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Uptake of an Oviductal Antigen by the Hamster Zona Pellucida[1]

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ABSTRACT

Using an antiserum raised against hamster oviductal zona pellucida, we observed specific immunogenic components of the reproductive tract on the zonae of oviductal eggs and in oviductal fluid. Results of immunohistochemical studies suggested that these oviductal components may originate from epithelial cells of the isthmus and, to a lesser extent, of the ampulla and fimbria. The oviductal immunogenic components have also been observed within the bursal cavity, which contains the ovary. These observations suggest that these oviductal components may play an important role in the first steps of the hamster reproductive process.

INTRODUCTION

In the mammalian ovary, the zona pellucida appears as a discrete extracellular glycoprotein structure that encloses the oocyte. As its assembly inside the ovary proceeds, the zona acquires specific molecular domains that possess important biological functions. Thus, the zona mediates species-specificity of sperm binding to the ovulated egg and provides the major block to post-fertilization polyspermy (Wassarman and Bleil, 1982). The zona also appears to protect the growing embryo as it passes down the oviduct (Mintz, 1962; Modlinski, 1970).

In the rabbit, structural transformations of the zona pellucida have been observed during the passage of the embryo through the oviduct. Deposition of a new layer outside the zona has been visualized by light microscopy around the ovulated egg as early as 6–9 h after ovulation (Dickmann, 1963). Forty-eight h after ovulation, this new layer reaches a diameter of 60 μm (Kane, 1975). Upon staining with toluidine blue, this layer exhibits a strong metachromatic reaction, whereas the zona pellucida shows only a weak reaction. Initially, this approach revealed the difference in the chemical composition of the two layers; subsequently, the use of many different histochemical stains showed that this new layer contained highly sulfated acid mucoproteins, whereas the zona appeared to be composed of neutral or weakly acidic mucoproteins (Braden, 1952; Denker and Gerbes, 1979). The appearance of this new layer has been ascribed to an oviductal secretion (Jansen and Bajpai, 1982). Shapiro et al. (1974) also have shown that fluorescent antibodies raised against a sulfated glycoprotein isolated from rabbit oviductal fluid resulted in fluorescence on the mucin coats that surround 3-day-old rabbit embryos. A similar type of fluorescence has been observed on ovulated eggs of the hamster after incubation with anti-uterus sera (Fox and Shivers, 1975a). Using electron microscopy, Baranska et al. (1975) have observed a similar deposition of material on the outer surface of zonae surrounding mouse embryos during the preimplantation period. Upon electrophoretic analyses of porcine zonae pellucidae it has been observed that ovulated eggs have one additional component that is absent in zonae isolated from homogenized porcine ovaries (Brown and Cheng, 1985). Although the existence of a distinct covering, such as the mucin coat of the rabbit egg, has not been described around zonae of

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other species, these last three reports indicate that an analogous phenomenon may exist in other mammalian species.

We have investigated the addition of oviductal material to hamster zona during the passage of the egg through the oviduct by using antibodies directed against heat-solubilized zonae isolated from hamster oviductal eggs.

MATERIALS AND METHODS

Collection of Oviductal Ova and Removal of Zonae Pellucidae

Sexually mature female hamsters (*Mesocricetus auratus*, 6–8 wk old, Charles River Inc., Montreal, Quebec, Canada) were superovulated by injecting (i.p.) 25 IU pregnant mare's serum gonadotropin (PMSG, Equinex, Ayerst Laboratories, Montreal, Quebec, Canada) followed 72 h later by 25 IU human chorionic gonadotropin (hCG, A.P.L., Ayerst). Seventeen to 42 h after hCG injection, the animals were killed by cervical dislocation, and their oviducts were excised. The oviducts were placed in a small volume of Dulbecco's phosphate-buffered saline (PBS-D, pH 7.4, Gibco Inc., Burlington, Ontario, Canada), and their ampullae were pierced with fine steel tweezers (17 or 24 h post-hCG) or flushed with PBS (42 h post-hCG) under a dissecting microscope. The cumulus masses thus collected were treated for 10–30 min in phosphate-buffered saline (PBS; 0.01 M phosphate, 0.15 M NaCl) containing 2 mM ethyleneglycol-bis-(β-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), 11 mM sodium citrate and 350 U/ml hyaluronidase (Gibco Labs., Grand Island, NY), final pH 6.7. After cumulus cell dispersal, the ova were isolated by using mouth-operated micropipets and washed three times in PBS-D. The zonae pellucidae were removed from ova either in an intact form (by using mouth-operated micropipets with a bore size smaller than the diameter of the ova) or in a soluble form by treating ova with PBS-D adjusted to pH 2.5 with concentrated HCl. In the latter case, washed ova were centrifuged at 15,000 × *g* for 5 min (Microcentrifuge, Fisher Scientific, Pittsburgh, PA), and the zonae were dissolved by resuspending the ova pellet in 25 μl of PBS-D, pH 2.5, per 100 zonae pellucidae. Solubilization was followed under a dissecting microscope (5–30 s.), and the ova were discarded by centrifugation. The buffer containing the solubilized zonae was collected and kept frozen at −20°C in siliconized tubes until use. The entire procedure for five pairs of oviducts took approximately 2 h and allowed the recovery of 125 zonae pellucidae. These two methods provided zona material that exhibited the same specificity of reaction with the antisera, only the intensity of the reaction differed.

Collection of Ovarian Oocytes and Removal of Zonae Pellucidae

Adult female hamsters in metestrous, as ascertained by examination for the postovulatory vaginal discharge (Orsini, 1961), were injected with 25 IU PMSG to increase ovarian follicular development (Chiras and Greenwald, 1978). Forty-eight h after PMSG injection, the animals were killed and their ovaries excised and placed in PBS-D. Fat and connective tissue were removed under a dissecting microscope. The ovaries were then minced with fine steel scissors (Fine Science Tools, Vancouver, B.C., Canada) and incubated for 30 min with stirring in hyaluronidase solution (identical to that used for the collection of the oviductal ova). Subsequently, to complete the dissociation process, the mixture was shaken on a Vortex mixer at high speed for another 30 s and allowed to settle for 10–20 min. The oocytes were recovered between the pellet of the ovarian tissue and the supernatant. The oocytes were harvested free of adhering granulosa cells by repeated pipetting using mouth-operated micropipets, with a bore size slightly larger than the diameter of the oocytes, and were washed three times in PBS-D. After the final wash, the zonae pellucidae were solubilized in PBS-D at pH 2.5, as described for the oviductal ova. To optimize recovery, the complete treatment of the ovarian pellets was repeated three to four times. The entire procedure for ten pairs of ovaries took approximately 8–10 hours and enabled the recovery of 425 zonae pellucidae. This method has been chosen over other enzymatic treatment, such as collagenase or DNAase, to duplicate, as closely as possible, the treatment applied to the oviductal ova.

