d'aspartate, glutamate et sérine. La localisation immunocytochimique de ces protéines a été effectuée sur les spermatozoïdes épидидymaires et testiculaires du rat, de l'homme et du bélier et sur les spermatozoïdes éjaculés du bélier, du taureau et de l'homme. Des déterminants antigéniques communs existeraient pour les protéines sécrétoires épидидymaires qui manifestent une affinité pour les spermatozoïdes au cours de la maturation chez les mammifères.

## References

- AMANN R. P., 1987. Function of the epididymis in bulls and rams. *J. Reprod. Fert., Suppl.* **34**, 115-131.
- BROOKS D. E., 1982. Purification of rat epididymal proteins « D » and « E », demonstration of shared immunological determinants and identification of regional synthesis and secretion. *Int. J. Androl.*, **5**, 513-524.
- BROOKS D. E., 1983. Effects of androgens on protein synthesis and secretion in various regions of the rat epididymis as analyzed by two-dimensional gel electrophoresis. *Mol. cell. Endocrinol.*, **29**, 225-270.
- BROOKS D. E., MEANS A. R., WRIGHT E. J., SINGH S. P., TIVER K. K., 1986. Molecular cloning of the cDNA for androgen-dependent sperm-coating glycoproteins secreted by the rat epididymis. *Eur. J. Biochem.*, **161**, 13-18.
- FAYE J. C., DUGUET L., MAZUCCA M., BAYARD F., 1980. Purification, radioimmunoassay and immunohistochemical localization of a glycoprotein produced by the rat epididymis. *Biol. Reprod.*, **23**, 423-432.
- FOURNIER-DELPECH S., BAYARD F., BOULARD Cl., 1973. Etude d'une protéine spécifique du sperme épидидymaire chez le rat ; hormonodépendance, relation avec l'acide sialique. *C. R. Soc. Biol.*, **163**, 1989-1996.

- FOURNIER-DELPECH S., COUROT M., 1987. Sperm-zona binding capacity in mammals. In CLARKE J., *Oxford reviews*. Clarendon Press. Oxford, Vol. **9**, 295-321.
- FOURNIER-DELPECH S., PISSELET Cl., GARNIER D. H., DUBOIS M. P., COUROT M., 1981. Mise en évidence chez le bélier d'une préalbumine sécrétée par l'épididyme sous l'action de la testostérone. *C. R. Acad. Sci. Paris, Sér. III Vie*, **193**, 589-594.
- FOURNIER-DELPECH S., VENIEN A., PISSELET Cl., COUROT M., 1988. Localisation sur la tête des spermatozoïdes de Bélier de sites d'affinité pour la préalbumine épididymaire androgène-dépendante 64 kD ; *C. R. Acad. Sci. Paris, Sér. III Vie* **306**, 333-338.
- GARBERI J. C., KOHANE A. C., CAMEO M. S., BLAQUIER J. A., 1979. Isolation and characterization of specific rat epididymal proteins. *Mol. cell. Endocr.*, **13**, 73-82.
- HAMMERSTEDT R. H., PARKS J. E., 1987. Changes in sperm surface associated with epididymal transit. *J. Reprod. Fert., Suppl.* **34**, 133-149.
- JONES R., FOURNIER-DELPECH S., WILLADSEN S. A., 1982. Identification of androgen-dependent proteins synthesized *in vitro* by the ram epididymis. *Reprod. Nutr. Develop.*, **22**, 495-504.
- KOHANE A. C., GARBERI J. C., CAMEO M. S., BLAQUIER J. A., 1979. Quantitative determination of specific proteins in rat epididymis. *J. Steroid. Biochem.*, **11**, 671-674.
- LEA O. A., PETRUSZ P., FRENCH F. S., 1978. Purification and localization of acidic epididymal glycoprotein (AEG) : A sperm coating protein secreted by the rat epididymis. *Int. J. Androl., Suppl.* **2**, 592-607.
- ORGBIN-CRIST M. C., 1986. Physiologie de l'épididyme et maturation du sperme : Etat actuel des connaissances ; *Contracept. Fert. Sex.*, **14**, 487-495.
- TEZÓN J. G., RAMELLA E., CAMEO M. S., VASQUEZ M. H., BLAQUIER J. A., 1985. Immunohistochemical localization of secretory antigens in the human epididymis and their association with spermatozoa. *Biol. Reprod.*, **32**, 591-597.
- WONG P. Y., TSANG A. Y. F., 1982. Studies on the binding of a 32 kD rat epididymal protein to rat epididymal spermatozoa. *Biol. Reprod.*, **27**, 1239-1246.
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