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Identification and Characterization of De Novo-Synthesized Porcine Oviductal Secretory Proteins[1]

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ABSTRACT

Oviductal secretory products provide a biochemical environment important for establishment of pregnancy. A previous study identified three de novo-synthesized glycoproteins by one-dimensional SDS-PAGE as well as increased incorporation of [^{3}H]Leu into secretory protein by whole oviduct and ampulla associated with proestrus, estrus, and metestrus only. Here, our objective was to further identify and characterize oviductal secretory proteins, specifically 115 000- and 85 000-M_r estrus-associated proteins (EAP). Two-dimensional SDS-PAGE resolved the 115 000-M_r protein into two proteins of 100 000 M_r, one basic and one acidic, and the 85 000-M_r protein into 75 000- and 85 000-M_r species (pI < 4.0). Differential secretion of proteins between ampulla and isthmus was indicated. The 100 000-, 75 000-, and 85 000-M_r proteins were synthesized by ampulla during estrus but not by isthmus nor by uterine endometrium. De novo-synthesized EAP were labeled with glucosamine, Leu, and Met, and the 75 000–85 000-M_r proteins from ampulla and a 30 000-M_r family from isthmus were labeled with fucose. Inorganic [^{35}S]sulfate labeled three EAP. Fractionation of culture medium by gel filtration demonstrated differences between products secreted by ampulla and isthmus and suggested that some EAP may be found as high-molecular weight forms in the native state. Results indicate that porcine oviductal tissue synthesizes specific EAP at the time of fertilization and early cleavage-stage embryonic development, that there are differences in the type and distribution of glycoproteins from ampulla and isthmus, and that post-translational modifications occur with the addition of glucosamine, fucose, and inorganic sulfate.

INTRODUCTION

The mammalian oviduct and its secretions provide a biochemical environment that facilitates or is essential for the establishment of pregnancy. This environment, in addition to the transport of gametes and embryos, allows capacitation of sperm, fertilization, and early cleavage-stage embryonic development. Oviductal epithelium [1], protein composition of oviductal fluids [2], and oviductal fluid volume [3] vary according to the hormonal status of the female and appear to be controlled by ovarian estrogen and progesterone. Proteins in oviductal fluid, while having similarities to serum, also include proteins assumed to be secretory products of the epithelium since they are not found in homologous serum. Recent studies have established that some oviductal secretory proteins (OSP) are de novo-synthesized and released. These specific de novo-synthesized OSP have been identified, defined, and described through use of radiolabeled precursors, explant culture, and one- (1D) and/or two-dimensional (2D) PAGE in swine [4, 5], cattle [6, 7], sheep [8], baboons [9, 10], and humans [11, 12]. Similar analyses using epithelial cell cultures have been performed in sheep [13] and rabbits [14, 15], and immunological localization of OSP in secretory epithelium has been demonstrated in rabbits [15], mice [16], hamsters [17], and baboons [18]. These studies suggest that OSP are produced in response to endogenous estrogen at estrus, during the follicular phase, or in ovariectomized (OVX) estrogen-treated animals. A study by Verhage and Fazleabas [10] showed that OVX estrogen-treated baboons produced two specific proteins and a third protein appeared to be enhanced by estrogen compared to control and progesterone-treated baboons. Together these studies identifying stage-specific proteins suggest that such proteins may influence or mediate the establishment or maintenance of pregnancy. Some studies have suggested distinct biochemical functions and ultrastructural differences between the different functional segments—the ampulla and isthmus. Differences have been described for rates of incorporation of radiolabeled precursors into OSP by ampullar and isthmic tissue from cyclic gilts [5] and rabbits [19, 20]. Studies have also reported differences in binding of lectins in mouse oviduct [21], changes in the presence of mucopolysaccharides in rabbit oviduct [22], the presence of biochemically distinct epithelial secretory product from ampulla but not isthmus in mice [16], and changes in electron density of secretory sulpho-granules in nonciliated epithelium of ampulla and isthmus [23]. Further, although estrogen-treated baboon oviduct produced three specific glycoproteins in fimbria, ampulla, and isthmus, there appears to be a synthetic gradient for their production with one protein greater in the isthmus and lowest in the fimbria and a second protein highest in the fimbia and lowest in the isthmus [10].

This study was designed to further identify and characterize total OSP, but more specifically to describe the 115 000-M_r and 85 000-M_r glycoproteins, their distribution and

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synthesis by porcine oviduct and by ampulla and isthmus during the estrous cycle. These studies are important for understanding the function of the oviduct and its functional segments.

MATERIALS AND METHODS

Materials

Acrylamide, *N, N'*-diallyltartardiamide, urea, and SDS were obtained from Gallard-Schlesinger, Carle Place, NY. *N,N,N',N'*-tetramethyl ethylenediamine and X-Omat AR film (XAR-5) were products of Eastman-Kodak, Rochester, NY. Amino acids and protein standards were purchased from Sigma Chemical, St. Louis, MO. All supplies and reagents for gel electrophoresis and chromatography were purchased from either Bio-Rad Laboratories, Richmond, CA, or Fisher Scientific, Orlando, FL. L-[4,5-^{3}H]leucine (sp. act. 120 Ci/mmol), D-[6-^{3}H]glucosamine-HCl (sp. act. 22 Ci/mmol), D-[2-^{3}H]mannose (sp. act. 20 Ci/mmol), L-[6-^{3}H]fucose (sp. act. 90 Ci/mmol), and L-[^{35}S]methionine (sp. act. 194 mCi/mmol on reference date) were obtained from Amersham Corporation, Arlington Heights, IL: Inorganic [^{35}S]sulfate (sp. act. 250–1 000 mCi/mmol) was obtained from DuPont Company, NEN Research Products, Boston, MA: Ampholines were from Pharmacia-LKB, Piscataway, NJ; and medium and culture supplies were purchased from GIBCO, Grand Island, NY.

Animals

Sexually mature, crossbred gilts were observed daily for behavioral estrus in the presence of intact boars, and the first day of estrus was designated Day 0. Gilts were assigned randomly to specific days of the estrous cycle for subsequent surgery and tissue collection. Day of estrous cycle, number of gilts/day, and number of cultures/day from which tissue was obtained and culture medium was examined are as previously described [5].

Preparation of Media

Eagle's leucine- and methionine-deficient (modified) minimum essential medium (MEM) was prepared according to the method of Basha et al. [24] and supplemented as previously described [5]. Content of L-leucine (Leu) and L-methionine (Met) was limited to 5.2 μg/ml and 1.5 μg/ml (0.1 normal), respectively, to enhance uptake of L-[^{3}H]-leucine ([^{3}H]Leu) and L-[^{35}S]methionine ([^{35}S]Met) added to oviductal explant cultures. For fucose, mannose, and glucosamine experiments, MEM was prepared as above except that it was not deficient in amino acids.

