BIOLOGY OF REPRODUCTION 43, 340–346 (1990)

Localization of Oviductal Glycoproteins within the Zona Pellucida and Perivitelline Space of Ovulated Ova and Early Embryos in Baboons (*Papio anubis*)[1]

MELINDA LEE BOICE,[4] THOMAS J. McCARTHY,[3,5] PATRICIA A. MAVROGIANIS,[4] ASGERALLY T. FAZLEABAS,[4] and HAROLD G. VERHAGE[2,4]

Department of Obstetrics and Gynecology[4] and Biologic Resources Laboratory[5]
University of Illinois College of Medicine at Chicago, Chicago, IL 60680

ABSTRACT

The estrogen-dominated baboon oviductal epithelium synthesizes and secretes a family of oviduct-specific glycoproteins. The objective of this study was to determine if these glycoproteins become associated with ova and early embryos. Ovarian and oviductal eggs obtained from superovulated baboons 72 h post-hCG were subjected to an indirect immunofluorescent assay that used a polyclonal antibody prepared toward the baboon oviduct-specific glycoproteins. Oviductal ova as well as 2-cell and 4-cell embryos showed intense, specific fluorescence within their zonae pellucidae. Ovarian ova did not exhibit fluorescence. Oviductal eggs were also fixed and processed for peroxidase-antiperoxidase immunocytochemistry and colloidal gold immunoelectron microscopy to confirm the immunofluorescent data and to determine the subcellular distribution of the antigens. Oviductal ova as well as 2-cell and 3-cell embryos exhibited immunolabeling localized within the zona. Gold particles were distributed uniformly throughout the width of the zona. Occasional groupings of gold particles were observed within the zona. Also, in most eggs, immunoreactivity was observed associated with flocculent material in the perivitelline space as well as the vitelline membrane. Furthermore, immunogold labeling above background level was noted in the cytoplasm of the eggs, particularly in the blastomeres of 3-cell embryos. Collectively, these results indicate that baboon estrogen-dependent oviductal secretory glycoproteins become intimately associated with oviductal ova and with embryos.

INTRODUCTION

The mammalian oviduct provides and modulates the fluid environment in which the fertilization process and early embryonic development occur. Oviductal fluid is composed predominantly of plasma derivatives (reviewed by Leese, 1988), but it also contains macromolecules that are synthesized and secreted under ovarian steroid regulation by the oviductal epithelium. Oviductal secretory glycoproteins have been characterized for several species including mice (Kapur and Johnson, 1985; 1986), hamsters (Léveillé et al., 1987; Oikawa et al., 1988, Robitaille et al., 1988), rabbits (Oliphant and Ross, 1982; Oliphant et al., 1984), sheep (Sutton et al., 1984, 1986; Gandolfi et al., 1989), and baboons (Fazleabas and Verhage, 1986; Verhage and Fazleabas, 1988). Other studies suggest that oviduct-specific proteins exist in pigs (Buhi et al., 1989), cattle (Joshi, 1988; Malayer et al., 1988), and humans (Verhage et al., 1988; Wagh and Lippes, 1989). Oviductal secretory macromolecules have been shown to intimately associate with oviductal eggs. Reports indicate that in hamsters (Araki et al., 1987; Léveillé et al., 1987; Kan et al., 1988, 1989; Oikawa et al., 1988) and sheep (Gandolfi et al., 1989), the characterized oviductal glycoproteins become associated with the zonae pellucidae of ovulated eggs and developing embryos. In mice, a high molecular weight oviductal glycoprotein becomes sequestered within the perivitelline (PV) space of postovulatory ova (Kapur and Johnson, 1986, 1988). Recently, Verhage et al. (1989) have characterized a family of high molecular weight, oviduct-specific glycoproteins synthesized and secreted by the estrogen (E_2)-dominated baboon *(Papio anubis)* oviduct (Verhage et al., 1990). The objective of the present study was to determine if these primate oviductal antigens become associated with oviductal ova and/or embryos.

MATERIALS AND METHODS

Materials

Immunochemical supplies were purchased from Organon Teknika-Cappell (Durham, NC). Lowicryl K4M embedding resin was obtained from Polysciences, Inc. (Warrington, PA) and electron microscopic grids were purchased from Ladd Research Industries (Burlington, VT). All other chemicals of reagent grade or better were products of Sigma Chemical Co. (St. Louis, MO).

Collection of Ova and Embryos

Ovarian and oviductal eggs were obtained from baboons in which multiple follicular development and superovulation were induced as described by McCarthy et al. (1989). In brief, starting on Day 1 of the menstrual cycle, baboons received 37.5 IU human menopausal gonadotropin (hMG;

Accepted April 6, 1990
Received February 14, 1990.
[1]This work was supported by NIH grant HD20571.
[2]Reprint requests: Dr. Harold G. Verhage, Department of Obstetrics and Gynecology, University of Illinois at Chicago, 840 South Wood Street, Chicago, IL 60612.
[3]Current address: Dr. Thomas J. McCarthy, Division of Comparative Medicine, Washington University School of Medicine, Box 8061, 660 S. Euclid Ave., St. Louis, MO 63110.