Collection of Oviductal Fluid

Oviductal fluid was collected from immature, 27-day-old hamsters to avoid contamination by residual ovarian material from previous ovulations. The excised oviducts were rinsed in PBS-D, blotted on filter paper, and flushed with 50 μl of PBS-D after insertion of a 30-gauge needle (Becton Dickinson, Lessard and Sons Inc., Montreal, Quebec, Canada)

into the infundibulum. The flushings obtained from 10–15 females were pooled, filtered (0.45 μm) and frozen (−20°C) until use. Protein concentration was measured by Bradford's method as modified by Macart and Gerbaut (1982) with bovine serum albumin (Fraction V, Sigma Chemical Co., St. Louis, MO) as standard. Pooled oviductal fluid was tested for contamination by proteinase by using Bio-Rad casein-agar plates (Bio-Rad Laboratories Ltd., Mississauga, Ontario, Canada); since no proteinase activity was detected, inhibitors were not added to the pool.

Preparation of Antibodies

Intact zonae pellucidae obtained from oviductal ova and solubilized by heating at 65–80°C in PBS-D were diluted in Complete Freund's adjuvant and used to immunize a male white rabbit, as previously described (Bousquet et al., 1981). The specificity of the antiserum obtained was determined by absorption methods on homogenized ovary, oviduct, kidney, liver or red blood cells (RBC). Oviducts and ovaries were excised from 25 hamsters that were not in metestrous, as ascertained by examination for the postovulatory vaginal discharge; this was to avoid contamination of the oviduct by ovarian material and to obtain ovaries rich in zona material. The organs were placed in PBS-D at 4°C, dissected free of fat and checked for purity under a dissecting microscope. The organs were minced, washed four times in PBS-D at 4°C, and homogenized on ice with a Potter-Elvehjem apparatus in anti-zona pellucida serum diluted 1/16 with PBS-D. To obtain a complete absorption, two volumes of the diluted antiserum were added to one volume of minced ovaries, and six volumes of diluted antiserum were added for each volume of minced oviducts. The homogenates were incubated for 24 h at 4°C with stirring. After centrifugation at 15,000 × *g* for 5 min at 4°C, the supernatants were collected, filtered (1.2 μm) and kept frozen at −20°C. Anti-zona serum was absorbed with liver or with kidney tissue using a similar method. For these two absorptions, an antiserum: organ ratio of 2:1 was used. Antiserum absorption on red blood cells was accomplished by using RBC pooled from adult female hamsters. Females were decapitated, blood was collected in cold heparinized tubes and centrifuged at 1000 × *g* for 10 min at 4°C. The centrifuged RBCs were washed five times in PBS-D at 4°C. Three volumes of antiserum (diluted 1/16) were added to two volumes of RBCs, and the mixture was incubated for 24 h at 4°C with stirring. After centrifugation, the supernatant was collected, filtered, and kept frozen as already described. Control sera included normal rabbit sera or sera from rabbits immunized with complete Freund's adjuvant alone.

Dot-Immunobinding Assay

Screening for the reactivity of anti-zona antibodies with oviductal fluid and specificity analyses of this interaction was accomplished with a solid-phase immunoassay system of high sensitivity described by Hawkes et al. (1982) and subsequently developed as a kit by Bio-Rad (Immuno-blot [GAR-HRP] Assay Kit, Bio-Rad Laboratories Ltd.). This type of assay was required because only small amounts of zonae pellucidae and oviductal fluid were available. One μl of a solution of acid-solubilized oviductal zonae (5 zonae/μl), 3 μl of a solution of acid-solubilized ovarian zonae (4 zonae/μl), 1 μl of a solution of pooled oviductal fluid (180 ng of protein), and 1 μl of a diluted solution of pooled sera from adult female hamsters (80 ng of protein) were dotted on each nitrocellulose filter. The nitrocellulose was blocked with 3% (w/v) gelatin in Tris-buffered saline (TBS, 20 mM Tris, 0.5 mM NaCl, pH 7.5) for 30 min. The blots were then incubated with the primary antisera diluted 1/256 in 1% gelatin-TBS. Primary antisera included control rabbit sera, anti-zona serum, and anti-zona serum absorbed either with ovary, oviduct, liver, kidney or RBCs. After an incubation period of 2 h, the blots were washed three times for 10 min in TBS containing 0.025% (v/v) Tween-20 (TTBS). After washing, affinity-purified peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG) (Bio-Rad Laboratories Ltd.), diluted 1/4000 in 1% gelatin-TBS, was reacted with the blots for 1 h. Excess second antibody was removed by 3 washes of 10 min in TTBS. Color was developed in the blot by incubation for 15–30 min in the developing solution (Bio-Rad kit). All steps were done at 23°C with gentle stirring.

Immunocytochemical Assay

The association of oviductal antigens with oviductal zonae was also studied by indirect immunofluorescence on ovarian oocytes and oviductal ova (collected as already described). Experiments were performed in spot plates, with 20 oocytes or ova in each well. Oocytes and ova were incubated in anti-

zona serum, anti-zona serum absorbed with ovarian tissue or with control rabbit sera diluted 1/128 in PBS-D. Incubations were carried out in 100 μl of the appropriate serum at 37°C for 1 h. The oocytes or ova were then washed three times for 5 min in PBS-D and incubated in 100 μl of fluorescein-conjugated goat anti-rabbit IgG (Miles Laboratories, Rexdale, Ontario, Canada) diluted 1/32 in PBS-D. After incubation for 1 h at 37°C, the oocytes or ova were washed three times with PBS-D, then transferred to gelatinized glass slides and mounted in PBS-D under coverslips supported with four spots of Vaseline at the corners. The slides were examined at 20 × or 40 × with an American Optical Microscope equipped with an Epi-illumination system. Color photomicrographs were taken using Kodak Ektachrome 400 ASA slide film.

