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Steroid-dependent oviduct secretions in the primate

Secreciones oviductales controladas por esteroides en los primates

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The primate oviduct is a target tissue for the ovarian steroids, and the most sensitive tissue compartment of the oviduct to the changing levels of the ovarian steroids appears to be the epithelium lining the lumen of the oviduct (1, 2). At the time of ovulation, the lining epithelium of the human and baboon oviduct consists of fully differentiated columnar ciliated and secretory cells (Fig. 1). Withdrawal of estradiol and/or the long term treatment with progesterone results in the dedifferentiation of this epithelium and the loss of cilia and secretory function (Fig. 1). In the human, approximately 10% of the ciliated cells in the fimbria and ampulla form new cilia during the follicular phase of the menstrual cycle, whereas the secretory cells undergo a complete cycle of differentiation during the follicular phase and dedifferentiation during the luteal phase (3, 4). These cyclic processes are much more dramatic in the nonhuman primate, especially in the fimbriae and ampulla, where virtually all of the ciliated cells form new cilia during the follicular phase (5-9). In nonhuman primates, the secretory cells are maximally developed during the periovulatory period of the menstrual cycle, and secretory activity is minimal or absent during the late luteal phase (8, 9). Thus, it has been clearly established that estradiol induces hypertrophy, hyperplasia, and the differentiation of the mature ciliated and secretory cell, whereas progesterone, in the presence or absence of estradiol, causes atrophy, deciliation and loss of secretory activity within the primate oviduct.

At the time of ovulation, approximately half of the epithelial cells lining the oviductal lumen are secretory (1, 2). The apical tips of these cells contain secretory granules (Fig. 1), and release of product from these granules by the process of exocytosis has been observed in the cow (10), sheep (11), cat (12), pig-tailed monkey (8) and baboon (9). Thus, the secretory cells of the mammalian oviduct contribute steroid-dependent macromolecules to oviduct fluid, a fluid which provides the optimal environment for the processes associated with fertilization and the development of the early embryo.

Explant culture systems have been successfully used to study the *in vitro* synthesis of proteins from different tissue types (13-15). When using these systems, one assumes that the tissue being cultured will continue to synthesize the same proteins that were being synthesized *in vivo.* We have used such systems in an attempt to detect and identify steroid-dependent secretory macromolecules in human (16) and baboon (14, 17) oviducts, macromolecules which are possibly synthesized by the secretory cells lining the oviductal lumen. Segments of oviducts were obtained from humans and baboons at different reproductive stages, minced, and cultured in the presence of radiolabelled amino acid precursors. Electrophoretic analysis (18) revealed that the pattern of proteins synthesized by both the human (16) and baboon (14, 17) oviduct changes during the menstrual cycle, and in the baboon, the pattern of protein synthesis is also dependent on the regimen of steroid treatment in ovariectomized animals.

Electrophoretic and fluorographic (19, 20) analysis of human oviduct culture media clearly showed one major area of