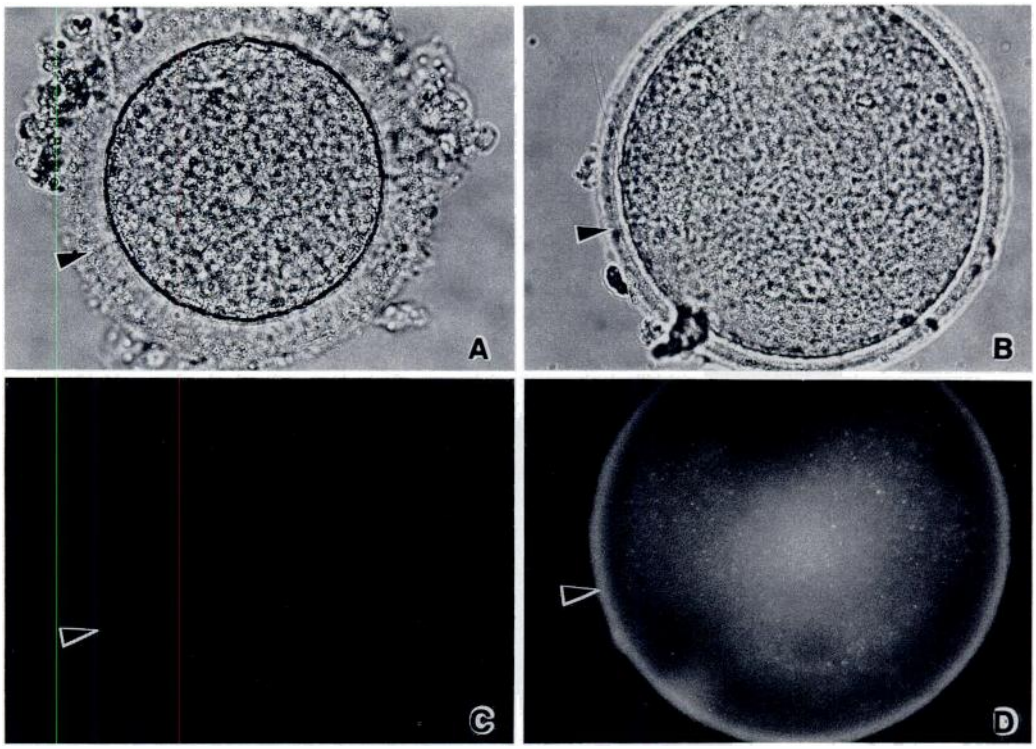


FIG. 1. Whole mounts of living ova collected 72 h post-hCG from superovulated baboons. Phase microscopy of (A) an ovarian ovum aspirated from a follicle that was greater than 5 mm in diameter and (B) an ovum collected by retrograde lavage of the oviduct. Arrowheads point to the zonae pellucidae. Indirect immunofluorescence of the same ova showed that (C) no localized fluorescence was observed with the ovarian ovum and (D) intense fluorescence was associated with the zona pellucida that surrounds the oviductal ovum. ×245.

Pergonal; generously provided by Serono Laboratories, Norwell, MA) administered i.m. twice a day (b.i.d.) on Days 1–4 and 75 IU hMG b.i.d. on Days 5–10. HCG (5 000 IU i.m.; Profasi, Serono) was administered on Day 11, and the females were placed with fertile males for 2–3 days. At 72 h post-hCG treatment, a midventral laparotomy was performed and the genital tract was exposed. Oviductal oocytes and/or embryos were obtained by retrograde lavage with Hanks' Balanced Salt Solution (HBSS), 37°C. Ovarian ova were obtained by aspiration of large follicles (>5 mm in diameter) using a 20-gauge needle attached to a heparinized 1-ml syringe. Ova and embryos isolated from the aspirates and lavages were rinsed several times with HBSS.