Immunohistochemical Localization

Experiments were performed using reproductive tissues obtained from prepubertal (23–26 days old), pubertal (32–38 days old) and adult (over 42 days old) cyclic female golden hamsters. Cyclicity was ascertained by examination for the postovulatory vaginal discharge; adult females were used for which at least two consecutive 4-day estrous cycles had been recorded. Cyclic females representing each day of the estrous cycle were studied. In addition, one superovulated female (PMSG-hCG treatment) killed 17 h after hCG injection was also used to render observations of oviductal ova in their surroundings more predictable. Organs, including ovary, ovarian bursa, oviduct and the adjacent part of the uterus, were excised from the animals and rapidly placed in cold, neutral, buffered formol (Biopharm, Montreal, Quebec, Canada). Control tissues, including spleen, liver, gut and lung, were also excised and were treated like the reproductive tissues. The specimens were cut into 10- 200 mm^3 pieces and kept in the fixative for 24 h at 4°C. They were then dehydrated and processed for embedding in paraffin (Tissueprep, Fisher Scientific, Montreal, Quebec, Canada) by using an automatic circulation system (Shandon Southern Apparatus, Johns Scientific, Montreal, Quebec, Canada). Paraffin sections were cut to a thickness of 5 μm and mounted on gelatinized glass slides. Some sections were stained with hematoxylin, phloxine and safranine (HPS) to verify tissue integrity. Unstained test sections were immunostained by using indirect immunofluorescence. Briefly, organ sections on slides were deparaffinized in toluene, rehydrated from absolute ethanol to 50% ethanol, and soaked 10 min in double distilled water. The sections were then covered with control rabbit sera, anti-zona serum, or anti-zona serum absorbed with ovarian or oviductal tissue, or RBCs; the antisera were diluted 1/32 in PBS (0.01 M phosphate, 0.15 M NaCl, pH 7.4). After an incubation of 1 h in a moist chamber at 23°C, the slides were washed three times for 15 min in PBS. The slides were then returned to the moist chamber and covered with diluted fluorescein-conjugated goat anti-rabbit IgG (Miles Laboratories) diluted 1/100 in PBS. After an incubation of 1 h at 23°C, the excess reagent was washed away as described above, and the sections were mounted under coverslips in 90% glycerol-10% PBS. Method controls included the use of PBS instead of the first antibody to check the specificity of the fluorescein-conjugated antibody and the use of nonspecific blocking agents—such as albumin (1% w/v), gelatin (1%-3% w/v) or normal goat serum (5% v/v)—in the washings or in the antibody solutions or as a preincubation step to eliminate nonspecific background staining. The slides were examined and color photomicrographs were taken as already described.

Mucoprotein Histochemistry

Experiments were performed by using sections of the oviducts from the superovulated female. Two methods were used to identify those cells producing mucoproteins: periodic acid-Schiff procedure coupled with a diastase treatment (PAS-diastase), and the alcian blue procedure (pH 2.5–3.0). The PAS produces a purple staining of neutral, sialylated (sialidase-labile) and highly sulfated epithelial mucins, whereas sialylated (sialidase-resistant), highly sulfated connective mucins and hyaluronic acid are not stained. Glycogen, which is also stained with the PAS procedure, was eliminated by pretreatment with diastase. In contrast, the alcian blue procedure at pH 2.5–3.0 results in the blue staining of the hyaluronic acid and the sialylated mucins, whereas highly sulfated epithelial or connective mucins remain unstained (Cook, 1977). Stained sections were examined with an American Optical microscope. Color photomicrographs were taken using Kodak Ektachrome 50 ASA slide film.

RESULTS

When oviductal zonae pellucidae were used for immunization of a rabbit, the antiserum obtained was

specific for the organs of the reproductive system, including the ovary, ovarian bursa, oviduct and uterus as judged by immunofluorescence on tissue sections. No reaction was observed on control tissues, including the liver, spleen, gut and lung. In the reproductive system, fluorescence has been observed over ovarian follicles, in the oviductal lumen and over the epithelial cells of the oviduct and on the lining of the inner surface of the uterus. This suggests that oviductal zona pellucida antigens may originate from two sources: the ovary and the oviduct.

To further substantiate the dual origin of oviductal zona antigens, antiserum absorption techniques were used and dot-immunobinding assays were conducted. As seen in Figure 1A, the anti-zona serum recognized the immunoreactive components in acid-solubilized ovarian and oviductal zonae and also in oviductal fluid. The intensity of the reaction on oviductal zonae did not increase as a function of time after hCG injection. No reaction was seen with hamster serum. Preabsorption of the anti-zona serum with ovarian tissue eliminated the reaction with ovarian zonae, but the reaction with oviductal zonae and oviductal fluid was retained--although the intensity of the interaction was diminished (Fig. 1B). Preabsorption of the anti-zona serum with oviductal tissue eliminated the reaction with oviductal fluid, but the reaction with ovarian and oviductal zonae was retained; again, the intensity of the reaction was reduced (Fig. 1C). The preimmune serum gave completely negative results. Control preabsorption on kidney or liver tissue did not alter the reaction of the anti-zona serum with any of the test antigens (not shown).

Immunofluorescence studies confirmed the results obtained by dot-immunobinding assay. When treated with the anti-zona serum, zonae pellucidae of ovarian and oviductal eggs gave an intense fluorescent reaction (Fig. 2A; the reaction with an oviductal egg is shown). Preabsorption of the antiserum with ovarian tissue only reduced the fluorescence observed on the oviductal eggs (Fig. 2B), whereas it eliminated the fluorescent staining of the ovarian eggs (Fig. 2C). No fluorescent reaction was observed after an incubation with normal rabbit serum (except for the autofluorescence of the vitelli).

Immunohistochemical studies were done to determine the cellular origin of the immunoreactive zona component(s) originating from the oviduct. Considering that the hamster ovary is enclosed within a membranous sac called the bursa ovarica, which completely isolates the ovary from the peritoneal cavity, and which is continuous with the oviduct (Clewe, 1965), immunohistochemical localization of oviductal material was performed on the ovarian bursa in addition to the oviduct itself. Figure 3A illustrates the close relative positioning of the ovary and its bursa. Immunolocalization with anti-zona serum using indirect immunofluorescence indicated that immunoreactive components were present at the surface epithelium of the ovary and also in the inner part of the bursa facing the ovary. No fluorescence was observed on the fat tissue that forms another part of the bursa (Fig. 3B). Figure 3C illustrates the accumulation of material secreted within the bursal