Collection of Oviductal Tissue

Gilts were subjected to surgery, and oviductal tissue was collected and prepared as previously described [5].

Oviductal Flushing

Before oviducts were dissected and collected, a surgical needle with a cutting edge was used to make a small incision in an avascular area of the isthmus above the tubouterine junction. A polyvinyl catheter (i.d. = 1.25 mm) was inserted 1–2 cm towards the ampulla. The infundibulum was clamped, and 10 ml 0.15 M NaCl was introduced at the infundibulum with a 10 ml syringe (20-gauge needle), massaged towards the isthmic catheter, and collected in a sterile bottle. The fluids were centrifuged (2 200 × *g*, 10 min, 4°C), and the supernatant was dialyzed and then stored frozen (−20°C) until analyzed. Protein concentrations of flushings were determined by the Bio-Rad microassay (Bio-Rad Laboratories), and aliquots of 500 μg were used for 2D-SDS-PAGE and silver staining.

In Vitro Oviductal Explant Cultures

For whole oviductal cultures, each oviduct was opened longitudinally, cut lengthwise again to provide two equal segments, and rinsed in MEM. For ampullar and isthmic tissues, the locations of the ampulla and isthmus was determined by gross examination, and the intervening sequence was cut out and discarded. The ampulla was treated as described for whole oviducts. The isthmus, because of its small size, was simply opened longitudinally and cultured. Cultures were carried out as described by Basha et al. [24]. Briefly, 500 mg whole oviductal tissue, ampulla, or isthmus was transferred into sterile 100-mm Petri dishes and cut into 1–3-mm^3 pieces. Either (1) modified MEM (15 ml) containing 100 μCi[^{3}H]Leu or 25–40 μCi[^{35}S]Met; or (2) complete MEM containing 50–100 μCi D-[2-^{3}H]mannose, L-[6-^{3}H]fucose, or D-[6-^{3}H]glucosamine; or (3) complete MEM containing 220 μCi inorganic [^{35}S]sulfate was added to each dish and transferred to a controlled atmosphere chamber (Bellco Biological Glassware, Vineland, NJ). Explants were gassed and incubated for 24 h, and tissues and supernatant were separated and treated as previously reported [5].

A histological comparison of tissue collected both before and after explant culture was made. Oviductal tissue was taken at surgery and after 24 h of culture, fixed in Bouin's fluid, embedded in paraffin, and stained with hematoxylin and eosin. Tissue appeared normal after 24 h of culture when compared to the noncultured specimen (data not shown).

De Novo Protein Synthesis and Secretion

The secretion of de novo-synthesized polypeptides into culture medium by oviductal tissue was determined by measuring incorporation of [^{3}H]Leu into nondialyzable macromolecules after a 24-h incubation. After cultures were terminated, medium was extensively dialyzed (molecular weight cut-off = 3 500) against 10 mM Tris-HCl buffer, pH 8.2 (two changes of 4 liters/24 h), and then against deionized water (two changes of 4 liters each); and radioactivity

of the retentate was determined for nondialyzable macromolecules. Dialyzed culture medium (100 μl) was mixed with 300 μl distilled water, 4 ml scintillation fluid was added, and radioactivity was determined by scintillation spectrometry. Incorporation was defined as dpm nondialyzable macromolecules/mg wet tissue.

Preparation of Samples for Electrophoresis

Aliquots of dialyzed conditioned culture medium or oviductal flushings were lyophilized for 2D-SDS-PAGE. These pellets were then dissolved in 5 mM K_2CO_3 solubilization buffer containing 9.4 M urea, 2% (v/v) Nonidet P-40, and 0.5% (w/v) dithiothreitol.

2D-SDS-PAGE

All conditioned culture media and oviductal flushings were analyzed as individual specimens by 2D-SDS-PAGE [25] to resolve acidic proteins (pI < 8.0) by isoelectric focusing in the first dimension and by 10% SDS-polyacrylamide slab gels in the second dimension. Gels were fixed, stained with Coomassie Brilliant Blue dye, destained, and photographed; those containing radiolabeled proteins were washed in water, soaked in 1 M sodium salicylate, and dried. Fluorographs were prepared by exposing the dried gels at −70°C to Kodak XAR-5 film as previously described [26]. Comparisons were made only between fluorographs containing the same amount of radioactivity and exposed for the same time period. Gels containing nonlabeled proteins from oviductal flushings were equilibrated with 50% (v/v) methanol and stained with silver [27].

Protein Fractionation

Aliquots of radiolabeled nondialyzable macromolecules from explant culture media were subjected to gel filtration chromatography on Sepharose CL-6B (1.8 × 92 cm) previously equilibrated in 10 mM Tris-HCl/0.4 M NaCl (pH 7.5) at 4°C. Protein standards for calibration included thyroglobulin, apoferritin, transferrin, ovalbumin, cytochrome C, and [^{3}H]Leu. Elution profiles were generated by determining the radioactivity in 100–400-μl aliquots of each effluent fraction by liquid scintillation spectrometry.

RESULTS

Culture Media Conditioned by Total Oviductal Tissue

Representative fluorographs from 2D-SDS-PAGE analysis of proteins de novo-synthesized and secreted by whole oviductal tissue (ampulla plus isthmus) in explant culture media from gilts on Days 0–20 of the estrous cycle are shown in Figure 1. Previous 1D-SDS-PAGE analysis [5] indicated the presence of three major glycoproteins of M_r 335 000, 115 000, and 85 000 during proestrus, estrus, and metestrus. One objective of this study was to further characterize specifically the 115 000-M_r and 85 000-M_r major proteins with respect to isoelectric point, subunits, and presence of other proteins of similar molecular weight. The 1D-PAGE band of 115 000 M_r was resolved into several different heterogeneous proteins including a major acidic protein of M_r 100 000 (Fig. 1, Arrow 2). When gels were exposed for short periods of time, this protein was found to be composed of at least three different isoelectric species (data not shown). There were also a slightly acidic minor protein (M_r 100 000, pI 6.0; Fig. 1, Arrow 5), and a major basic protein (M_r 100 000, pI > 8; Fig. 1, Arrow 3). Only a fraction of the 335 000-M_r protein band (pI 6.0) appeared to enter the 10% running gel (Fig. 1, Arrow 4), suggesting that the majority of this protein was retained in the stacking gel. The 85 000-M_r band identified by 1D-SDS-PAGE [5] was resolved into two molecular-weight species of about 75 000 and 85 000 M_r, both with pI < 4 (Fig. 1, Arrow 1; seen more clearly in Fig. 3A). This group of proteins was detected only on Days 0–4 and Days 18–20 of the cycle when the estrogen:progesterone ratio is high.

