Estrogen- and Progestosterone-Dependent Secretory Changes in the Uterus of the Sheep

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ABSTRACT

This study was undertaken to determine the effects of 17β-estradiol (E) and progesterone (P) on polypeptide synthesis and release from the uterus of the sheep. Uterine flushings (UF) and endometrium were obtained from ovariectomized untreated animals, ovariectomized animals treated with E (5–10 pg/ml) for 6 days (6E) and ovariectomized animals primed with E for 6 days then treated with P (~1.5–3 ng/ml), in the continued presence of E, for an additional 6 days (6EP). Endometrium was cultured (24 h) in the presence of [3H]leucine ([3H]-leu) or [3H]-glucosamine ([3H]-glcN), and newly synthesized and released proteins were detected in culture media by fluorography of 10% SDS gels. The quantity of proteins in UF and radiolabeled proteins in explant culture media did not change between treatment groups (p < 0.05). Qualitative changes in the synthesis and release of proteins were observed depending on the steroid treatment. An M, 57 000 protein was present in UF and [3H]-leu-labeled culture media obtained from animals treated only with E and an M, > 200 000 was present in [3H]-leu-labeled culture media of endometrium obtained from 6E and 6EP animals. An M, 44 000 protein was present only in UF from 6EP animals but could not be detected in endometrial culture media from animals undergoing this steroid treatment. These data show that the endometrium of the ovariectomized sheep undergoes alterations in secretory protein patterns which depend on the presence of E and P.

INTRODUCTION

In several mammalian species, 17β-estradiol (E) and progesterone (P) modulation of endometrial secretory activity has been documented morphologically [1–3] and biochemically [4–6]. It is postulated that steroid-regulated secretory alterations take place in a manner compatible with implantation and placenta. In the ewe, two P-regulated glycoproteins, the uterine milk proteins (M, 57 000 and 55 000) have been extensively studied and found to share sequence homology to a serpine superfamly of serine protease inhibitors [4, 5]. These glycoproteins are synthesized and released from the endometrial epithelium in copious quantities after implantation in the pregnant animal and when ovariectomized animals are treated with P. The biological role of the uterine milk proteins has not been elucidated. Using light and electron microscopy we have shown that the uterine epithelial cells of the sheep undergo steroid-dependent alterations in protein-synthesizing organelles indicative of enhanced biosynthetic activity [3].

E treatment to ovariectomized ewes induces maturation of the Golgi complex and rough endoplasmic reticulum in glandular epithelial cells. The coadministration of E and P to E-primed ewes maintains the differentiation of the secretory apparatus and results in an extensive interlayering network of microvilli at the luminal margins of the epithelium. Our morphological data extend biochemical observations in other laboratories [4, 5] regarding the secretory activity of the sheep uterus during ovarian hormone replacement therapy; however, the identity of polypeptide(s) whose production is altered during the treatment regimen used in our studies is not known.

Embryo transfer experiments using ovariectomized steroid-treated sheep have shown that normal preimplantation embryo development depends upon changes in uterine secretions that are induced by a precise sequence of E and P [7, 8]. These data in the sheep as well as asynchronous embryo transfers in sheep [7, 9] and other species [10, 11] support the hypothesis that an appropriate steroid-induced biochemical milieu of the preimplantation uterus is essential for normal early embryonic development. The role of E-priming and subsequent P administration to ovariectomized ewes in altering the quantity and quality of uterine polypeptide synthesis in preparation for the conceptus is not clear. This information is essential for further understanding the nature of the interactions between the uterine luminal environment and the conceptus and which characterize the uterine secretory alterations associated with pregnancy success and loss can begin to be defined.

Since our structural data show that E alone for 6 days or in combination with P for 6 days can result in maturation of secretory organelles in the uterine epithelium [3], the objective of the present study was to determine whether this experimental paradigm also alters in vivo and in vitro polypeptide synthesis and release from the uterus of the sheep. In these studies, long-term ovariectomized ewes were treated with E (~5–10 pg/ml) and P (~1.5–3 ng/ml) and quantitative and qualitative changes in secretory protein composition were assessed in uterine flushings (in vivo secretions) and endometrial explant culture media (in vitro secretions).