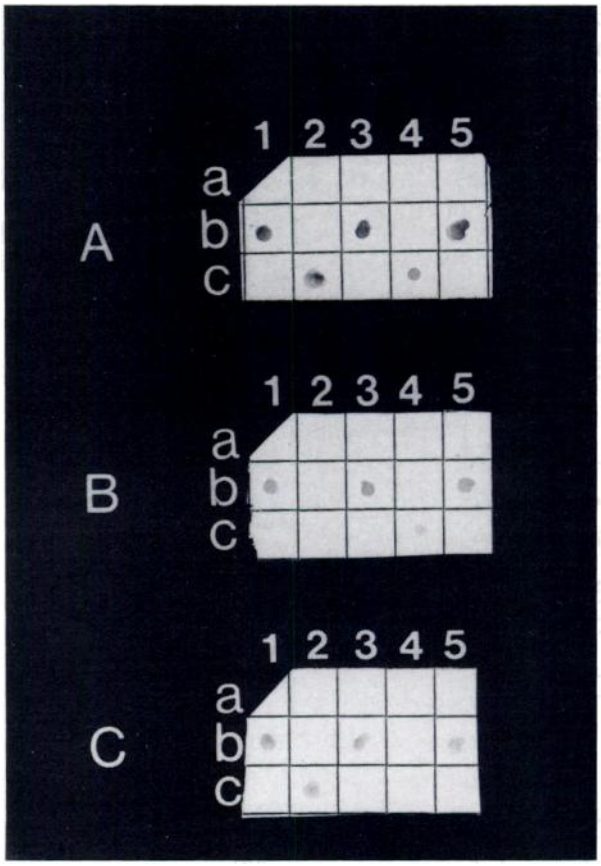


FIG. 1. A dot immunoassay for serum antibodies against oviductal hamster zonae pellucidae. *Division a2:* hamster serum (80 ng of protein); *division b1:* 5 oviductal zonae, 17 h post-hCG injection; *division b3:* 5 oviductal zonae, 24 h post-hCG injection; *division b5:* 5 oviductal zonae, 42 h post-hCG injection; *division c2:* 12 ovarian zonae; *division c4:* oviductal fluid (180 ng of protein). *A)* Incubation with anti-zona serum. *B)* Incubation with anti-zona serum absorbed with ovarian tissue. *C)* Incubation with anti-zona serum absorbed with oviductal tissue.

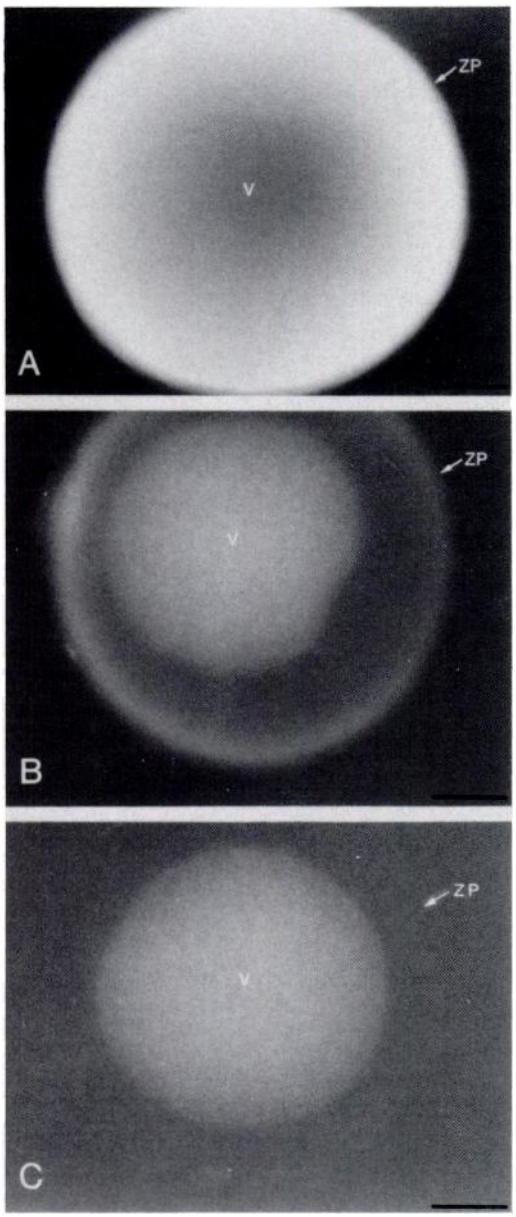


FIG. 2. Indirect immunofluorescence on ovarian and oviductal eggs. Interaction between an oviductal egg and the anti-zona serum (*A*) or the anti-zona serum absorbed with ovarian tissue (*B*). (*C*) Interaction of an ovarian egg and the anti-zona serum absorbed with ovarian tissue. *v*: vitellus; *zp*: zona pellucida. Bars = 25 μm.

cavity which was particularly copious in PMSG-stimulated females. When treated with anti-zona serum, these secretions showed intensive fluorescent staining (Fig. 3D). The origin of these secretions was established with the use of anti-zona serum absorbed with oviductal or with ovarian tissue. When adjacent sections were reacted with anti-zona serum absorbed with oviductal tissue, no reaction occurred at the surface epithelium of the ovary, on the bursa or in the secretions enclosed within the bursal cavity. A reduction in the intensity of the staining was noted when adjacent sections were incubated with the anti-zona serum absorbed with ovarian tissue (result not shown). In the fimbria, which is enclosed in the bursal cavity, a fluorescent staining was observed only in some of the epithelial cells (Fig. 4A). In the ampulla, a reaction was seen in most of the epithelial cells and in the lumen. In addition, fluorescent material also formed a continuous film on the wall of the oviduct (Fig. 4B). The highest reactivity was seen in the isthmus; at this level, fluorescent staining was observed in all of the epithelial cells lining the lumen and also within the lumen (Figs. 4C,D). In the uterus, fluorescence was present only along the uterine wall, and no reaction was observed in the epithelial cells (Fig. 4E). Preabsorption of the anti-zona serum with oviductal tissue completely abolished the fluorescent reaction along the entire oviduct and also along the uterus (Fig. 4F). On the other hand, when adjacent sections were treated with anti-zona serum absorbed with ovarian tissue, a reduction in the intensity of the staining was noted (results not illustrated).