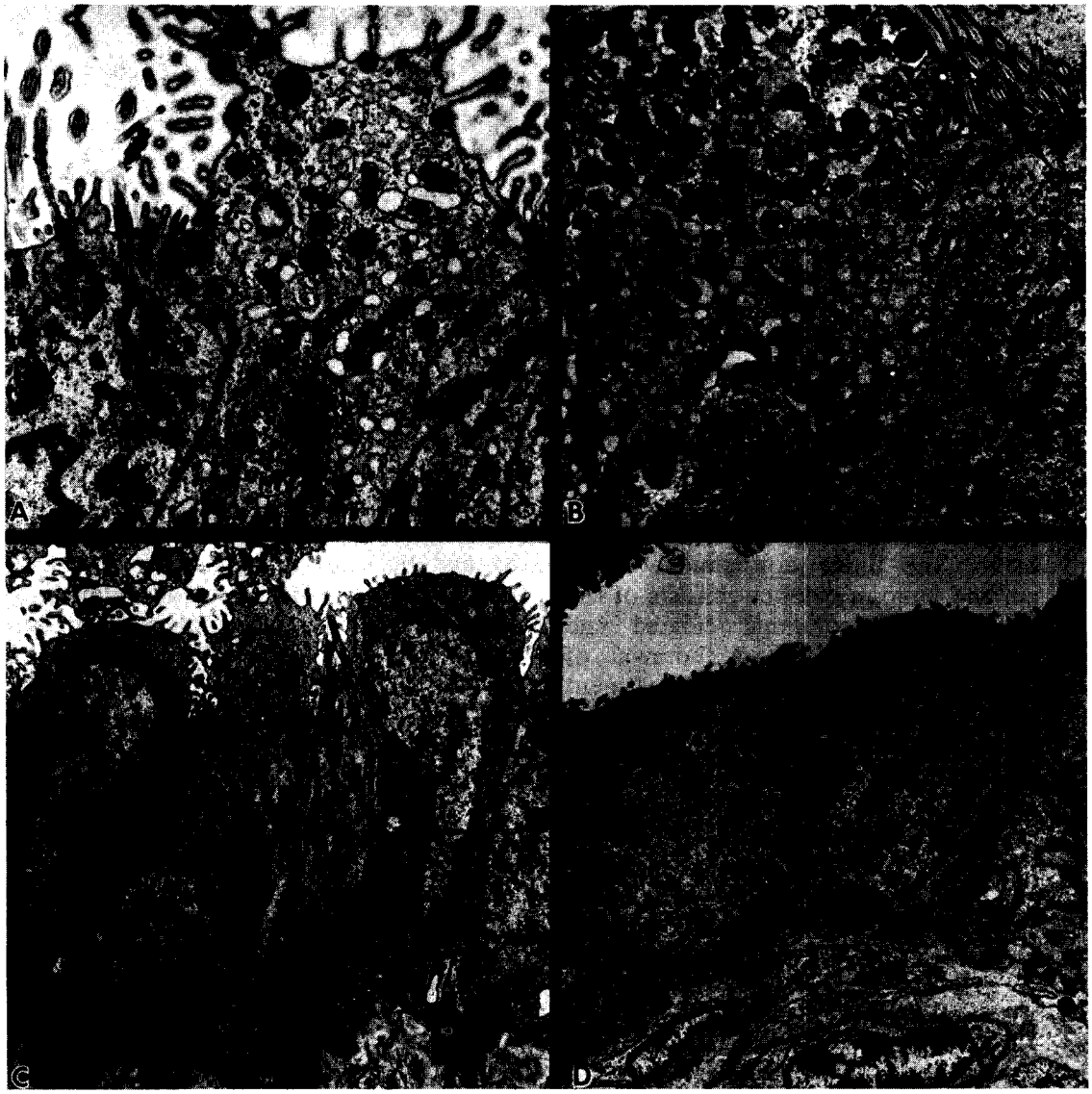

Fig. 1: Comparison of the oviductal epithelium in the human and baboon. Panel A, Ampulla of the human oviduct obtained during the periovulatory period. Note the presence of secretory granules in the apical tip of the secretory cell. x 8,500. Panel B, Ampulla of the baboon oviduct obtained from an estradiol treated animal. x 7,000. Panel C, Ampulla of the human oviduct obtained immediately postpartum. The nonciliated cells lack secretory granules. x 4,000. Panel D, Ampulla of the baboon oviduct obtained from an ovariectomized animal. There is no apparent evidence for secretory activity. x 4,000.

stage-specific label intensification (16). This was the 120,000 M_r region which was intensely labelled in all midcycle oviduct samples when either labelled glucosamine or methionine was included in the culture media (Fig. 2). On 2-D PAGE (21) fluorographs the 120,000 M_r region was resolved into two major glycoproteins, one basic with a pI = 8 and the other acidic with a pI = 4.5. The basic glycoprotein appeared to incorporate more labelled leucine and methionine than the acidic glycoprotein. Labelling in the 120,000 M_r region was either greatly reduced in intensity or absent at all other stages of the menstrual cycle and also in media from postpartum oviducts (Fig. 2).