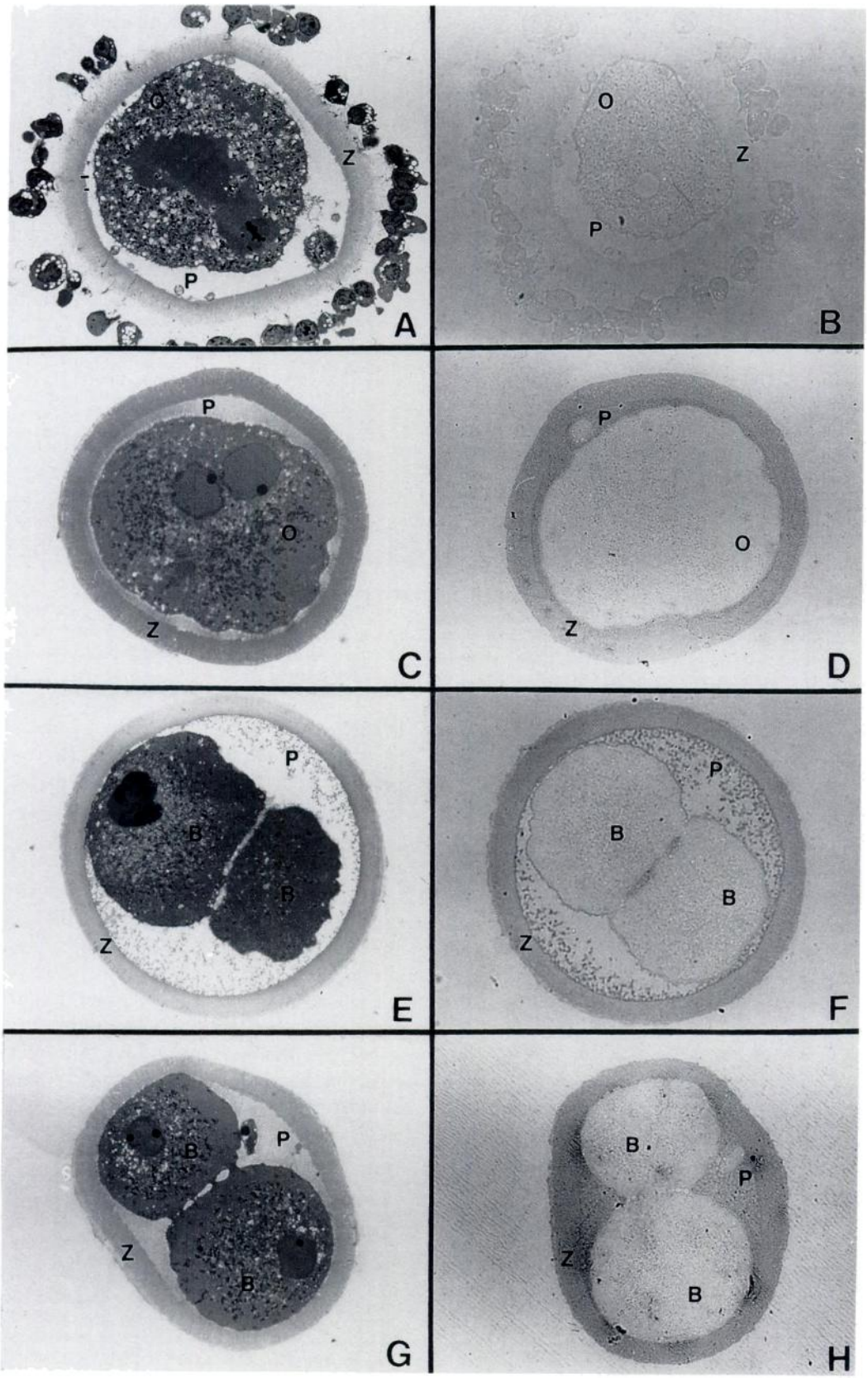
Immunofluorescence Microscopy

Immediately after recovery, ova and embryos were subjected to an indirect immunofluorescent assay as described by Kapur and Johnson (1986), with the following modifications. The polyclonal antiserum (anti-PA ovd.) previously prepared toward the family of oviduct-specific glycoproteins characterized in baboons (Verhage et al., 1989) was utilized at a 1:250 dilution as the primary antibody. Fluorescein-conjugated goat anti-rabbit IgG was diluted 1:200 for use as the second antibody. Ovarian (n = 8) and ovulated (n = 4) ova, as well as 2-cell (n = 2) and 4-cell (n = 2) embryos recovered from four females were subjected to the immunofluorescent assay. Whole mounts of the eggs were examined by fluorescence microscopy and photographed with Tri-X film (Eastman-Kodak, Rochester, NY) using 2-min exposures.

Light Immunocytochemistry

Immunocytochemical techniques were employed to confirm the immunofluorescence data. Ova isolated from the ovarian aspirates and retrograde lavages from four additional females were fixed in a HEPES-buffered solution containing 3% paraformaldehyde/1% gluteraldehyde (Verhage et al., 1979) for 3 h and then stored in 1% sodium cacodylate/4.5% sucrose buffer overnight at 4°C. Ova were processed and embedded in Lowicryl K4M as described by Bendayan (1984). Sections (0.5 μm) were cut with a glass knife on a Sorvall MT-5 000 ultramicrotome (Sorvall, Nor-