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MATERIALS AND METHODS

Reagents

All tissue culture supplies were purchased from Gibco Laboratories (Grand Island, NY). The radiochemicals L-[1-\textsuperscript{3}H]-leucine (\(\textsuperscript{3}H\)-leu; specific activity 60 Ci/mmole) and D-[\textsuperscript{3}H]-glucosamine (\(\textsuperscript{3}H\)-glcN; specific activity 42.5 Ci/mmole) were purchased from ICN Radiochemicals (Irvine, CA). Electrophoresis supplies were purchased from Bio-Rad Laboratories (Richmond, CA). XAR-5 film was from Eastman-Kodak (Rochester, NY). P, E, phenylmethylsulfonylfluoride (PMSF), rabbit anti-sheep serum (IgG fraction, Cat. \#S-6758 Lot 20488843), and cyanogen bromide (CN-Br)-activated Sepharose 4B were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals of reagent grade or better were purchased from Sigma Chemical Co.

Animals and Tissue Collection

Steroid-treated ovariectomized sheep were used in these studies. The sequence and duration of E and P treatment was identical to that previously shown to induce secretory organelle maturation [3]. Sexually mature purebred Dorset ewes were bilaterally ovariectomized. To establish a baseline to compare steroid-induced changes in secretory macromolecules, animals received no steroid treatment for 6 wk. After this time, animals either received no additional treatment (ovariectomized, controls), were treated with one 4-cm E Silastic implant for 6 (6E) days, or were primed with E for 6 days and treated with six 13-cm P implants, in the continued presence of E, for an additional 6 (6EP) days. We have shown that this steroid treatment produces serum E (~5–10 pg/ml) and P (~1.5–3 ng/ml) concentrations similar to that reported in the intact ewe [3]. Uterine horns were obtained at the time of hysterectomy from 3 animals in each of the treatment groups.

Collection and Preparation of Uterine Secretions

Changes in the secretory activity of the uterus were analyzed by collecting in vivo uterine flushings and in vitro uterine explant culture media proteins. At the time of hysterectomy, the uterine horns were placed in sterile Earle’s modified Eagle’s minimal essential medium (MEM), transferred to a laminar flow hood, and trimmed of the excess fat and mesentery.

In vitro secretions-uterine flushings. At laparotomy, the uteri were rapidly exposed and clamped at the uterotubal and uterocervical junctions. Uterine contents were immediately collected in situ by flushing the lumen of one horn with 10 ml of sterile physiological saline and recovering the fluid from the contralateral horn at its most proximal end. Uterine flushings were immediately placed on ice and PMSF was added to a final concentration of 10 mM; the sample was centrifuged (5000 \(\times\) g, 20 min), dialyzed extensively against distilled water at 4°C (\(M_1\), cutoff 3500), and stored at ~20°C. Protein concentration in dialyzed flushings was determined by the method of Bradford [12].

In vitro secretions-explant culture. For explant culture, the endometrium was cultured in MEM according to the procedure of Basha et al. [15]. Briefly, uteri were opened longitudinally, the endometrium was separated from the myometrium with the aid of a dissecting microscope and cut into 1–3-mm\(^3\) pieces. Endometrial tissue (150 mg tissue/6 ml medium) was placed in MEM and cultured at 37°C in a gas-tight chamber (Forma Scientific, Marietta, OH) under an atmosphere of 50% N\(_2\)-45% O\(_2\)-5% CO\(_2\) for 24 h. To detect newly synthesized and released protein, \(\textsuperscript{3}H\)-leu (25 \(\mu\)Ci) or \(\textsuperscript{3}H\)-glcN (20 \(\mu\)Ci) was added to the culture media. To enhance the incorporation of \(\textsuperscript{3}H\)-leu and \(\textsuperscript{3}H\)-glcN into secreted proteins, the concentration of unlabeled leucine or glucose in the culture media was reduced tenfold. After the 24-h incubation, explant cultures were harvested, culture media were placed on ice, and PMSF was added to a final concentration of 10 mM. Culture media were centrifuged (5000 \(\times\) g, 20 min), dialyzed (\(M_1\), cutoff 12 000–14 000) extensively against 10 mM Tris, pH 8.3, at 4°C, and stored at ~20°C. Newly synthesized and released proteins were detected in dialyzed culture media by trichloroacetic acid (TCA) precipitation and 10% SDS gels followed by fluorography. Radioactivity in dialyzed culture media was determined by scintillation spectrometry.