The 2D-SDS-PAGE analyses further resolved other de novo-synthesized OSP described by Buhi et al. [5]. The 43 000–46 000-M_r 1D bands separated into two and possibly three heterogeneous protein families (Fig. 1, Arrow 6). These increased in intensity after metestrus, became most intense during diestrus, and then decreased during proestrus, i.e., Days 18–20. The 60 000-M_r protein band appeared to be composed of three isoelectric subunits (pI ≤ 7), the intensity of which was low during estrus and increased during diestrus (Fig. 1, Arrow 7). Two other protein groups, a basic 30 000-M_r family (Fig. 1, Bracket 8) and a 30 000–40 000-M_r complex (Fig. 1, Bracket 9) were present throughout the cycle but with some slight modulation by ovarian steroids, increasing during diestrus.

Cell-free oviductal flushings from gilts on Days 1 and 8 of the estrous cycle were analyzed by 2D-SDS-PAGE to identify estrus-associated proteins (EAP). As shown in Figure 2, the three major EAP were detected in oviductal fluid collected on Day 1, but not on Day 8, of the estrous cycle.

Culture Media Conditioned by Oviductal Ampulla

To determine whether two distinct functional regions of the oviduct synthesize similar or different secretory proteins during the estrous cycle, ampullar and isthmic tissues were cultured separately, and explant culture media were then analyzed. Representative fluorographs of 2D-SDS-PAGE analyses of secreted proteins found in explant culture medium conditioned by ampullar tissue cultured in the presence of [^{3}H]Leu are shown in Figures 3A and 3B. Although ampullar tissues from throughout the estrous cycle were studied, only results from Day 1 (estrus) and Day 15 (diestrus) explant cultures are shown. On Day 1, the major proteins included the 100 000-M_r acidic protein (Fig. 3A, Arrow 2), which consisted of several subunits: the acidic 75 000–85 000-M_r subunits, the basic 100 000-M_r protein, and an acidic complex (M_r 28 000–30 000) that appeared intermit-

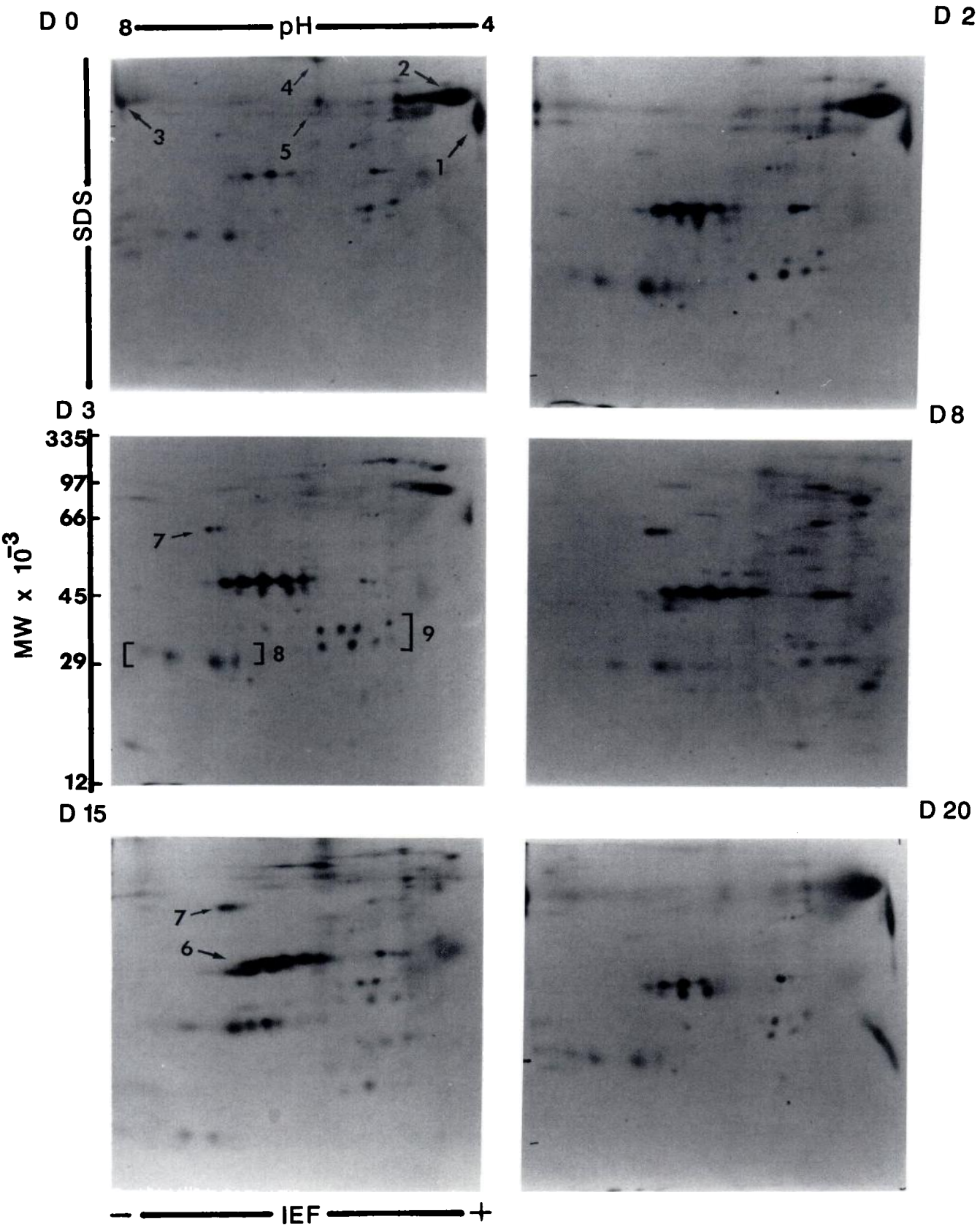

FIG. 1. Representative fluorographs from 2D-SDS-PAGE analysis of explant culture medium (25 000 cpm) conditioned by total oviductal tissue taken from gilts on Days 0, 2, 3, 8, 15, and 20 of the estrous cycle. Major [^{3}H]Leu-labeled proteins are indicated by Arrows 1–7 and Brackets 8 and 9.