The electrophoretic analysis of baboon oviduct culture media suggested that the protein synthetic pattern was more com-

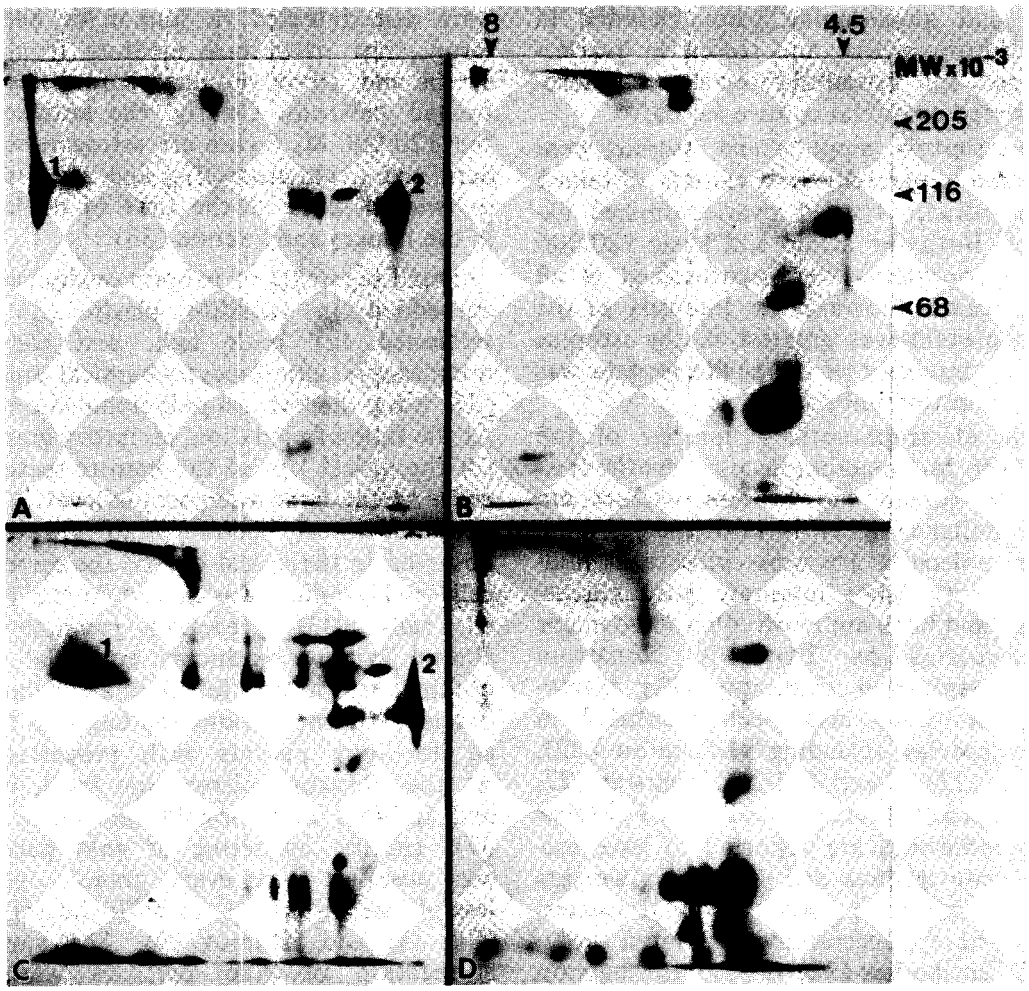

Fig. 2: Fluorographs of dried 2-D gels (7.5%) of culture medium (100,00 cpm) of oviducts. Panel A, Human late follicular stage. Panel B, Human immediately postpartum. Panel C, Baboon late follicular stage. Panel D, Ovariectomized baboon. The basic protein (1) and the acidic protein (2) in the 120 kD region appeared to be estrogen-dependent in both the human and baboon oviduct.

plex than that observed in the human. On 1-D PAGE fluorographs three major regions with M_r's of 160,000, 120,000 and 90,000 showed intense label when radioactive methionine was included in the culture media (17). The 160,000 M_r protein was present during all stages of the menstrual cycle, but appeared to decrease in intensity during the late luteal stage. The 120,000 M_r region was the most intense during the late follicular and mid-luteal stages, forming an intense diffuse band around the time of ovulation. The 90,000 M_r region intensity appeared to be similar to the 160,000 M_r macromolecules. Analysis of culture media from both cycling and ovariectomized steroid-treated animals suggested the 120,000 M_r macromolecules were the most stage-specific and sensitive to the levels of estradiol.