Serum Immunoaffinity Chromatography of Uterine Secretions

Serum proteins are normal components of uterine secretions. To distinguish between uterine-derived secretory proteins and serum transudates, serum proteins present in culture media and uterine flushings were removed by passage through an affinity column containing the IgG fraction of a rabbit anti-sheep serum. Fifty milligrams of rabbit immunoglobulins (IgG) to sheep serum proteins was coupled to CN-Br-activated Sepharose 4B according to the manufacturer’s directions. The immunoaffinity column (1.5 \(\times\) 3 cm) adsorbed approximately 90% of sheep serum proteins (data not shown). Aliquots of dialyzed \(\textsuperscript{3}H\)-leu- and \(\textsuperscript{3}H\)-glcN-labeled endometrial culture media (200 000 cpm/300–500 \(\mu\)g protein) or uterine flushings (600 \(\mu\)g protein) were lyophilized and resuspended in column running buffer (PBS, pH 7.0). Resuspended culture media or uterine flush proteins were applied to the serum affinity column and the column was washed extensively with PBS at pH 7.0. The adsorbed proteins were eluted with 0.05 M glycine, 0.15 M NaCl, pH 2.3, and immediately neutralized with 100 \(\mu\)l of 1 M Tris, pH 8.0. Each fraction (1 ml) was monitored for radioactivity by scintillation spectrometry and protein concentration by the method of Bradford [12]. The fractions contained in material that did not bind (peak I) or material that adsorbed (peak II) to the serum affinity column were pooled, extensively dialyzed against distilled water at 4°C (\(M_1\), cutoff 12 000–14 000), and analyzed on 10% SDS gels.
To detect radiolabeled proteins in culture media, gels were fluoroenhanced, dried, and exposed to XAR-5 film. SDS gels of fractionated uterine flush proteins present in peak I and peak II material were stained with silver nitrate.

**In Vitro Protein Synthesis and Release-TCA Precipitation**

Radioactivity incorporated into protein was determined by TCA precipitation of endometrial explant culture media obtained from animals in each of the treatment groups. Aliquots of dialyzed culture media (10 μg) were blotted onto TCA-treated Whatman 3 MM paper and dried. TCA-coated papers were washed in 20% (w/v) TCA containing 20 mM pyrophosphate (TCA/PPI) (30 min), boiled in 5% (w/v) TCA/PPI (2 min), washed twice in 5% (w/v) TCA/PPI (20 min, 10 min), washed twice in 95% ethanol (5 min each), and dried under a heat lamp. Radioactivity incorporated into protein was determined by scintillation spectrometry. As a measure of the amount of proteins synthesized and released by the endometrium, the ratio of the fmol of 3H-glcN or 3H-leu incorporated/μg protein was determined for explant culture media in each of the treatment groups.

**Electrophoresis**

Proteins present in endometrial culture media and uterine flushings were analyzed by SDS-PAGE according to the procedure of Laemmli [14]. To detect radiolabeled proteins, gels were soaked in 1 M sodium salicylate (15 min, room temperature) as a fluoroenhancer prior to drying [15]. Equal counts (33 000 cpm) of dialyzed culture media were applied to 10% SDS gels for fluorographs. Dried gels were exposed to XAR-5 film at −70°C. Ten micrograms of uterine flush proteins was applied to 10% SDS gels and stained with silver nitrate.

**Statistics**

Protein concentration in uterine flushings and radioactivity incorporated into culture media proteins were expressed as the mean ± SEM. Significant differences between group means were determined by Kruskal-Wallis and Mann-Whitney U tests (p < 0.05) [16].

**RESULTS**

**In Vivo Secretions-Uterine Flushings**

Protein concentration in uterine flushings did not significantly change (p < 0.05) between treatment groups (Fig. 1). Qualitative differences in uterine flush proteins were examined using SDS-PAGE. Proteins present in uterine flushings were separated on 10% SDS gels and stained with silver nitrate (Fig. 2). The protein-staining pattern of uterine flushings obtained from animals in the three treatment groups indicated that a large number of the proteins were proteins present in serum. Serum proteins were removed by passage of uterine flushings over an affinity column con-

![Graph](image1.png)

**FIG. 1.** Protein concentration in uterine flushings. No significant (p < 0.05) differences were detected among the treatment groups. Untreated, ovariectomized animals (OVX); ovariectomized animals treated with E for 6 days (6E); ovariectomized animals treated with E for 6 days followed by E and P for an additional 6 days (6EP).