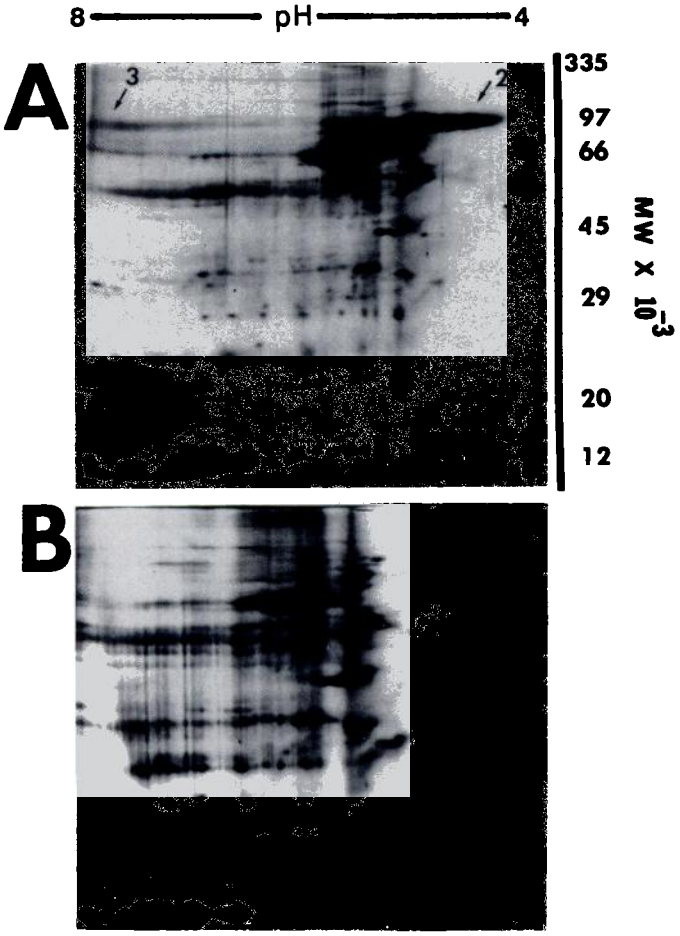

FIG. 2. Two-dimensional polyacrylamide gels of oviductal flushings (500 μg protein/gel) stained with silver from gilts on (A) Day 1 and (B) Day 8 of the estrous cycle. Note the major estrus-associated proteins indicated by arrows during estrus (Day 1), but not diestrus (Day 8), which correspond to those in *Figure 1*, Arrows 1, 2, and 3.

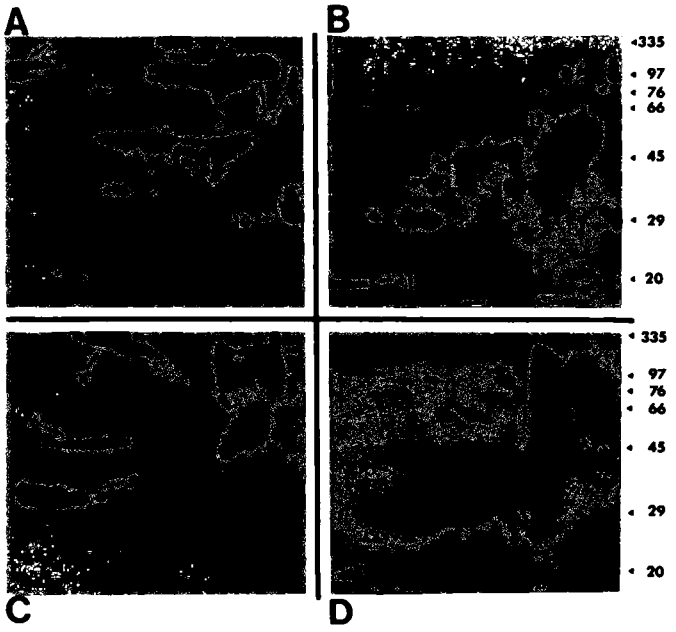

FIG. 3. Representative fluorographs from 2D-SDS-PAGE analysis of explant culture medium (100 000 cpm) conditioned by ampulla (A, B) and isthmic (C, D) tissue from porcine oviducts taken on Day 1 (A, C) and Day 15 (B, D). Major [^{3}H]Leu-labeled proteins are indicated by arrows.

tently and was made up of at least three subunits as determined by short autoradiographic exposures (Fig. 3A, Arrows 1, 3, and 10, respectively). This complex also appeared very faintly in whole oviductal explant medium. The 335 000-M_r protein appeared as a relatively minor protein here. By Day 15, EAP were no longer present (Fig. 3B) but a basic 20 000-M_r protein and a 45 000–50 000-M_r acidic protein complex were noted (Arrows 11 and 12, respectively). An acidic protein (arrowhead) was present, with an isoelectric point similar to that of Protein 1 (Fig. 3A) but having an M_r of >95 000. Small amounts of the 28 000–30 000-M_r acidic complex were also present on Day 15.

Culture Media Conditioned by Isthmic Tissue

Representative fluorographs of 2D-SDS-PAGE analyses of proteins synthesized de novo and secreted into medium by oviductal isthmic tissue on Day 1 and Day 15 of the estrous cycle are presented in Figures 3C and 3D, respectively. On Day 1, the major proteins included the 335 000-M_r protein, whose migration appeared to be slightly retarded in the isoelectric dimension; the 43 000–46 000-M_r heterogeneous protein families; and the basic 30 000-M_r protein family (Fig. 3C, Arrows 4, 6, and 8, respectively). An acidic protein (arrowhead) with an isoelectric point similar to that of Protein 2 (Fig. 3A) but a molecular weight in excess of 100 000 was also present. By Day 15, the 43 000–46 000-M_r proteins were more dominant; the 335 000-M_r protein was absent, and the basic 30 000-M_r protein family was much less significant.

Fractionation of Oviductal Culture Media

Fractionation on a Sepharose CL-6B column of explant culture media conditioned by total oviductal tissue resulted in four peaks of radioactivity. Considerable radioactivity eluted at the void volume (Peak 1) when media conditioned by tissue taken at estrus, metestrus, and proestrus, Days 0–3 and 20 (Fig. 4), were analyzed. This peak, with a molecular weight of several million, was present in media from all days of the estrous cycle, but appeared to vary with the stage of the cycle. As much as 20–40% of total incorporated [^{3}H]Leu was found in Peak 1 on Days 0–3 and Day 20 compared to 4–9% on Days 4–15 (Fig. 4; Table 1).

The broad second peak consisted of two major overlapping peaks (Peaks 2 and 3) with an estimated molecular weight ranging from 669 000 to 12 500. Similar to Peak 1, radioactivity in Peak 2 was highest during estrus and proestrus and lowest during diestrus (Fig. 4). Radioactivity in Peak 3 appeared constant throughout the cycle. Radioactivity in Peak 4 appeared to be associated with low-molecular-weight polypeptides or free label.