FIG. 2. Light micrographs of serial cross sections of baboon eggs. Panels A, C, E, and G are toluidine blue-stained sections of an ovarian ovum, a 1-cell fertilized ovum, a 2-cell embryo, and a 3-cell embryo, respectively. Panels B, D, F, and H represent sections of the same eggs (in the same order) subjected to the peroxidase-antiperoxidase immunocytochemical localization procedure. Note that only nonspecific background staining was seen in the zona pellucida (Z), perivitelline space (P), and ooplasm (O) of the ovarian ovum (Panel B). In contrast, immunostaining was observed to various degrees in the Z and P of the 1-cell (Panel D), 2-cell (Panel F), and 3-cell (Panel H) eggs. Immunoreactivity was not detected in the ooplasm or within the blastomeres (B). ×390.



walk, CT), placed on microscope slides, and stained with 0.1% toluidine blue for morphological examination. Serial 1-μm sections were then cut, placed on poly-L-lysine-coated, acid-cleaned slides and subjected to (without etching) a modification (Murray and Verhage, 1985) of the peroxidase-antiperoxidase immunocytochemical localization procedure of Sternberger et al. (1970) to detect the E_2-dependent oviductal glycoproteins. The IgG fraction of anti-PA ovd., which was isolated sequentially by ammonium sulfate precipitation and ion-exchange chromatography on diethylaminoethyl cellulose (DE52; Whatman, Kent, United Kingdom), was used as the primary antibody at 22 ng/ml. Serial sections incubated in buffer containing IgG isolated from preimmune rabbit serum instead of anti-PA ovd. served as negative controls. Ovarian (n = 2) and oviductal (n = 6) ova, as well as a 2-cell and a 3-cell normal embryo were examined and the results of the immunocytochemical analysis were photographed.

Colloidal Gold Immunolabeling

The oviductal eggs that were fixed and embedded in Lowicryl (see above) were also subjected to colloidal gold immunoelectron microscopy to determine distribution of the glycoprotein antigens. One hundred nanometer sections were collected on 300-mesh formvar/carbon-coated nickel grids and were subjected to the indirect immunogold labeling technique as described by Varndell and Pollack (1984), with the following modifications. The sections were not etched and they were exposed to a primary antiserum for 48 h at 4°C. The IgG fraction isolated from anti-PA ovd. as described above was used at 12 ng/ml. Negative controls were treated with preimmune IgG under the same conditions. Sections from oviductal ova (n = 4) and embryos (n = 2) were observed and photographed with a Philips 300 electron microscope.

RESULTS

When viewed by fluorescence microscopy, ovulated 1-cell ova subjected to the indirect immunofluorescent assay exhibited intense fluorescence that appeared to be localized within the zona pellucida (Fig. 1), whereas ovarian ova showed no localized fluorescence. Two-cell and 4-cell embryos collected from superovulated baboons along with a 2-cell embryo obtained from a normally cycling animal displayed fluorescence associated with the zona that was similar to that seen in 1-cell oviductal ova (data not shown).