![Image](image2.png)

**FIG. 2.** Gel electrophoresis of uterine flushings. Proteins (10 μg) present in uterine flushings were separated on 10% SDS gels and gels were stained with silver nitrate. Many of the proteins present in uterine flushings were serum proteins (S). Refer to Figure 1 for explanation of abbreviations.
sisting of rabbit IgG to sheep serum proteins (Figs. 3 and 4). When 800 µg of sheep serum proteins was applied to the affinity column, some serum proteins were present in the flow-through material (Fig. 5). However, the majority (~80–90%) of total serum proteins applied to the affinity column bound to the column and were eluted under low pH conditions (Fig. 5). An M₆ 57,000 protein was present in peak I fractions of uterine flushings obtained from 6E animals (Fig. 4, arrow 1). This protein was not present in uterine flushings obtained from untreated, ovariectomized animals, nor in 6EP animals. However, a protein of similar electrophoretic mobility was detected in peak I material of sheep serum proteins fractionated on affinity columns (Fig. 5).

It is possible that E treatment alone selectively allows the transudation of an M₆ 57,000 serum protein into the uterine lumen. In addition, an M₆ 44,000 protein was enhanced in peak I fractions of uterine flushings obtained from 6EP animals (Fig. 4, arrow 2). The M₆ 44,000 protein was not present in peak I material of sheep serum proteins fractionated on affinity columns (Fig. 5).

**In Vitro Secretions-Explant Culture Media**

The ratio of the fmol of ³H-glcn incorporated/µg protein and fmol ³H-leu incorporated/µg protein was determined in TCA precipitates of explant culture media and used as an index of the amount of newly synthesized and released endometrial proteins (Fig. 6). The in vitro incorporation of ³H-leu and ³H-glcn into protein did not significantly (p < 0.05) change between treatment groups (Fig. 6), even though qualitative differences in the in vitro synthesis and release of radiolabeled proteins were observed in fluorographs of explant culture media (Figs. 7 and 9).
Culture media of endometrium obtained from steroid-treated sheep were separated on 10% SDS gels, and newly synthesized and released proteins were detected by fluorography of dried gels. Figure 7 is a representative fluorograph of 3H-leu- (Fig. 7A) and 3H-glcN- (Fig. 7B) labeled culture media proteins of endometrium obtained from animals in each of the treatment groups. Qualitative differences in radiolabeled proteins were observed depending on the steroid treatment. The labeling intensity of two proteins changed depending upon the steroid treatment of the animal. An M, 57 000 protein was synthesized and released from endometrium obtained from 6E animals (Fig. 7A, arrow 2). The labeling intensity of this M, 57 000 protein was not enhanced in 3H-glcN-labeled culture media (Fig. 7B). A protein of similar molecular weight was detected in uterine flushings obtained from 6E animals (Fig. 4, arrow 1). An M, > 200 000 protein was present in 3H-leu-labeled culture media of endometrial explants obtained from both 6E animals and 6EP animals (Fig. 7A, arrow 1). The synthesis and release of the M, > 200 000 protein were reduced in 6EP endometrial explants. High molecular weight (M, > 200 000) 3H-glcN-labeled macromolecules were present in endometrial culture media obtained from animals in all the treatment groups (Fig. 7B). It is unclear whether this band represents the M, > 200 000 3H-leu-labeled protein detected in endometrial culture media of steroid-treated animals. Two low molecular weight proteins were also detected in 3H-leu-labeled culture media from 6E and 6EP animals (Fig. 7); however, their banding patterns were very diffuse and indistinct, making accurate molecular weight estimates impossible (Fig. 7A, arrows 3 and 4). The M, 44 000 protein detected in fractionated uterine flushings obtained from 6EP animals (Fig. 4) was not detected in fluorographs of 3H-leu- or 3H-glcN-labeled culture media from animals in any of the treatment groups.

To confirm that the 3H-leu M, 57 000 and M, > 200 000 proteins were endometrium-derived secretory products,
and in vitro synthesis and release of hormonally dependent proteins from the sheep uterus. The present study has identified both E-modulated and EP-modulated alterations in endometrial protein biosynthesis.

The concentration of proteins present in uterine flushings did not change between treatment groups \( (p < 0.05) \). Endometrium obtained from steroid-treated animals did not incorporate more \(^3\text{H}\)-leu or \(^3\text{H}\)-glcN into protein when compared to untreated animals \((p < 0.05)\). The lack of statistically significant changes may be due to the number \((n = 3)\) of animals in each of the treatment groups. The inherent biological variability within this random population of ewes may be high so that significant differences in protein synthesis could not be detected. Even though no quantitative differences were noted, qualitative changes in the synthesis and release of uterine-derived proteins were observed during E treatment alone or when E was coadministered with P.