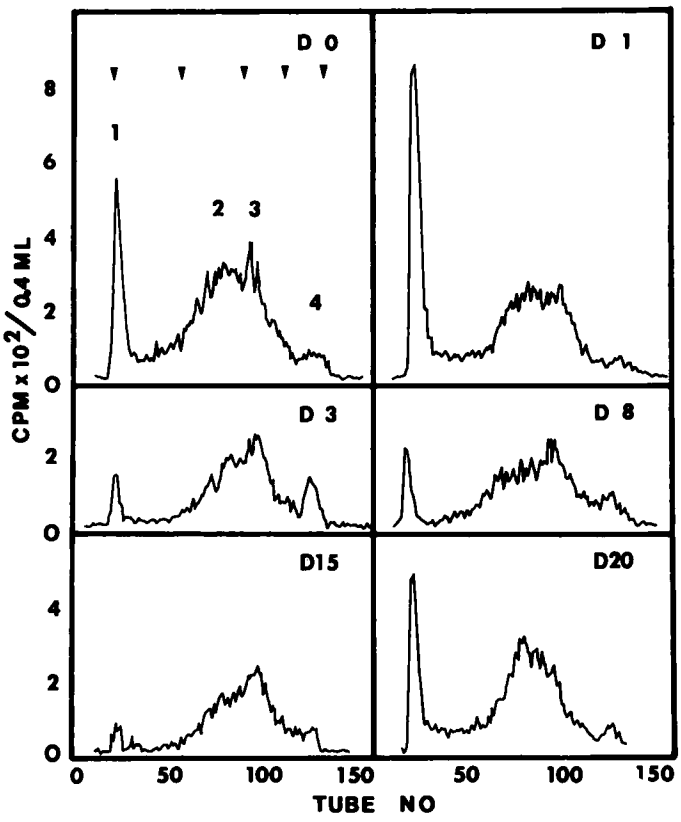

FIG. 4. Fractionation of oviductal explant culture medium labeled with [^{3}H]Leu by Sepharose CL-6B. Tissues cultured were taken from gilts on Days 0, 1, 3, 8, 15, and 20 of the estrous cycle. Eluted fractions are subdivided into four peaks; Peak 1 corresponds to the void volume. Arrowheads shown in DO panel are standards (left to right): blue dextran (2 × 10^6), thyroglobulin (665 000), ovalbulmin (45 000), cytochrome C (12 500) and [^{3}H]leucine (131).

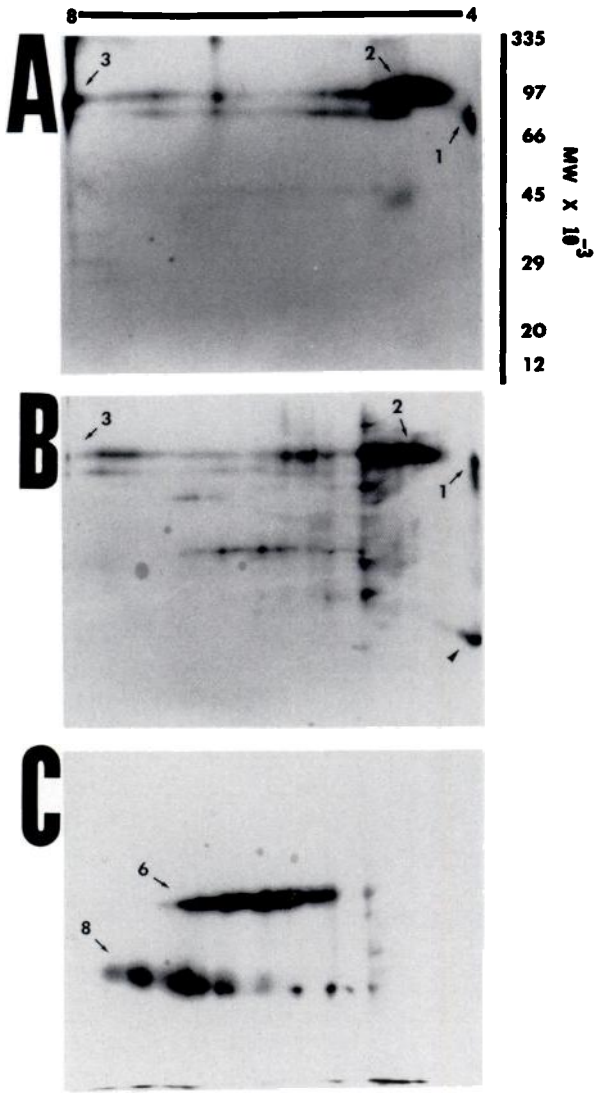

FIG. 5. Representative fluorographs from 2D-SDS-PAGE analysis of pooled fractions (100 000 cpm) (A) Peak 1, (B) Peak 2, and (C) Peak 3 from Sepharose CL-6B fractionation of explant culture medium conditioned by oviductal tissue collected from a gilt on Day 1 of the estrous cycle (see *Figure 4*). Arrows 1, 2, and 3 indicate estrus-associated proteins as identified in *Figure 1* labeled de novo with [^{3}H]Leu.

Pooled fractions from Sepharose CL-6B separations of oviductal explant culture media were analyzed by 2D-SDS-PAGE and fluorography (see Fig. 5). The major radiolabeled EAP (Arrows 1–3, Fig. 1) were present in Peaks 1 and 2 (Fig. 5A and 5B). The presence of these three proteins in both peaks suggests that some portions of these glycoproteins are present in culture medium (native state) in a higher molecular weight, undissociated complex state. The 28 000–30 000-M_r acidic complex (arrowhead) found in the ampulla (Fig. 2A, Arrow 10) was also present in Peak 2. The 335 000-M_r protein, when detected, was present in Peak 1. Peak 3 (Fig. 5C) contained the radiolabeled, basic 30 000-M_r and the 43 000–46 000-M_r families of proteins.

Fractionation of explant culture media from ampullar and isthmic tissue by gel filtration resulted in elution patterns represented in Figure 6. The elution pattern of radioactivity for medium conditioned by ampullar tissue (Day 1 of estrus) was similar to that for total oviductal tissue taken at

TABLE 1. Percent [^{3}H]Leu incorporated into Peak 1 macromolecules from explant culture media on different days of the estrous cycle fractionated by Sepharose CL-6B.

	Day of the estrous cycle										
Parameter	0	1	2	3	4	8	10	12	15	18	20
Mean[a]	18.7	27.1	16.8	14.3	7.5	7.1	8.0	6.0	5.4	13.8	20.2
SEM	2.2	2.8	2.9	5.0	1.3	0.8	2.0	1.3	0.3	1.5	—
N[b]	7	7	6	5	4	4	3	4	2	3	1

[a]Mean percent radioactivity in Peak 1.
[b]Number of individual gilts from which a single explant culture medium was examined.