The labelled proteins present in baboon oviduct culture media were also analyzed on 2-D PAGE fluorographs (17). The 1-D 120,000 M_r region was resolved into three major proteins, two of which appeared to be sensitive to the plasma levels of estradiol (Fig. 2). One of these proteins was basic with a pI = 8, and the other acidic with a pI = 4.5. Both regions stained intensely in oviducts obtained from mid-cycle baboons and estradiol-treated ovariectomized baboons when labelled methio-

nine and glucosamine were included in the culture media, and absent in ovariectomized and estradiol-primed, progesterone-treated oviductal culture media.

The fimbria, ampulla and isthmus were analyzed separatelly in oviducts obtained from estradiol-treated ovariectomized baboons. Both the acidic and basic 120,000 M_r glycoproteins were synthesized in all three regions, however the intensity of the basic protein was greatest in the isthmus and the intensity of the acidic protein was greatest in the fimbria.

The electrophoretic properties of the 120,000 M_r macromolecules synthesized by the human and baboon oviduct are very similar (Fig. 2). In both species these macromolecules are most intense at midcycle, both label intensely with glucosamine, and both are resolved into two major glycoproteins on 2-D gels. Nonserum estrus associated oviduct-specific macromolecules have also been identified in other species including the rabbit (22), mouse (23), sheep (24-26), hamster (27, 28), pig (29) and cow (30, 31). All of these macromolecules are reported to have carbohydrate moities and molecular weights characteristics for each individual species.

We have successfully generated polyclonal antibodies (32) toward the baboon oviductal proteins found in the 120,000 M_r region. One of the polyclonal antibodies was generated against the 120,000 M_r region separated on 1-D gels (33). Polyclonal antibodies were also generated toward the acidic and basic components of the 120,000 M_r region separated on 2-D gels. The antibodies against the acidic and basic forms each cross-reacted with both the acidic and basic components suggesting that at the very least, the basic and acidic glycoproteins share antigenic sites, and may be isoelectric variants of the same macromolecule. Western blot analysis (34) of oviductal flushings and culture media obtained from both humans and baboons showed that the 120,000 M_r antigens were only present in oviductal flushings obtained from follicular stage human (unpublished data) and baboon oviducts, and estradiol-treated ovariectomized baboon oviducts (33). These antigens were not detected in human or baboon serum, nor in culture medium from the uterus and cervix obtained from estradiol-treated baboons. Clearly, the antigens in the 120,000 M_r region are estrogen-dependent and oviduct-specific, and are present in oviduct fluid at the time of ovulation in the human and baboon (33).

Small segments of baboon oviducts were embedded in Araldite plastic (3) and sectioned for both light and electron microscopic immunocytochemical analysis (35) using the baboon polyclonal antibody. At the time of ovulation, secretory granules fill the apical tips of the mature secretory cells. At the light microscopic level, immunocytochemical reaction product was only observed in the apical tips of the secretory cells and product was absent in the ciliated cells (33). Reaction product was absent in the oviductal epithelial cells obtained from late luteal phase baboons, ovariectomized baboons and baboons treated for long periods with progesterone. At the electron microscopic level, using and indirect immunogold technique (36, 37), specific clustering of gold particles was observed over every granule regardless of size or density, in both the ampulla (Fig. 3) and isthmus (9). The specific aggregation of gold particles was not observed over any other cytoplasmic compartment or organelle of either the secretory or ciliated cells. The clustering of gold particles over the secretory granules was absent when preimmune serum was substituted for antiserum. A few secretory granules were still present in the isthmus of the oviducts obtained from late luteal phase baboons and from ovariectomized estradiol-primed baboons treated for two weeks with progesterone. The specific clustering of gold particles was also observed over the secretory granules remaining the these progesterone-dominated animals. The immunocytochemical localization of oviduct-specific antigens has also been reported for the mouse (38), hamster (27), rabbit (39) and cow (31). Without exception the oviductal antigens were localized to the secretory granules of the secretory cells. However, some regional variation in the localization of the antigens