An \( M_r \), 57 000 protein was detected in uterine flushings and explant culture media obtained from 6E animals. A

![Graph showing protein concentration and radioactivity vs. fraction number](image)

**Fig. 8.** Representative immunoaffinity chromatography of \(^3\text{H}\)-leu-labeled proteins \((200 000 \text{ cpm/300–500 \mu g})\) present in endometrial explant culture medium obtained from an ovariectomized animal. The column was washed extensively with PBS, pH 7.0, and the adsorbed proteins (peak I) eluted under conditions of low pH. Protein concentration and radioactivity were determined in unbound (peak I) and bound (peak II) fractions. Similar chromatographic profiles were obtained when culture media from steroid-treated animals was fractionated.

serum proteins present in \(^3\text{H}\)-leu-labeled endometrial culture media from animals in each of the treatment groups were removed by passage through an affinity column containing the IgG fraction of rabbit anti-sheep serum (Figs. 8 and 9). As expected, material that did not bind to serum affinity columns (peak I) contained the majority of radioactivity and relatively less total protein than material that bound to serum affinity columns (peak II) (Fig. 8).

The proteins present in peak I and peak II \(^3\text{H}\)-leu-labeled culture media were separated on 10% SDS gels and analyzed by fluorography (Fig. 9). The \( M_r > 200 000 \) (arrow 1) and \( M_r, 57 000 \) (arrow 2) proteins were detected in \(^3\text{H}\)-leu-labeled culture media that did not bind to serum affinity columns (Fig. 9). The two low molecular weight proteins detected in unfractionated media (Fig. 7A; arrows 3 and 4) were barely discernable in these fluorographs (Fig. 9; arrows 3 and 4). As expected, neither the \( M_r > 200 000 \) nor the \( M_r, 57 000 \) protein was present in culture media material that bound to the affinity column. This fraction (peak II) contained serum proteins and no evidence of newly synthesized proteins even after exposure of gels to film for 70 days (data not shown).

**DISCUSSION**

We have previously shown that the sequence and duration of E and P treatment used in this study is accompanied by the maturation of protein-synthesizing organelles in uterine epithelial cells \( [3] \). The data presented here show that this same experimental paradigm altered endometrial protein-synthesizing capabilities and resulted in the in vivo

![Fluorographs of endometrial culture media](image)

**Fig. 9.** Representative fluorographs of endometrial culture media affinity fractions (peak I). Aliquots \((33 000 \text{ cpm/peak I})\) of \(^3\text{H}\)-leu-labeled proteins were separated on 10% SDS gels. The \( M_r > 200 000 \) protein was present in peak I \(^3\text{H}\)-leu-labeled culture media of endometrium obtained from animals treated only with E and E-primed animals treated with P (arrow 1) and the \( M_r, 57 000 \) protein in culture media of endometrium obtained during E treatment alone (arrow 2). Two lower molecular weight proteins were faintly labeled in culture media obtained from steroid-treated animals (arrows 3 and 4). Refer to Figure 1 for explanation of abbreviations.
protein of similar electrophoretic mobility was present in immunoaffinity-fractionated sheep serum. It is possible that the \( M_5 \) 57 000 protein is an endometrium-derived protein that is selectively released into the systemic circulation during periods of E dominance. Serum proteins of endometrial origin have been described [17]. In humans, it has been suggested that the endometrial insulin-like growth factor binding protein (IGF-BP) is the major source of IGF-BP in the serum during early pregnancy [17].

In the ewe, the \( P \)-modulated uterine milk glycoproteins \((M_5 57 000 \text{ and } M_5 55 000)\) are synthesized during pregnancy and when \( E \)-primed animals are treated with \( P \) [4]. The \( M_5 57 000 \) protein described in our study was an \( E \)-modulated protein and did not incorporate \(^3\)H-glCN in explant culture; therefore, it is unlikely that it is the higher molecular weight form of the uterine milk proteins. Neither an \( M_5 57 000 \) or \( M_5 55 000 \) protein was detected in uterine secretions from \( 6\text{EP} \) animals. Using Western blotting, Ing et al. [4] reported low levels of the uterine milk proteins in endometrial explant culture media and uterine flushings obtained from \( 6\text{EP} \) animals. It is possible that the uterine milk proteins were present in uterine secretions obtained from \( 6\text{EP} \) animals at extremely low levels such that they could not be detected in SDS-silver stained gels and fluorographs. The Silastic implants used in our study release steroids in a steady-state fashion rather than the fluctuating manner observed in cycling and pregnant animals. It is also conceivable that chronic rather than fluctuating levels of \( E \) could have suppressed the synthesis and release of the \( P \)-modulated uterine milk proteins. Estrogen antagonism of \( P \)-regulated uterine protein production has been reported for utroferrin in the pig [18].