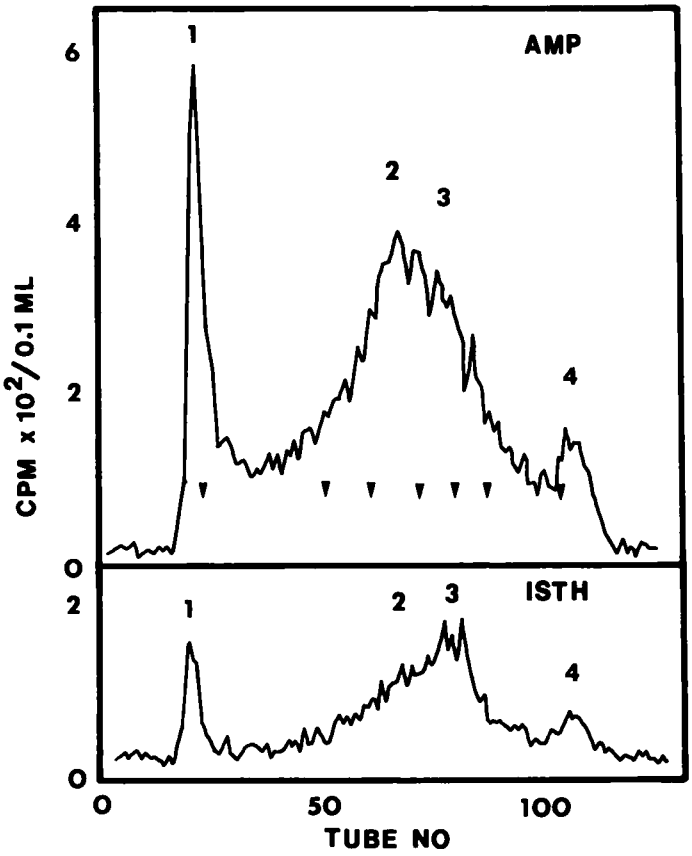

FIG. 6. Sepharose CL-6B fractionation of explant culture medium conditioned by ampullar and isthmic tissue from a gilt on Day 1 of the estrous cycle and labeled with [^{3}H]Leu. Peaks 1, 2, 3, and 4 correspond to those identified in *Figure 4*. Arrowheads represent standards (left to right): blue dextran (2 × 10^6), thyroglobulin (665 000), apoferritin (443 000), transferrin (76 000), ovalbumin (45 000), cytochrome C (12 500), and [^{3}H]leucine (131).

estrus (Fig. 4), whereas the pattern for isthmus (Day 1 of estrus)-conditioned medium indicated diminished Peaks 1 and 2 similar to those detected for whole oviduct during diestrus (Day 8 and Day 15). Peak 1 contained about 20% of total incorporated label for ampullar tissue compared to about 10% for isthmic tissue. Further, Peak 1 from ampulla culture medium contained nearly 7-fold more radioactivity than that from isthmus culture medium. Gel filtration results substantiated those from 2D-SDS-PAGE analyses of ampulla and isthmus culture media (Figs. 3A and 3C). That is, the ampulla produced the majority of the high molecular weight proteins compared to the isthmus except for the 335 000-M_r protein; and the major EAP, as defined as those found in total oviductal explant culture medium (see Fig. 1), were found in the ampulla in Peaks 1 and 2 after Sepharose CL-6B.

Labeled Precursors

Various radiolabeled precursors were used to define protein and carbohydrate composition, post-translational modifications, and distribution of de novo synthesized and secreted proteins from the ampulla and isthmus. The major EAP produced by the ampulla and isthmus incorporated [^{3}H]Leu (Fig. 3), [^{35}S]Met (Figs. 7A and 7B), and [^{3}H]-glucosamine (Figs. 8A and 8B), suggesting that all were glycoproteins. Tritiated fucose was incorporated primarily into the 75 000–85 000-M_r acidic proteins (Fig. 9A, Arrow 1) from ampullar tissue and the basic 30 000-M_r family (Arrow 8) from isthmic tissue (see Fig. 3). Other proteins were present that appeared to contain lower amounts of labeled fucose. Inorganic ^{35}S-sulfate was incorporated into the acidic 75 000–85 000-M_r, the basic 100 000-M_r, and the 335 000-M_r proteins (Fig. 9B, Arrows 1,3, and 4, respectively). Gel filtration (Sepharose CL-6B) fractionation of [^{35}S]sulfate-labeled macromolecules in oviductal culture medium indicated that the majority of [^{35}S]sulfate was in Peak 1 (data not shown). Incorporation of [^{3}H]mannose into secreted proteins was not detected as determined by 2D-SDS-PAGE and fluorography.

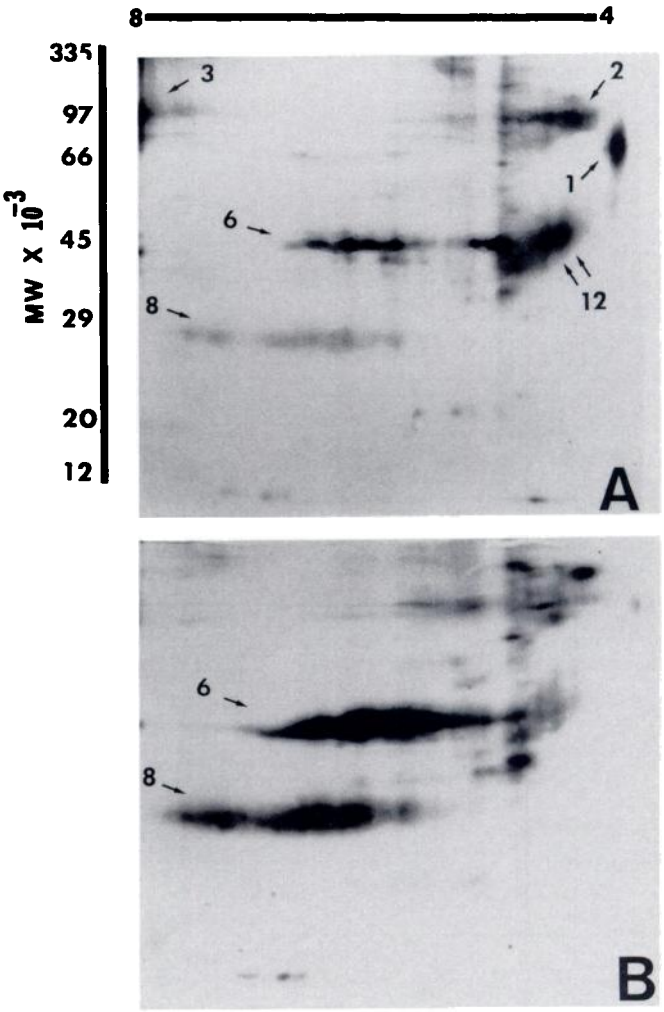

FIG. 7. Representative fluorographs from 2D-SDS-PAGE analysis of explant culture medium (100 000 cpm) conditioned by (A) ampullary and (B) isthmic tissue from porcine oviducts taken on Day 1 of the estrous cycle. Proteins labeled with [^{35}S]Met are indicated by numbers corresponding to those in *Figures 1* and *3*.