In addition to their positive staining observed with the immunohistochemical technique, the epithelial cells stained purple with the PAS-diastase procedure (Fig. 4G) but were not stained by alcian blue (pH 2.5–3.0) (Fig. 4H). Figures 4D and G show serial sections stained immunohistochemically and with PAS-diastase, respectively. As can be seen, there was excellent correlation between the areas that were stained by the two techniques. In addition to staining the epithelial cells of the isthmus, the PAS-diastase procedure also stained immunoreactive cells of the fimbria and of the ampulla. The zonae pellucidae and the secretions within the lumen of the ampulla were also stained by the PAS-diastase procedure (Fig. 5A). Immunohistochemical staining at this level, using anti-zona serum, indicated that immunoreactive components were preferentially localized on the inner and outer surfaces of the zona as well as adherent to the cumulus cells and the extracellular matrix in which they were enclosed (Fig. 5B). Anti-zona serum absorbed with oviductal tissue produced no reaction on the cumulus cells and their extracellullar matrix but retained a reaction on the outer surface of the zona (Fig. 5C). The absorption of anti-zona serum with ovarian tissue led to a decrease in the intensity of the reaction over the inner and outer surfaces of

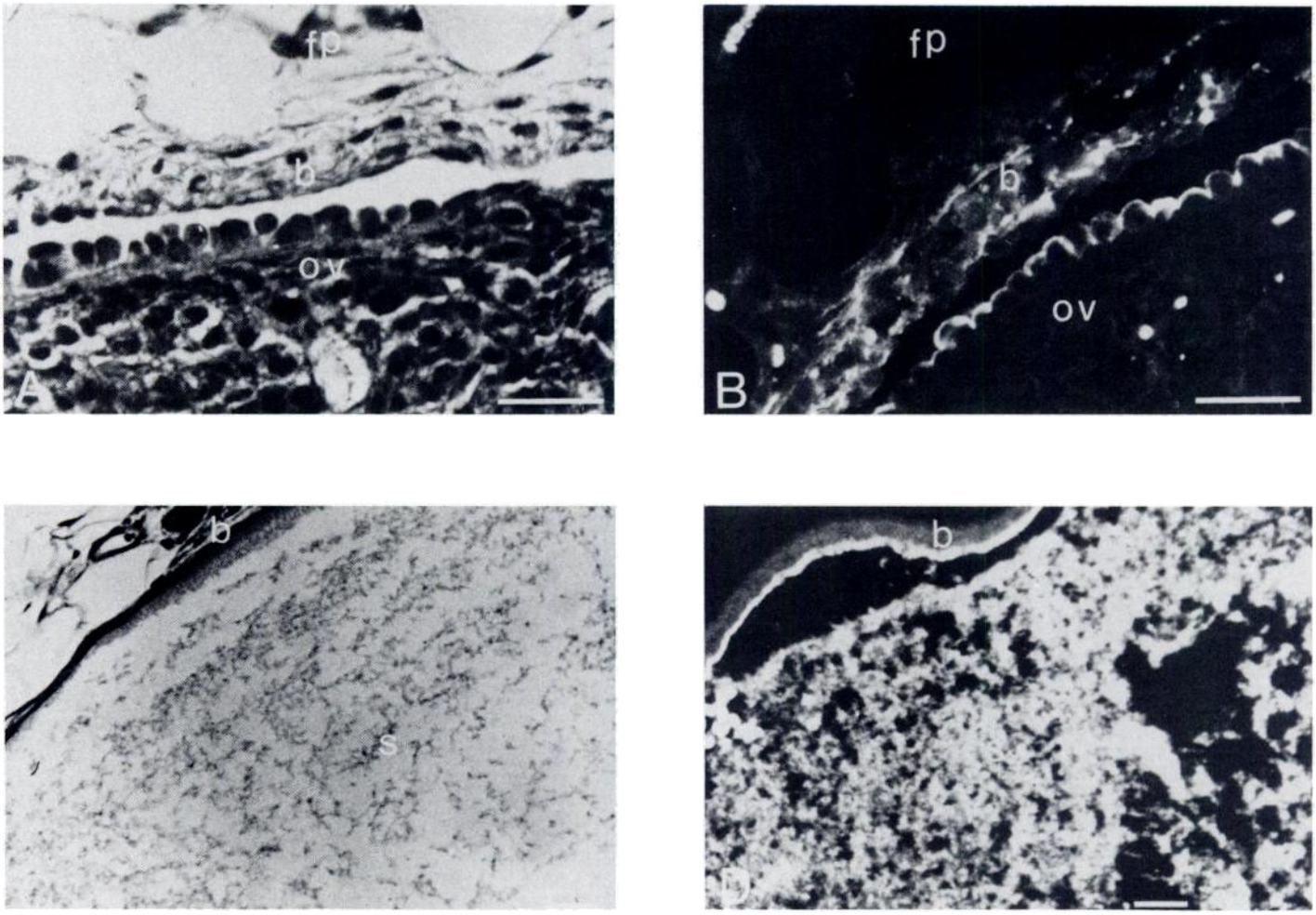


FIG. 3. Immunoreactivity within the ovarian bursa treated with anti-zona serum. *A,B*) Sections of ovary and bursa. *C,D*) Sections through the bursal cavity. HPS-stained section (*A*). PAS-stained section (*C*). *B,D*) Adjacent sections treated for indirect immunofluorescence with anti-zona serum. *b*: bursa; *fp*: fat pad; *ov*: ovary; *s*: secretions. *Bars* = 30 μm.

the zona and also on the cumulus cells and their extracellular matrix (Fig. 5D). In these two cases, the absorbed anti-zona serum produced a reaction that was clearly distinguishable from that obtained after an incubation with normal rabbit serum (Fig. 5E).

