Secretory Proteins of the Bovine Conceptus Alter Endometrial Prostaglandin and Protein Secretion in Vitro


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ABSTRACT

The conceptus is believed to produce factors that regulate endometrial function and prevent luteolysis during early pregnancy. Endometrial tissues were collected from cyclic (n = 8) and pregnant (n = 2) cows at Day 17 post-estrus and cultured for 24 and 48 h with bovine conceptus secretory proteins (bCSP) (0%, 10%, 100%), where the amount of protein produced by a bovine conceptus during 24 h of culture is 100%. Incorporation of [3H]leucine into secreted proteins was determined and examined qualitatively by trichloroacetic acid precipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography. Levels of an intracellular endometrial inhibitor of prostaglandin synthesis were determined with a cotyledonary microsomal test system. Treatment with 10% and 100% bCSP reduced incorporation of [3H]leucine into secreted proteins. However, bCSP selectively induced two secreted proteins (13 and 10 kDa) from endometrium of cyclic cows. Prostaglandin F (PGF) secretion was decreased by bCSP treatment while prostaglandin E \textsubscript{2} secretion was unaltered. An intracellular endometrial inhibitor of prostaglandin synthesis was induced by bCSP; synthesis of PGF by the cotyledonary prostaglandin-generating system was decreased when incubated with cytosol of endometrium treated with bCSP, but unaltered by cytosol from control tissues. In conclusion, products produced by the bovine conceptus are capable of regulating endometrial protein and prostaglandin biosynthesis in a fashion that could act to prevent luteolysis in vivo and provide endometrial secretory products for embryonic development.

INTRODUCTION

In cattle, the sequence of events leading to luteolysis must be blocked or attenuated because maintenance of the corpus luteum (CL) is necessary for early pregnancy (Hansel et al., 1973; Thatcher et al., 1984). Indeed, increased plasma concentrations of 13, 14-dihydro-15-keto-prostaglandin F \textsubscript{2\alpha} (PGFM), usually associated with luteolysis, are reduced during early pregnancy (Kindahl et al., 1976; Betterridge et al., 1984). Furthermore, in vitro endometrial synthesis of prostaglandin F (PGF) and PGFM are lower for pregnant than for cyclic cows at Day 17 post-estrus (Thatcher et al., 1984; Gross et al., 1988b). In cattle, intracellular inhibitors of prostaglandin synthesis have been reported for placental and uterine tissues (Shemesh et al., 1984; Wlodawer et al., 1976). Basu and Kindahl (1987) and Gross et al. (1988a) recently demonstrated the presence of an endometrial inhibitor of prostaglandin synthesis during the estrous cycle that increases in inhibitory activity during early pregnancy. It has been hypothesized that this inhibitor functions to decrease endometrial prostaglandin synthesis and therefore prevents luteolysis during early pregnancy.

Secretory proteins produced by Day 17 bovine conceptuses (bCSP) lengthen the luteal phase when administered into the uterus during the mid-luteal phase of the estrous cycle (Knickerbocker et al., 1986b). Furthermore, estradiol-induced prostaglandin secretion is reduced in cows receiving uterine intraluminal infusions of bCSP (Knickerbocker et al., 1986a). Endometrial protein synthesis may also be altered by bCSP treatment, as has been reported for ovine endometrium treated with ovine trophoblast protein-1 (Godkin et al., 1984). Therefore, it is likely that changes in endometrial prostaglandin and protein secretion during early pregnancy are regulated by secretory products from the conceptus. The current experiment examined the effect of bCSP on endome-
trial protein and prostaglandin secretion and on induction of the intracellular inhibitor of prostaglandin synthesis in endometrium from cyclic and pregnant cows at Day 17 post-estrus.