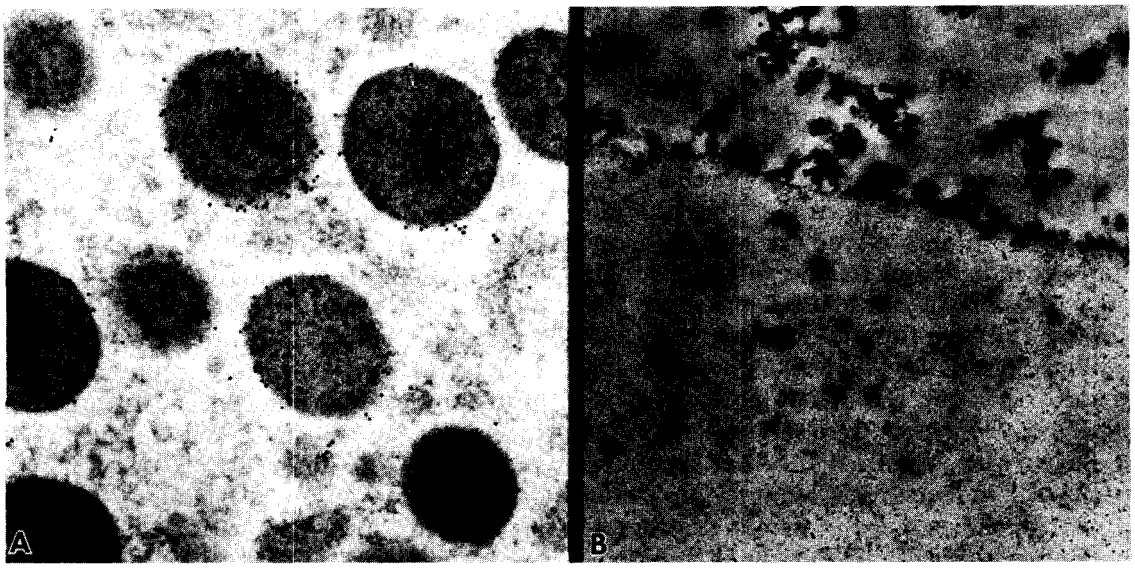

Fig. 3: Panel A, Secretory granules present in the apical tip of oviductal secretory cells obtained from a baboon during the late follicular stage. Note the specific clustering of gold particles over every secretory granule. x 27,000. Panel B, the zona pellucida (zp) and perivitelline space (pv) of a 2-cell baboon ovum. Note the colloidal gold particles within the zona pellucida and associated with flocculent material within the perivitelline space. x 11,000.

has been observed. In the mouse (38), the antigen appeared to be absent in the isthmic secretory cells whereas in the hamster (27) most intense immunoreactive staining was observed in the isthmus.

The oviduct-specific antigens also appear to become associated with oviductal ova and embryos. Ovulated 1-cell baboon ova analyzed with an indirect immunofluorescent assay exhibited intense fluorescence that appeared to be localized within the zona pellucida, whereas ovarian ova showed no localized fluorescence (40). Two-cell and 4-cell embryos collected from superovulated baboons along with a 2-cell embryo obtained from a normally cyclic animal displayed fluorescence associated with the zona that was similar to that seen in 1-cell oviductal ova. When baboon ova and embryos were subjected to the colloidal gold technique and examined with the electron microscope, specific localization of colloidal gold particles was observed in the zona pellucida of all oviductal eggs and embryos (Fig. 3). Gold particles were generally distributed homogeneously throughout the width of the zonae. Frequently flocculent material was present within the perivitelline space. This flocculent amorphous material was heavily immunolabelled as well as the vitelline membrane (Fig. 3). The hamster oviductal antigens (41-46) become associated with the zona pellucida whereas the mouse antigens (38, 47) become sequestered within the perivitelline space. In the sheep (26), the oviductal antigens appear to bind to the zona pellucida, associate with the perivitelline space and may even migrate within the cytoplasm of individual blastomeres. The data demonstrating the association of oviductal antigens with oviductal ova and embryos in the baboon (40), hamster (41-46), mouse (38, 47) and sheep (26) suggest that this may be a general mammalian phenomenon.