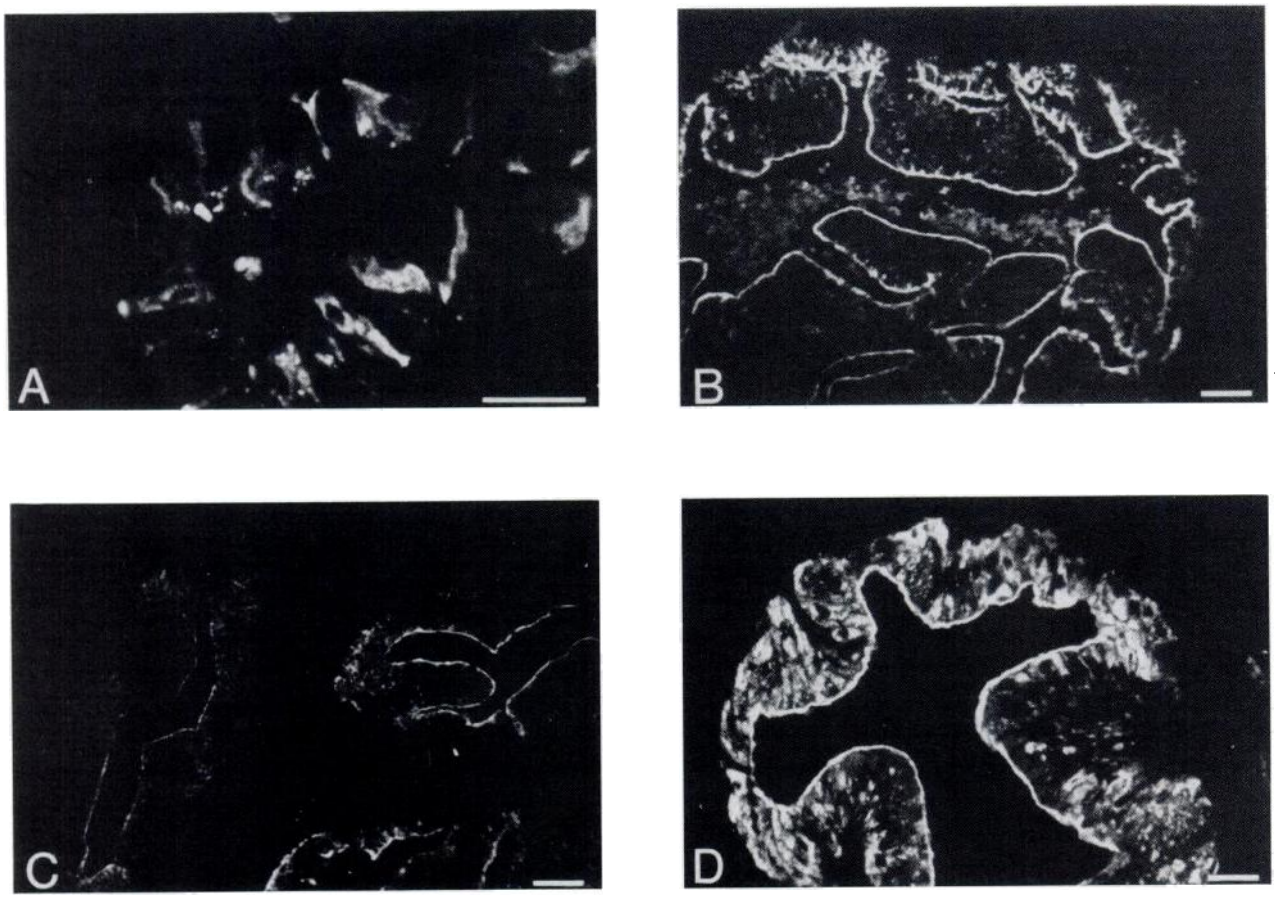
The anti-zona serum reacted with the oviductal tissue of adult cyclic females as well as of prepubertal and pubertal females. Detailed studies of the hormonal control of these secretions have not been made, but an increased reactivity was noted after PMSG stimulation of immature or adult females.

Control absorption of the antiserum with RBCs did not affect the immunohistochemical staining, nor did any of the method controls where the blocking agents albumin, gelatin or normal goat serum were added during incubation. In addition, no reaction was ever observed in the use of normal rabbit sera, sera from rabbits immunized with Freund's adjuvant alone, or with PBS as the first antiserum.

DISCUSSION

In the present study, a polyclonal antiserum directed against hamster zonae pellucidae isolated from oviductal ova has been used to investigate the addition of oviductal material to hamster eggs after ovulation. As revealed by indirect immunofluorescence on organ sections, the anti-zona serum appears to be specific towards the reproductive system; no reaction occurred on any of the control tissues such as spleen, lung, liver and gut.

The possibility exists that the hamster oviductal zonae pellucidae are composed of two distinct groups



of immunogenic components—one group synthesized by the ovary and the other by the oviduct; it is also possible that the same group of immunogenic components is synthesized by these two organs. The presence of two distinct groups of immunogenic components in the hamster oviductal zona, i.e. one from the ovary and the other from the reproductive tract, was proposed by Fox and Shivers (1975a). These investigators used an antiserum produced against homogenized hamster uterine tissue and absorbed with gut, liver and lung tissues to render it specific towards the reproductive tract.

To distinguish the ovarian vs. oviductal immunogenic components in the oviductal zonae, the anti-zona serum that was polyspecific towards the ovary and the oviduct was treated by using absorption methods to render it monospecific towards each organ. As seen in the dot-immunobinding assay or in the immunofluorescent assay on eggs, the anti-zona serum that was monospecific towards oviductal material (absorption with ovarian tissue) or towards ovarian material (absorption with oviductal tissue) still reacted with the oviductal zonae. This suggests that the oviductal zona is composed of immunogenic components specific to each organ. In each case, the absorption procedure eliminated the reaction with the homologous antigens, which excluded the possibility of incomplete absorption. On the other hand, the reduction in the intensity of the interaction of the ovarian zona with the anti-zona serum absorbed with oviductal tissue and a similar degree of reduction in the interaction of the oviductal fluid with the anti-zona serum absorbed with ovarian tissue would suggest that immunogenic determinants common to the ovary and the oviduct were present in the ovarian zona and in the oviductal fluid. The results demonstrated the double origin of the oviductal zona as proposed by Fox and Shivers (1975a) who suggested that oviductal material is associated with the zona during the passage of the egg through the