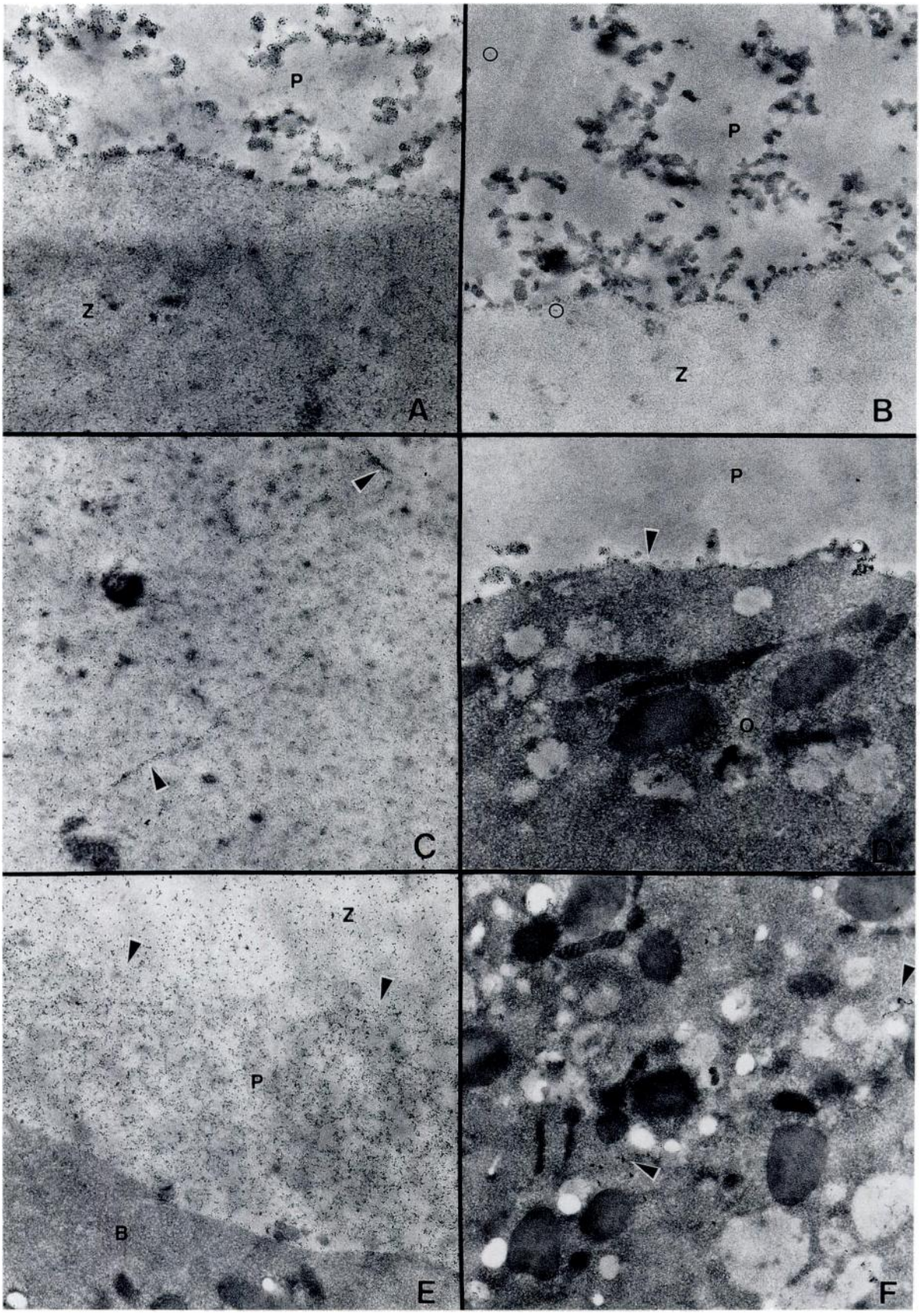
Immunocytochemical analysis at the light microscopic level confirmed and extended the immunofluorescence data. Ovarian ova (visible in toluidine blue-stained sections, Fig. 2A) did not display specific immunoperoxidase staining in any region of the ovum (Fig. 2B). Also, immunolabeling was not observed in control sections of oviductal ova treated with the IgG fraction isolated from preimmune rather than immune serum (data not shown). Immunoperoxidase staining was present in the zonae pellucidae of all oviductal eggs examined, regardless of the stage of development (Fig. 2D, F, H). However, as development of the embryo progressed, the intensity of staining within the zona appeared to increase. Immunolabeling was also observed within the PV space of three of the four oviductal eggs and both embryos. The immunoreactivity appeared to be associated with flocculent material in the PV space of the unfertilized as well as fertilized ova. The PV space of the 3-cell embryo appeared to be homogeneously and intensely labeled. Little or no immunoperoxidase staining was noted within the cytoplasm of any of the eggs or polar bodies.

At the ultrastructural level, colloidal gold immunolabeling was evident in the zona pellucida of all oviductal eggs assayed (Fig. 3). Gold particles appeared distributed homogeneously throughout the width of the zonae (Fig. 3A, E), with occasional groupings of particles observed (Fig. 3C). Very few gold particles were observed scattered singly in sections that were treated with the IgG fraction isolated from the preimmune serum (Fig. 3B). The flocculent material, which was present as discrete islands in the PV space in the 1-cell unfertilized egg (Fig. 3A) and the 2-cell embryo obtained from one baboon, was distinctly labeled with gold particles. Additionally, in the 3-cell embryo obtained from a different animal, the PV-space material that appeared amorphous in structure was heavily immunolabeled, as was the zona (Fig. 3E). Immunoreactivity was associated with the vitelline membrane of all oviductal ova (Fig. 3D). Some gold particles could be seen scattered in the cortical region of the ooplasm of all eggs. In the 3-cell embryo, clusters of immunolabeling above background level were detected within the cytoplasm of the blastomere (Fig. 3F).

DISCUSSION

This study provides evidence that in baboons, E_2-dependent oviduct-specific glycoproteins previously characterized by Verhage and colleagues (Fazleabas and Verhage, 1986; Verhage and Fazleabas, 1988; Verhage et al., 1989, 1990) become associated with oviductal ova and early embryos. Although this is the first report documenting this phenom-

FIG. 3. Electron micrographs of baboon oviductal eggs subjected to colloidal gold immunoelectron microscopy. (A) The zona pellucida (Z) and perivitelline space (P) of an unfertilized ovum. Note colloidal gold particles within the Z and associated with flocculent material within the P. ×17 442. (B) A section of the same unfertilized ovum incubated with preimmune instead of immune IgG. Gold particles (within O) were seldom observed. ×14 569. (C) The Z of an unfertilized ovum. Note groupings of particles (arrowheads) that were occasionally observed. ×13 760. (D) The vitelline membrane (arrowhead) of an unfertilized ovum (O). ×17 442. (E) A 3-cell embryo. Note heavy immunolabeling within the P and Z. Arrowheads mark the border between the Z and P. B = blastomere. ×16 302. (F) The cytoplasm of a blastomere of a 3-cell embryo. Clusters of immunolabeling (arrowheads) above background level were detected. ×16 302.