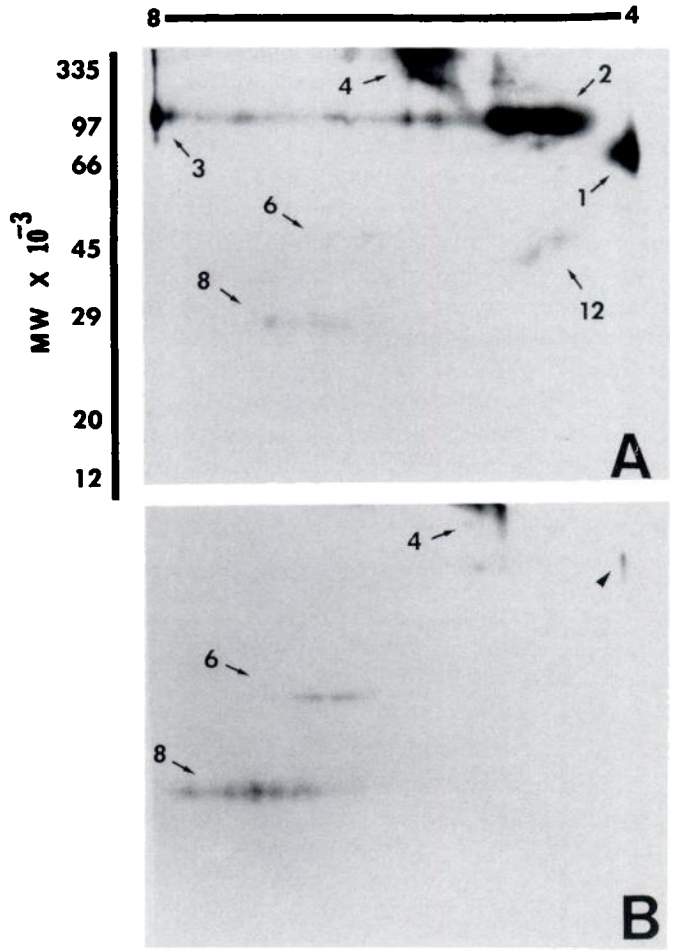

FIG. 8. Representative fluorographs from 2D-SDS-PAGE analysis of explant culture medium (100 000 cpm) conditioned by (A) ampullary and (B) isthmic tissue from porcine oviduct taken on Day 1 of the estrous cycle. Proteins labeled with [^{3}H]glucosamine are indicated by numbers corresponding to those in *Figures 1* and *3*. The arrowhead in (B) indicates a protein with an isoelectric point similar to that of Protein 1 but which has a higher molecular weight M_r (>95 000 M_r).

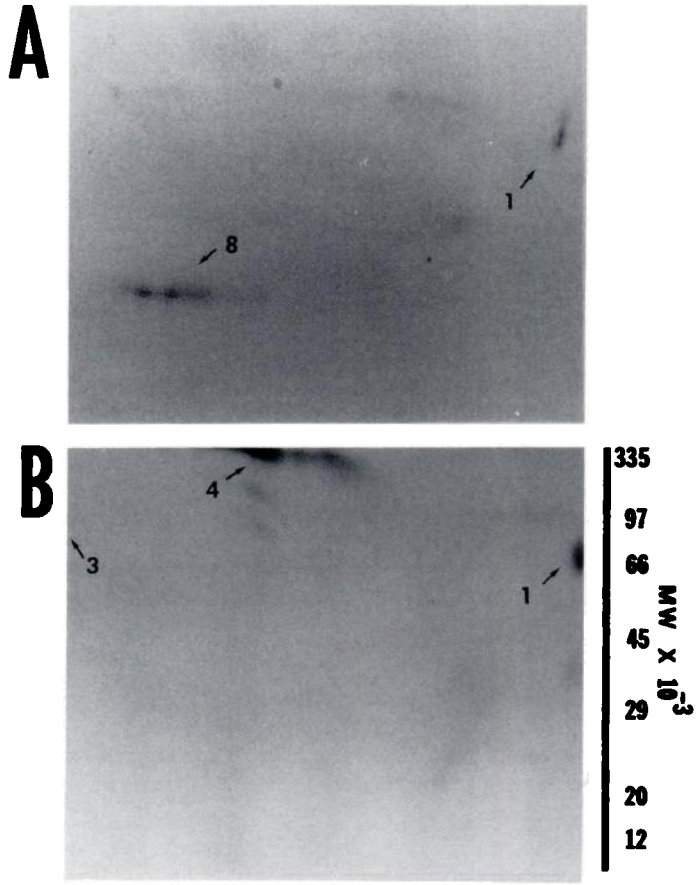

FIG. 9. Fluorographs representing 2D-SDS-PAGE analysis of explant culture medium (100 000 cpm ^{3}H and 5 000 cpm ^{35}S) from porcine oviductal tissue collected on Day 1 of the estrous cycle. Tissues were cultured in the presence of (A) ^{3}H-fucose or (B) inorganic [^{35}S]sulfate. Arrows 1 and 8 in (A) indicate major fucose-labeled proteins and arrows 1, 3, and 4 in (B) represent proteins modified by addition of [^{35}S]sulfate.

Uterine Endometrial Explant Cultures

Proteins synthesized de novo and secreted into explant culture medium by uterine endometrium from estrous gilts were examined by 2D-SDS-PAGE. Oviductal EAP did not appear to be synthesized and secreted by uterine endometrium. However, minor acidic proteins of 30 000–40 000 M_r found in oviductal culture medium were similar to a prominent group of proteins in the uterine endometrial-conditioned culture medium (data not shown).

DISCUSSION

Analysis of porcine OSP by 1D-SDS-PAGE in a previous study [5] indicated synthesis and secretion of three major glycoproteins with molecular weights of 335 000, 115 000, and 85 000 during proestrus, estrus, and metestrus. In this study, high-resolution 2D-SDS-PAGE was used to further resolve isoelectric and molecular-weight variants of radiolabeled complex secretory proteins initially detected by 1D-SDS-PAGE. The major 115 000-M_r band was clearly resolved into two major glycoproteins, one basic (M_r 100 000, pI > 8) and one acidic (M_r 100 000, pI 4.5–5.5). The more dominant acidic protein was present as three or more isoelectric species. The basic and acidic proteins had electrophoretic properties (pI and M_r) similar to those of a glycoprotein produced by cultured oviductal epithelium [13] and oviductal explants [8] of sheep and present in oviductal fluid of sheep [8, 28]. The 85 000-M_r protein was resolved into two very acidic (pI < 4.0) proteins with molecular weights between 75 000 and 85 000. The basic (M_r 100 000), acidic (M_r 100 000), and very acidic (M_r 75 000–85 000) oviductal proteins synthesized by gilts in this study appear to be very similar to the de novo- synthesized proteins produced by baboon [9, 10] and human [11] oviducts, in terms of appearance at mid-cycle, the temporal association with elevated levels of estrogen, electrophoretic behavior, and radiolabeling with Leu, Met, and glucosamine. However, the molecular weights of glycoproteins from gilts were slightly lower than those reported for baboons and humans, al-

though their isoelectric points appeared to be similar. The three estrus-associated glycoproteins were present, based on 2D-SDS-PAGE analysis, in oviductal flushings only from estrous or metestrous gilts, suggesting their synthesis and secretion in vivo. The presence of all three proteins was, as previously suggested [5], temporally associated with elevated levels of estrogen. The 335 000-M_r protein (pI 6) was also associated with elevated levels of estrogen; however, this protein, while dominant in the isthmus, was also present in the ampulla; and much of it appeared to remain in the stacking gel, indicating a protein of much higher molecular weight, an undissociated protein complex, or a protein containing a large amount of carbohydrate.