In a recent preliminary study we have reported the isolation of a partial cDNA coding for the baboon oviductal glycoprotein from a recombinant cDNA library prepared to poly(A) + RNA isolated from oviducts obtained from estradiol-treated baboons (48). Northern blot hybridization indicated a single message of 2.8 kb was present in oviducts obtained from estradiol-treated baboons and absent in oviducts obtained from ovariectomized baboons and estradiol-primed ovariectomized baboons treated with progesterone. An mRNA of comparable size was also found in oviducts

obtained from periovulatory women. This partial cDNA was sequenced and a search of the GenBank data base revealed no significant degree of homology between the oviductal glycoprotein cDNA and other previously sequenced proteins. This preliminary data suggests that the 120,000 M_r glycoprotein of the baboon is estradiol-dependent and unique to the oviduct, thus supporting our data from more definitive electrophoretic and immunogold studies. Also, it would not be surprising to find that the cDNA coding for the baboon glycoprotein has significant homology with the mRNAS for proteins packaged in secretory granules in other primates, and perhaps in mammals generally. Future studies will determine the extent to which that is true.

It remains to be determined whether the oviduct-specific proteins synthesized by the secretory cells of the oviduct of vertebrate animals play a role in the reproductive process. However, several lines of evidence suggest that may be true. As discussed previously, the hamster oviductal antigens become associated with the zona pellucida. When a monoclonal antibody to the hamster oviductal glycoprotein was tested in an *in vitro* fertilization system, addition of the monoclonal antibody prevented *in vitro* fertilization (49). The monoclonal antibody formed a "zona precipitation band" and inhibited sperm binding to the zona pellucida. Several physiological parameters of the mature ovarian and oviductal oocytes were recently compared in the hamster, and the most significant difference was found in the zona pellucida (27, 50). The zona of the oviductal oocyte was heterogenous in its optical density and had a stronger acrosome reaction-inducing ability than that of the ovarian oocyte. The ovarian oocytes attained these properties when they were cultured in oviductal extracts and oviductal fluid. Perhaps the glycoproteins synthesized by the hamster oviduct are responsible for this physiological difference.

Significantly more sheep (51, 52), cow (53), pig (54, 55) and goat (56) ova or embryos were reported to develop into compact morulae or blastocysts when co-cultured with either minced oviduct tissue, on oviduct tissue monolayers, or in oviduct tissue culture conditioned medium. In humans (57), significantly more fertilized embryos cleaved to the compacted embryo stage when co-cultured with ampullary epithelial cells. Also, none of the co-cultured human embryos showed unequal-sized blastomeres whereas approximately one-fourth of the controls did. This effect of co-culture on embryonic development does not appear to be species specific, since hamster 2-cell embryos (58) and pig 1-cell embryos (59) were successfully cultured in mouse oviducts maintained in organ culture. However, this effect does appear to be specific for oviduct tissue since ova or embryo development when co-cultured with other somatic cell types was not different from those cultured in control media (51, 54). Collectively, these studies suggest that some oviductal factor(s) enhances *in vitro* embryonic development.

The oviductal sulfated glycoproteins of the rabbit have been purified and are known to be localized within the secretory granules found in the apical tips of the secretory cells. Based on data from *in vitro* studies, it is suggested that this protein may play a role in the regulation of the maternal immune system by suppressing complement-mediated cellular lysis in the oviduct (60). Since. sperm, the fertilized egg and the early embryo all express paternal antigens, some oviductal component which inhibits the classical pathway of complement activation may have physiological relevance. This topic is addressed in more detail by Thomas *et al.* in this volume.

The lower isthmus is thought to be the functional reservoir for the fertilizing pool of sperm, at least in domestic animals (61), and oviductal glycoproteins may be critical for the final events associated with maintaining these sperm and their ability to fertilize an egg. In the human, a 54,000 M_r oviductal glycoprotein has been reported to bind to the head of the human sperm (62, 63). These investigators also reported that sperm placed in periovulatory or

preovulatory human oviductal fluid displayed a marked increase in motility. In sheep, spermatozoa incubated with oviductal fluid lose specific membrane macromolecules and selectively absorb high molecular weight macromolecules (64, 65). Thus, one can postulate that during sperm sequestration within the lower isthmus, oviductal glycoproteins may affect motility, the acrosome reaction, and/or the final events associated with fertilization.

Thus, many studies suggest that some factor of oviductal origin enhances oocyte maturation, early embryonic development and/or sperm capacitation. We now know that the mammalian oviduct synthesizes and secretes a major periovulatory glycoprotein. Future research will show whether that glycoprotein may be the factor playing a key role in the oviductal stages of reproduction, or whether some as yet unidentified factor may be responsible for the reported enhancement of embryonic development.

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