enon in primates, other studies indicate that oviductal glycoproteins characterized in mice (Kapur and Johnson, 1986; 1988), hamsters (Kan et al., 1988, 1989; Oikawa et al., 1988), and sheep (Gandolfi et al., 1989) also interact with postovulatory ova and early embryos. Furthermore, other reports in pigs (Brown and Cheng, 1986; Hedrick et al., 1987) and rabbits (Bacsich and Hamilton, 1954; Greenwald, 1958) suggest that oviductal macromolecules are added to the egg investments as they traverse the oviduct. Collectively, these data suggest that the addition of oviduct-specific macromolecules to oviductal eggs is a phenomenon common to all mammalian species.

There appears to be variability among the mammalian species as to the localization and distribution pattern of oviductal antigens in postovulatory ova and embryos. In the present study, the colloidal gold immunolabeling data clearly demonstrated that the E_2-dependent oviductal glycoproteins were distributed throughout the zona pellucida. These results are similar to those reported by Kan et al. (1988, 1989) for hamsters, where the distribution of gold particles within the zona was found to be uniform and homogeneous. Although Fox and Shivers (1975) described oviductal antigen localization within only the peripheral region of the zona of hamster ova, the indirect immunofluorescent techniques that they utilized are limited in resolution. Furthermore, the antisera used by Fox and Shivers recognizes both oviductal and uterine antigens, whereas the antigens characterized in the baboon (Verhage et al., 1989) and the hamster (Kan et al., 1988; 1989) are oviduct-specific.

Immunocytochemical staining is also evident in the PV space of baboon ova, with the antigen appearing to be specifically associated with flocculent material within the PV space. A glycoprotein (GP 215) that is secreted by the mouse oviductal epithelium becomes sequestered within the PV space of ovulated ova and developing embryos (Kapur and Johnson, 1986, 1988). GP 215 is not found in the zona, but appears to be associated with amorphous material within the PV space. The nature and origin of the material in the PV space is unknown. Sathananthan et al. (1986) showed that the PV space in a mature human ovum contains flocculent material and shreds of zona. Also, reticular and semiparticulate components have been described for pig and opposum PV space (Talbot and DiCarlantonio, 1984a, b). In this study, the flocculent material was present in the PV space of unfertilized as well as fertilized ova, indicating that the material is probably not released by cortical granules. The possibility that the flocculent matter within the PV space is an artifact of the fixation, dehydration, and embedding process can not be eliminated.

The baboon oviductal antigens also become associated with plasmalemmae of the ovulated ovum and the blastomeres of the early embryo. Gandolfi and colleagues (1989) reported that a sheep oviductal glycoprotein that binds the zona of oviductal eggs also translocates to the developing embryo. However, they did not determine whether the antigen is associated with the embryonic membrane and/or the cytoplasm.

Although the evidence that specific mammalian oviductal antigens associate with oviductal ova and embryos is accumulating, it is not yet known if this sequestration process is specific. Studies have shown that zonae of follicular oocytes cultured in vitro in medium containing sheep oviductal glycoproteins and 10% fetal calf serum bind the antigens but not any of the serum proteins (Gandolfi et al., 1989). Furthermore, Kapur and Johnson (1986) suggested that sequestration of the mouse oviductal glycoprotein appears to be a selective process since other proteins such as albumin and immunoglobulins are not retained in an analogous manner. However, it is known that the zona is freely permeable to many molecules including ferritin (500 kDa) and horseradish peroxidase (40 kDa; Hastings II et al., 1972). Thus, the possibility that oviductal antigen association in vivo with oviductal eggs is merely a consequence of equilibrium dynamics can not be dismissed.

Regardless of the mechanism, intimate association of oviductal antigens with mammalian eggs may have biological significance. Gamete interaction and subsequent fertilization may be affected by specific oviductal secretory macromolecules. In hamsters, sperm binding to ova is reduced and in vitro fertilization is inhibited if the ovulated ova are treated with a monoclonal antibody prepared toward the characterized hamster oviductal glycoprotein (Sakai et al., 1988). However, it is known that follicular and ovulated oocytes can be fertilized in vitro without exposure to the oviduct. This suggests that oviductal antigens may not be essential to the fertilization process, but rather may facilitate the event in vitro.