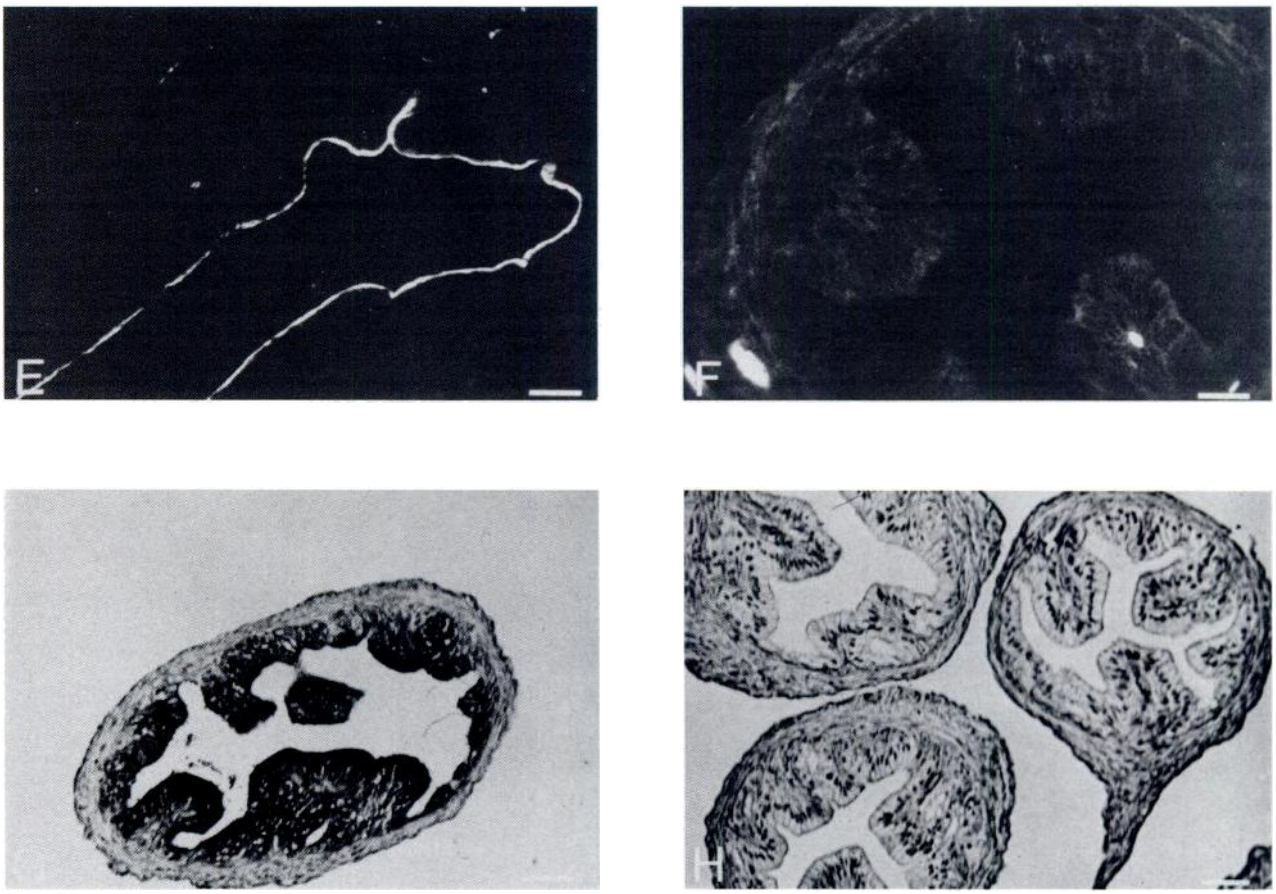


FIG. 4. Immunoreactivity along the reproductive tract when treated with anti-zona serum. *A,B,C,D,E*) Sections treated for indirect immunofluorescence with anti-zona serum. *A*) Estrous fimbria. *B*) Estrous ampulla. *C*) Estrous isthmus. *D*) Metestrous isthmus from PMSG/hCG-stimulated female. *E*) Estrous uterus. *F,G,H*) Adjacent sections from the PMSG and hCG-stimulated female treated with anti-zona serum absorbed with oviductal tissue (*F*), stained with the PAS-diastase procedure (*G*), stained with the alcian blue procedure (*H*). *Bars* = 30 μm for *A,D,E* and *F*; 60 μm for *B,C,G* and *H*.

oviduct; they also suggested the presence of common immunogenic determinants of the ovary and the oviduct associated with the zona pellucida and the oviductal fluid. Moreover, Sacco and Shivers (1973) reported the existence of immunogenic components in the rabbit that are specific to the reproductive system but common to many organs of this system.

Several investigators developed antisera against the zona pellucida in the hope of developing a method of immunocontraception. Certain groups used homogenized ovaries as the source of antigen. In these cases, antisera were specific towards the ovary only after absorption on many other tissues (Ownby and Shivers, 1972; Jilek and Pavlok, 1975; Trounson et al., 1976; Sacco, 1977). To eliminate these cross-reactivities, zonae isolated from ovaries were used instead of the entire ovary. In this manner, antisera were developed against porcine zonae (Sacco and Palm, 1977; Dunbar and Raynor, 1980; Dietl and Mettler, 1982), bovine zonae (Gwatkin and Williams, 1978) and rabbit zonae (Wood and Dunbar, 1981). When intact zonae were used, the resulting antisera did not exhibit complete specificity towards the ovary, but preabsorption with follicular fluid apparently led to ovarian specificity (Palm et al., 1979). On the other hand, when solubilized zonae rather than intact zonae were used, specificity against the ovary was observed. Dunbar and Raynor (1980) suggested that the solubilization process destroyed the antigenicity of the contaminating cellular components, or that these contaminating components were not solubilized and subsequently were removed by centrifugation during the preparation of solubilized zona material.