**MATERIALS AND METHODS**

**Materials**

Radioisotopes of L-[4, 5-3H]leucine (sp. act.: 150 Ci/mmol), [5, 6, 8, 11, 12, 14, 15-3H]PGF$_2\alpha$ (sp. act.: 160–180 Ci/mmole) and [5, 6, 8, 12, 14, 15-3H]PGE$_2$ (sp. act.: 140–170 Ci/mmole) were purchased from Amersham Corporation (Arlington Heights, IL). Arachidonic acid was purchased from Sigma Chemical Company (St. Louis, MO). Antiserum to PGF was provided courtesy of T. G. Kennedy and antiserum to PGE$_2$ was a gift from The E. L. Lilly Co. (Indianapolis, IN). Dialysis tubing was purchased from Spectrum Medical (Los Angeles, CA). Supplies for polyacrylamide gel electrophoresis (PAGE) were as follows: tris(hydroxymethyl)aminomethane (Tris) and N, N, N', N'-tetramethyl ethylenediamine (TEMED) were purchased from Sigma Chemical Co., sodium salicylate, 2-mercaptoethanol, glycine, and ammonium peroxydisulfate were purchased from Fisher Scientific (Orlando, FL); acrylamide, urea, dithiothreitol, and sodium dodecyl sulfate (SDS) were purchased from Research Organics (Cleveland, OH); and bis-acrylamide, gelatin, and Tween-20 were purchased from Bio-Rad (Richmond, CA). Dulbecco's Modified Eagle's Medium was purchased from Sigma Chemical Co.

A modified minimum essential medium (MEM; custom formula #87-5007) and other medium ingredients were purchased from Gibco (Grand Island, NY). Medium was prepared as described by Basha et al. (1980), except that phenol red was included and 10 ml of Gibco MEM vitamin solution was added per liter of medium. For cultures in the presence of [3H]leucine, medium was prepared at a reduced leucine content (0.1 × normal concentration).

**Preparation of Bovine Conceptus Secretory Proteins**

Beef cattle (primarily Angus or Brangus) were observed for estrous behavior and either bred by natural service or not bred (cyclic, n = 4). Cows were slaughtered at Day 17 post-estrus. Reproductive tracts were removed rapidly (within 30 min after stunning) and flushed with 40 ml of MEM into a petri dish to collect conceptus tissues. Tissue was transferred into another petri dish containing 15 ml of MEM and incubated for 48 h at 39°C on a rocker platform under an atmosphere of 47.5% O$_2$, 50% N$_2$, and 2.5% CO$_2$. Medium was collected after 24 h of incubation, and fresh MEM (15 ml) was added. Medium was again collected after an additional 24 h of incubation.

Medium from the second 24 h of culture was pooled from 24 conceptuses and dialyzed extensively against Dulbecco's Modified Eagle's Medium (DME) (two changes of 1 liter) using sterile dialysis tubing with a 6000–8000 molecular weight exclusion limit. Additional dialysis was performed against leucine-deficient MEM (two changes of 400 ml using sterile dialysis tubing with a 6000–8000 molecular weight exclusion limit) to yield bCSP in leucine-deficient medium. The leucine content of a portion of the bCSP medium was restored to the normal leucine concentration (41.6 mg/l by addition of supplemental leucine). These bCSP-containing media were used for subsequent endometrial explant cultures for determination of protein secretion (leucine-deficient medium) and prostaglandin secretion (leucine-complete medium). The protein content of bCSP medium was 3.86 mg/15 ml. Control medium (MEM) for endometrial explant cultures was processed similarly to the conceptus protein medium.

**Experiment 1:**

**Effects of bCSP on Endometrial Tissue**

Beef cows (primarily Angus or Brangus) were observed for estrous behavior and either bred by natural service (pregnant, n = 2) or not bred (cyclic, n = 4). Cows were slaughtered at Day 17 post-estrus. Reproductive tracts were removed rapidly (within 30 min after stunning) and flushed with 40 ml MEM to collect conceptus tissues and confirm pregnant or cyclic statuses. Endometrium from the uterine horn ipsilateral to the corpus luteum (CL) was isolated from myometrium and cut into small pieces (1–3 mm$^3$). Tissue (500 mg) was placed into duplicate petri dishes containing 0.2 mg arachidonic acid for each of the following treatments: 1) 0% bCSP (15 ml of MEM), 2) 10% bCSP (13.5 ml MEM and 1.5 ml bCSP medium), and 3) 100% bCSP (15 ml bCSP medium). Tissue (500 mg) also was placed into a petri dish containing 0.2 mg arachidonic acid and 0.1 mCi L-[4, 5-3H]leucine and 15 ml of 0, 10, or 100% bCSP
(prepared as described above, but with leucine-deficient medium). Tissues were incubated for 24 h at 39°C on a rocker platform under an atmosphere of 47.5% O₂, 50% N₂, and 2.5% CO₂. At the conclusion of incubation, tissue and medium were separated by centrifugation (3500 × g, 4°C, 30 min) and stored at -70°C until analyzed.