It is also tempting to speculate that oviductal glycoproteins play a role in embryonic development and survival. Studies have demonstrated that embryonic development in vitro is enhanced by coincubation with isolated oviducts (Minami et al., 1988; Krisher et al., 1989), oviductal cells in suspension (Eyestone and First, 1989), or monolayer culture (Gandolfi and Moor, 1987; Rexroad and Powell, 1988), as well as with medium previously incubated with oviductal cells (Eyestone and First, 1989). Also, in vivo development is enhanced if sheep embryos are cocultured with oviductal cells prior to transfer as compared to embryos cultured with other cell types or media alone (Rexroad and Powell, 1988). Further research will be needed to answer the question of the functional significance of the intimate association of oviductal glycoproteins with zonae pellucidae of oviductal ova and embryos. It may ultimately be demonstrated that while oviduct-specific molecules facilitate fertilization/development in vitro, they may play a critical role in the normal in vivo reproductive process.

ACKNOWLEDGEMENT

The authors thank Dr. Jeffrey D. Fortman for his assistance in surgery.

REFERENCES

Araki Y, Kurata S, Oikawa T, Yamashita T, Hiroi M, Naiki M, Sendo F, 1987. A monoclonal antibody reacting with the zona pellucida of the oviductal egg but not with that of the ovarian egg of the golden hamster. J Reprod Immunol 11:193–208

Bacsich D, Hamilton WJ, 1954. Some observations on vitally stained rabbit ova with special reference to their albuminous coat. J Embryol Exp Morphol 2:81–86

Bendayan M, 1984. Protein A-gold electron microscopic immunocytochemistry: methods, applications, and limitations. J Electron Microsc Tech 1:243–70

Brown CR, Cheng WKT, 1986. Changes in composition of the porcine zona pellucida during development of the oocyte to the 2- to 4-cell embryo. J Exp Morphol 92:183–91

Buhi WC, Vallet JL, Bazer FW, 1989. De novo synthesis and release of polypeptides from cyclic and early pregnant porcine oviductal tissue in explant culture. J Exp Zool 252:79–88

Eyestone WH, First NL, 1989. Co-culture of early cattle embryos to the blastocyst stage with oviducal tissue or in conditioned medium. J Reprod Fertil 85:715–20

Fazleabas AT, Verhage HG, 1986. The detection of oviduct-specific proteins in the baboon *(Papio anubis)*. Biol Reprod 35:455–62

Fox LL, Shivers CA, 1975. Immunologic evidence for addition of oviductal components to the hamster zona pellucida. Fertil Steril 26:599–608

Gandolfi F, Brevini TAL, Richardson L, Brown CR, Moor RM, 1989. Characterization of proteins secreted by sheep oviduct epithelial cells and their function in embryonic development. Development 106:303–12

Gandolfi F, Moor RM, 1987. Stimulation of early embryonic development in the sheep by co-culture with oviduct epithelial cells. J Reprod Fertil 81:23–28

Greenwald GS, 1958. Endocrine regulation of secretion of mucin in the tubal epithelium of rabbits. Anat Rec 130:477–95

Hastings II RA, Enders AC, Schlafke S, 1972. Permeability of the zona pellucida to protein tracers. Biol Reprod 7:288–96

Hedrick JL, Wardrip NJ, Berger T, 1987. Differences in the macromolecular composition of the zona pellucida isolated from pig oocytes, eggs, and zygotes. J Exp Zool 241:257–62

Joshi MS, 1988. Isolation, cell culture, and immunocytochemical characterization of oviduct epithelial cells of the cow. J Reprod Fertil 83:249–61

Kan FWK, St-Jacques S, Bleau G, 1988. Immunoelectron microscopic localization of an oviductal antigen in hamster zona pellucida by use of a monoclonal antibody. J Histochem Cytochem 36:1441–47

Kan FWK, St-Jacques S, Bleau G, 1989. Immunocytochemical evidence for the transfer of an oviductal antigen to the zona pellucida of hamster ova after ovulation. Biol Reprod 40:585–98

Kapur RP, Johnson LV, 1985. An oviductal fluid glycoprotein associated with ovulated mouse ova and early embryos. Devel Biol 112:89–93

Kapur RP, Johnson LV, 1986. Selective sequestration of an oviductal fluid glycoprotein in the perivitelline space of mouse oocytes and embryos. J Exp Zool 238: 249–60

Kapur RP, Johnson LV, 1988. Ultrastructural evidence that specialized regions of the murine oviduct contribute a glycoprotein to the extracellular matrix of mouse oocytes. Anat Rec 221:720–29

Krisher RL, Petters RM, Johnson BH, Bavister BD, Archibong AE, 1989. Development of porcine embryos from the one-cell stage to blastocyst in mouse oviducts maintained in organ culture. J Exp Zool 249:235–39

Leese HJ, 1988. The formation and function of oviduct fluid. J Reprod Fertil 82: 843–56