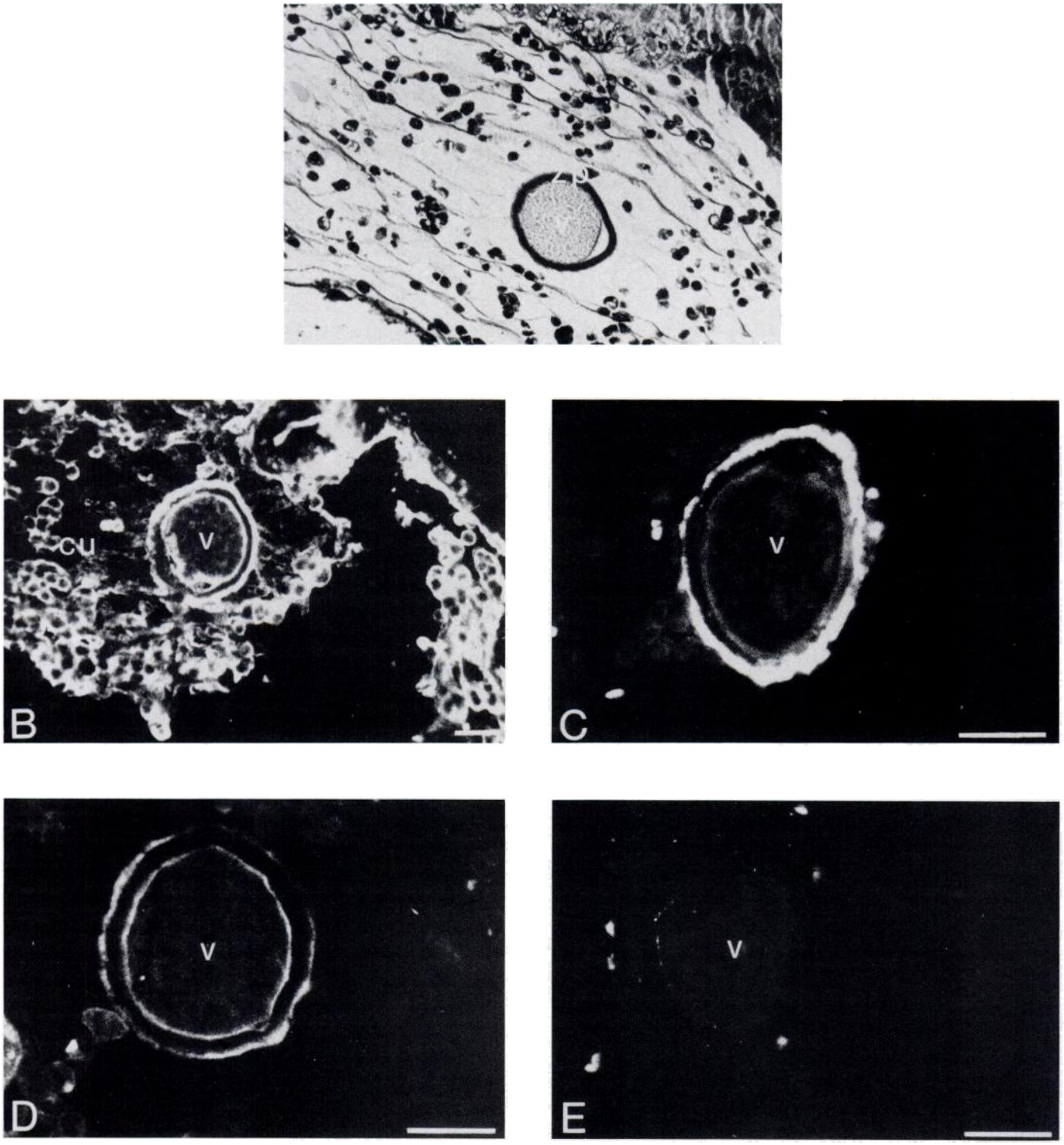


FIG. 5. Immunoreactivity of the cumulus masses present in the oviduct of a PMSG/hCG-stimulated female. *A*) Section stained with the PAS-diastase procedure. *B*) Section treated with the anti-zona serum. *C*) Section treated with anti-zona absorbed with oviductal tissue. *D*) Section treated with anti-zona serum absorbed with ovarian tissue. *E*) Section treated with serum from a rabbit immunized with Freund's adjuvant alone. *cu*: cumulus cells; *zp*: zona pellucida, *v*: vitellus. *Bars* = 30 μm.

Antisera against oviductal zonae also have been developed (Trounson et al., 1976; Gwatkin et al., 1977; Tsunoda and Chang, 1978; Sacco, 1979; Aitken and Richardson, 1981; Aitken et al., 1982; Tsunoda and Whittingham, 1982), but cross-reactivity with oviductal material was not evaluated in any of these reports. Therefore, until now, the similarity or dissimilarity with our data concerning cross-reactivity was impossible to evaluate.

Our results showed an accumulation of secretions within the bursal cavity. These results and those of Battalia and Yanagimachi (1979), which demonstrated fluid transport from the isthmus toward the ovary during the periovulatory period, and those published by Martin et al. (1981a,b), which reported the absence of fluid discharge from the bursal cavity towards the oviduct during the preovulatory period, all agreed with the hypothesis of fluid accumulation within the bursal cavity at ovulation. In addition, with the use of the anti-zona serum, we demonstrated that the intrabursal fluid partly originated from the secretory activity of the oviductal epithelial cells.

In 1969, Weakley presented an anatomical description of the surface epithelium of the hamster ovary as a part of the peritoneal epithelium and composed of only one continuous layer of cells varying in shape from squamous to low columnar. Our results showed that oviductal material within the bursal cavity might adhere to these cells. Moreover, oviductal material was also observed in the inner tissue layers of the bursa ovarica up to the surrounding fat pad. This result agreed with the recent finding that the adipose cells appeared to form an impermeable layer than was not penetrated by dyes injected into the bursal cavity (Martin et al., 1981a).

Oviductal immunogenic components detected in the bursal cavity by use of the anti-zona serum were also observed on the oviductal zonae; they were also visualized within the cumulus masses adhering to the cumulus cell surface and coating the uterine wall. These immunogenic components appeared to be synthesized along the entire oviduct but predominantly in the isthmus. This might reflect the increased number of secretory cells in this particular segment, as was proposed to occur in many species (Crespeau and Mialot, 1979). The immunostained cells were also stained with the PAS-diastase procedure, but not with alcian blue. Combination of these two results implies that the oviductal immunogenic components detected by use of anti-zona serum are likely to be neutral or highly sulfated mucoproteins (Cook, 1977). Similar material was also demonstrated to occur in the rabbit oviduct (Shapiro et al., 1974; Denker and Gerdes, 1979; Jansen and Bajpai, 1982; Oliphant et al., 1984).

The restricted localization of the immunogenic components to the epithelial cells was different from that reported by Fox and Shivers (1975b), even though both antisera recognized oviductal components associated with the oviductal zonae. Therefore, the two sera would appear to recognize different immunogenic determinants. On the other hand, the type of localization that we observed was more in accord with the cellular localization described for the rabbit oviductal mucoproteins (Oliphant et al., 1984).

In our investigation, the adherence of the oviductal immunogenic components to the surface of many distinct types of cell would suggest that their association with the zona pellucida is not a specific one. It is likely that these components might adhere to any particle contacted. This assumption is supported by the fact that in the rabbit, mucus similar to that observed on the zona of this species, has been also noted to occur on cumulus cells present in the oviduct (Bacsich and Hamilton, 1954) and on goat eggs transplanted to the rabbit oviduct (Rao et al., 1984).

In the oviductal zona, oviductal immunogenic components were found predominantly on the inner and outer surfaces of this structure. This agreed with a previous finding which indicated that the zona pellucida is permeable to high molecular weight molecules (Hastings et al., 1972). The observation of oviductal immunogenic components at the inner surface of the zona might also represent an association of these components with the plasma membrane of the ovum; this recently was reported for the mouse by Kapur and Johnson (1985).

The oviductal fluid provides the optimal environment for the freshly ovulated oocyte, for sperm capacitation, for fertilization and for early preimplantation development. The specific contribution of the mucoproteins to these processes remains to be demonstrated.

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