Two additional polypeptides \((M_5 > 200 000 \text{ and } M_5 44 000)\) identified in this study were released during \( E \) treatment alone and \( EP \) treatment to ovariecotimized ewes. The labeling intensity of the \( M_5 > 200 000 \) protein was reduced during \( EP \) treatment. Neither of these were detected in both uterine flushings and explant culture media. The high molecular weight protein \((M_5 > 200 000)\) was synthesized and released by endometrial explants obtained from ewes treated only with \( E \) and from \( EP \)-treated animals. This protein was not detected in silver-stained gels of uterine flushings. Perhaps the \( M_5 > 200 000 \) protein is released in such low quantities in vivo that it can not be identified in SDS gels or—in the intact animal—the endometrium is capable of synthesizing this protein but not secreting it into the uterine lumen due to other physiological regulatory factors absent in explant culture conditions. Without the influence of in vivo negative regulators, in vitro release would be permitted. The \( M_5 44 000 \) protein present in immunoaffinity-fractionated uterine flushings obtained from \( EP \) animals was not detected in endometrial explant culture media obtained from \( EP \)-treated animals. Cycle- and pregnancy-specific enzymatic activity has been reported in uterine flushings [19, 20]. It is possible that the \( M_5 44 000 \) protein may result from proteolytic cleavage of other uterine proteins as a consequence of the activation of luminal or apical plasma membrane proteases when \( P \) is administered to \( E \)-treated animals. Uterine luminal processing may explain the absence of in vitro synthesis since this would not occur in explant culture. Alternatively, the \( M_5 44 000 \) protein may be synthesized and released from the uterus of the intact animal in response to physiological influences that are not present in vitro. The relationship between this \( M_5 44 000 \) protein and uterine-derived enzymes or other secretory macromolecules is not known.

A normal menstrual or estrous cycle consists of an \( E \)-dominated phase followed by a \( P \)-dominated phase. If pregnancy occurs, cyclicity ceases and the hormonally differentiated uterus provides the appropriate environment for implantation. The role of \( E \) priming in this process is not fully understood. The results of this study show that the biosynthetic abilities of the endometrium differ between ewes treated with \( E \) alone and ewes treated with \( E \) and \( P \) in combination. Synergistic effects of \( E \) and \( P \) on \( E \)-modulated endometrial secretory proteins has been described in the pig [18] and baboon [6]. Our data show that a synergistic effect of \( E \) and \( P \) is to shift off the synthesis and release of one protein \((M_5 57 000)\) and to maintain the synthesis and release of two proteins \((M_5 > 200 000; M_5 44 000)\).

The biological role of uterine secretory proteins described in this study and others [5, 6, 17] is speculative. It has long been suggested that uterine fluids are synthesized and released in a steroid- and time-specific manner compatible with implantation and placentation. Uteroferin, a \( P \)-induced glycoprotein, has been extensively studied and functions in iron transport to the pig conceptus [18, 21]. The physiological relevance of an IGF-BP in the baboon [22] and human [23] endometrium is not clear. One proposed function is that IGF-BP prevents proliferation of trophoblast cells to restrict placental development [23]. A \( P \)-dependent cathepsin L-like protein is synthesized by the uterine epithelial cells of the cat and postulated to be involved in endometrial remodeling necessary for the establishment of pregnancy [24, 25]. The reproductive significance of steroid-regulated uterine proteins is not well defined and continues to await the use of purified proteins in vivo and in vitro studies.

In summary, the present study has shown that treatment of ovariecotimized sheep with \( E \) and \( P \), which mimic peripheral serum steroid levels reported in the intact animal, altered endometrial protein production and resulted in vivo and in vitro synthesis and release of uterine-derived proteins. It will be necessary to determine the cellular localization, stage of the estrous cycle, and time during early pregnancy that these macromolecules are synthesized by the endometrium. In addition it will be of interest to know whether these proteins are produced in a region-specific manner by the sheep endometrium—in other words, whether the caruncular and intercaruncular endometrium.
are similar in their steroid-regulated synthesizing capabilities. The ability to regulate the local biochemical milieu of the uterus in preparation for an ensuing pregnancy by treatment with endogenous hormones will allow us to design experiments to eventually understand the role of these macromolecules in reproduction in the sheep as well as in other species. This will be useful in further elucidating embryo-endometrial interactions preceding and during the implantation phase of development.

REFERENCES