Two groups of proteins with molecular weights of 43 000–46 000 and 60 000 [5] were resolved into at least three heterogeneous families of proteins and two or three isoelectric species, respectively, by 2D-SDS-PAGE. These proteins were present throughout the estrous cycle, becoming more intense during diestrus, but this may have been due to a decrease in presence of the higher molecular weight EAP. The 43 000–46 000-M_r protein families appeared to be electrophoretically similar to those produced by sheep oviduct [8, 13]. At least one of the three families of oviductal proteins from gilts incorporated glucosamine, suggesting that it may be a glycoprotein. Sutton et al. [28] found periodic acid-Schiff staining of proteins with similar molecular weights and isoelectric point values in sheep oviduct. A major difference between ewes and gilts, however, is that 43 000–46 000-M_r families of proteins are produced throughout the estrous cycle and early pregnancy in gilts but only during the first 4–5 days after estrus in ewes.

Metabolic labeling studies in gilts [5] and rabbits [19] and results from ELISA studies [20] indicate significant differences between the ampulla and isthmus in synthesis and secretion of proteins. In gilts and rabbits, the ampulla secretes both greater amounts of radiolabeled proteins and specific glycoproteins than does the isthmus. In rabbits, the estrogen-induced sulfated oviductal glycoprotein (SOG) is produced by both the ampulla and the isthmus, but the ampulla secretes more SOG [19, 20]. Synthesis and secretion of proteins is also greater for the ampulla compared to the isthmus in gilts [5]. Further, both 1D and 2D-SDS-PAGE analyses of explant culture media revealed three major EAP (M_r 100 000, pI > 8; M_r 100 000, acidic; M_r 75 000–85 000, pI < 4), the minor 28 000–30 000-M_r acidic polypeptide complex, and a 335 000-M_r protein from the ampulla of gilts that are not expressed during diestrus. This suggests that either these proteins are induced by estrogen or synthesis is suppressed by progesterone. The gilt isthmus synthesized and secreted three groups of proteins that were different from those synthesized by the ampulla. The major 335 000- and 30 000-M_r proteins from the isthmus appeared to be induced by estrogen, whereas the third, present during estrus, was enhanced during diestrus. A mouse oviductal glycoprotein, GP215, has been immunocytochemically localized to the oviductal infundibulum and ampullar epithelium, but not isthmic epithelium [16]. With OVX estrogen-treated baboons, using immuno-gold labeling, Verhage et al. [18] showed that oviductal-specific glycoproteins are present in both the ampulla and isthmus. However, electrophoretic analyses indicated that although all segments produced the oviductal proteins, a synthetic gradient was present with the acidic protein dominant in the fimbria and with the basic protein dominant in the isthmus.

The results presented here indicate that the ampulla and the isthmus in gilts each secrete unique proteins that correlate with specific hormonal control. The presence and distribution of biochemically distinct de novo-synthesized glycoproteins in the ampulla and isthmus of gilts, however, remains to be shown by use of specific antibodies and immunocytochemical techniques.

Comparison of de novo-synthesized and secreted products from porcine oviduct and uterine endometrial explant culture media analyzed by 2D-SDS-PAGE and fluorography suggests that each has unique secretory products. Similar results have been reported for baboon [9] and human [11] endometrial and oviductal secretions. The difference, as suggested, probably reflects different functional activities of these two tissues.

In this Sepharose CL-6B gel filtration study, Peaks 1 and 2 contained the major estrus-associated glycoproteins, which were decreased after Day 3 of the estrous cycle. Further results also suggest that EAP may be found in the native state in higher molecular weight forms. These complex macromolecules, once reduced and denatured, exist as 100 000-M_r and 75 000–85 000-M_r subunits. This characteristic is shared with rabbit SOG [14] and sheep estrogen-associated glycoprotein [29], which are also found as higher molecular weight complexes that can be reduced to smaller subunits.

The three major EAP incorporated [^{3}H]Leu, [^{35}S]Met, and [^{3}H]glucosamine, suggesting that they are glycoproteins. Two EAP, one from the ampulla and one from the isthmus, were radiolabeled with fucose, which suggests post-translational modification similar to that reported for the estrus-associated glycoprotein (EGP) in sheep by Sutton et al. [28]. [^{3}H]Mannose was not incorporated into any porcine oviductal protein in this study, a finding consistent with lectin studies of EGP in sheep [28]. These studies together suggest that these glycoproteins may not be *N*-linked glycoproteins, but instead may be mucins.

Inorganic [^{35}S]sulfate was incorporated into three de novo-synthesized glycoproteins in this study. A previous study [14] demonstrated inorganic sulfate post-translational modification of rabbit SOG, the only other OSP examined for sulfate incorporation. Of these three sulfate-containing glycoproteins in pigs, two are products of the ampulla and one of the isthmus. Whether the porcine sulfated glycoprotein is similar and can inhibit complement activity as described for rabbit SOG [30] remains to be investigated.

Although functional significance of porcine OSP has not yet been addressed, numerous functions can be postulated for these glycoproteins from the ampulla and the isthmus. These include sperm and ovum transport, sperm capacitation, fertilization, early cleavage-stage embryonic development [31], delay of enzymatic digestion of zona pellucida [32], and inhibition of the maternal humoral immune system [30]. Oviductal secretory proteins have been shown immunologically to be associated with the zona pellucida in rabbits, mice, and hamsters [33–35] and the perivitelline space in mice [16, 36]. In pigs, new macromolecules are associated with the zona pellucida after passage of the oocyte or zygote through the oviduct, suggesting addition of oviductal elements [37, 38] that may function to inhibit protease activity [32]. The specific synthesis of proteins by the oviduct and their localization in the oviduct, the zona pellucida, and the perivitelline space of mice suggest that these proteins affect or contribute to an environment facilitating or essential for fertilization and early cleavage-stage preimplantation development.

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