Léveillé MC, Roberts KD, Chevalier S, Chapdelaine A, Bleau G, 1987. Uptake of an oviductal antigen by the hamster zona pellucida. Biol Reprod 36:227–38

Malayer JR, Hansen PJ, Buhi WC, 1988. Secretion of proteins by cultured bovine oviducts collected from estrus through early diestrus. J Exp Zool 248:345–53

McCarthy TJ, Boice ML, Fazleabas AT, Verhage HG, 1989. Induction of multiple follicular development and superovulation in the olive baboon, *Papio anubis*. Biol Reprod 40(Suppl 1):358A

Minami N, Bavister BD, Iritani A, 1988. Development of hamster two-cell embryos in the isolated mouse oviduct in organ culture system. Gamete Res 19:235–40

Murray MK, Verhage HG, 1985. The immunocytochemical localization of a cat uterine protein that is estrogen dependent (CUPED). Biol Reprod 32:1229–35

Oikawa T, Sendai Y, Kurata S, Yanagimachi R, 1988. A glycoprotein of oviductal origin alters biochemical properties of the zona pellucida of hamster egg. Gamete Res 19:113–22

Oliphant G, Reynolds AB, Smith PF, Ross PR, Marta JS, 1984. Immunocytochemical localization and determination of hormone-induced synthesis of the sulfated oviductal glycoproteins. Biol Reprod 31:165–74

Oliphant G, Ross PR, 1982. Demonstration of production and isolation of three sulfated glycoproteins from the rabbit oviduct. Biol Reprod 26:537–44

Rexroad CE Jr, Powell AM, 1988. Co-culture of ovine ova with oviductal cells in medium 199. J Anim Sci 66:947–53

Robitaille G, St-Jacques S, Potier M, Bleau G, 1988. Characterization of an oviductal glycoprotein associated with the ovulated hamster oocyte. Biol Reprod 38:687–94

Sakai Y, Araki Y, Yamashita T, Kurata S, Oikawa T, Hiroi M, Sendo F, 1988. Inhibition of in vitro fertilization by a monoclonal antibody reacting with the zona pellucida of the oviductal egg but not with that of the ovarian egg of the golden hamster. J Reprod Immunol 14:177–89

Sathananthan AH, Trunson AO, Wood C, 1986. Atlas of Fine Structure of Human Sperm Penetration, Eggs, and Embryos Cultured In Vitro. New York: Praeger, pp. 26–27

Sternberger LA, Hardy PM Jr, Cueulis JJ, Meyer HG, 1970. The unlabeled antibody enzyme method of immunocytochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase antihorseradish peroxidase) and its use in identification of spirochetes. J Histochem Cytochem 18:315–33

Sutton R, Nancarrow CD, Wallace ALC, 1986. Oestrogen and seasonal effects on the production of an oestrus-associated glycoprotein in oviductal fluid of sheep. J Reprod Fertil 77:645–53

Sutton R, Nancarrow CD, Wallace ALC, Rigby NW, 1984. Identification of an oestrus-associated glycoprotein in oviductal fluid of the sheep. J Reprod Fertil 72:415–22

Talbot P, DiCarlantonio G, 1984a. The oocyte-cumulus complex: ultrastructure of the extracellular matrix in hamsters and mice. Gamete Res 10:127–42

Talbot P, DiCarlantonio G, 1984b. Ultrastructure of the opposum oocyte investing coats and their sensitivity for trypsin and hyaluronidase. Dev Biol 103:159–67

Varndell IM, Polak JM, 1984. Double immunostaining procedures: techniques and applications. In: Varndell IM, Polak JM (eds.), Immunolabeling for Electron Microscopy. Amsterdam: Elsevier Science Publishers, pp. 155–77

Verhage HG, Bareither ML, Jaffe RC, Akbar M, 1979. Cyclic changes in ciliation, secretion, and cell height of the oviductal epithelium in women. Am J Anat 156:505–21

Verhage HG, Boice ML, Mavrogianis PA, Donnelly K, Fazleabas AT, 1989. Immunological characterization and immunocytochemical localization of oviduct-specific glycoproteins in the baboon *(Papio anubis)*. Endocrinology 124:2464–72

Verhage HG, Fazleabas AT, 1988. The in vitro synthesis of estrogen dependent proteins by the baboon *(Papio anubis)* oviduct. Endocrinology 123:552–58

Verhage, HG, Fazleabas AT, Donnelly K, 1988. The in vitro synthesis and release of proteins by the human oviduct. Endocrinology 122:1639–45

Verhage HG, Mavrogianis PA, Boice ML, Li W, Fazleabas AT, 1990. Oviductal epithelium of the baboon: hormonal control and the immunogold localization of oviduct-specific glycoproteins. Am J Anat 187:81–90

Wagh, PV, Lippes J, 1989. Human oviductal fluid proteins. III. Identification and partial purification. Fertil Steril 51:81–88