**Experiment 2:**

**Time Course of Effects of bCSP on Endometrium**

Additional cows (primarily Angus or Brangus) were observed for estrous behavior (cyclic, n = 4) and slaughtered at Day 17 post-estrus. Reproductive tracts were removed within 30 min after stunning and flushed with 40 ml MEM. Endometrium from the uterine horn ipsilateral to the CL was isolated from myometrium and minced. Tissue (500 mg) was placed into quadruplicate petri dishes containing 0.2 mg arachidonic acid and bovine serum albumin (BSA, 15 ml MEM with 5 μg BSA/ml) or 10% bCSP (13.5 ml MEM and 1.5 ml bCSP medium). BSA was used as a control treatment because preliminary results indicated that BSA at concentrations of 4.8, 24, 120 μg/ml stimulated incorporation of [³H]leucine into secreted (229 ± 17%) and tissue (252 ± 21%) proteins without affecting PGF or PGE₂ secretion (S. D. Helmer, T. S. Gross, P. J. Hansen, and W. W. Thatcher; unpublished observation). BSA was therefore used as a control to compare bCSP to nonspecific effects caused by increased protein concentration in the medium.

For both treatments, two dishes were incubated for 24 h before tissue was harvested, and the other two dishes were incubated for 48 h before tissue was harvested. Medium samples (0.5 ml) were collected from each dish at 3, 6, 12, 18, 24, and when applicable, at 30, 36, 42, and 48 h of incubation. For the 48-h incubations, medium was collected after 24 h of incubation, and fresh MEM (15 ml) of the appropriate treatment was added. Tissue (250 mg tissue) was also placed into duplicate petri dishes containing 0.1 mg arachidonic acid, 50 μCi L-[4, 5³H]leucine and either BSA (7.5 ml leucine-deficient MEM with 5 μg BSA/ml) or 10% bCSP (6.75 ml leucine-deficient MEM and 0.75 ml leucine-deficient bCSP medium) for a 24-h incubation. Samples of medium (0.2 ml) were collected from each dish at 3, 6, 12, 18, and 24 h of incubation. All incubations were conducted at 39°C on a rocker platform under an atmosphere of 47.5% O₂, 50% N₂, and 2.5% CO₂. At the conclusion of incubation (24 or 48 h), tissue and medium were separated by centrifugation and stored at -70°C until analyzed.

**Radiolabeled Protein Determination**

Incorporation of radiolabeled leucine into secreted (medium) and intracellular (tissue) proteins was determined by trichloroacetic acid (TCA) precipitation. Tissue from incubations with [³H]leucine was solubilized in 50 mM Tris-acetate buffer (2 ml buffer/500 mg tissue) containing 1 mM phenylmethylsulfonyl-fluoride, 1 mM ethylenediamine-tetraacetic acid and 2% (v/v) Nonidet P-40. Aliquots (0.05 ml) of medium and solubilized tissue were each placed and dried onto Whatman 3MM paper that previously had been saturated with 20% TCA (wt/vol). Precipitation of proteins onto the filter paper and removal of nonproteinaceous compounds was accomplished by serial washing of filter paper with 20% and 5% TCA followed by 95% ethanol as described by Mans and Novelli (1961). Radioactivity of precipitated protein was quantified by scintillation spectrometry.

**Electrophoresis**

Medium from endometrial explants containing [³H]leucine was dialyzed extensively (three changes of 4 liters) against distilled water using dialysis tubing with a molecular weight exclusion limit of 6000–8000 to remove low molecular weight compounds and unincorporated radiolabeled precursors. Dialyzed samples of medium and samples of solubilized tissue were each examined qualitatively by SDS-PAGE. One-dimensional (1-D) SDS-PAGE was performed using the buffer system of Laemmli (1970), with 12.5% (w/v) polyacrylamide gels. Samples containing 50,000 cpm were lyophilized, dissolved in 0.1 ml of dissociating buffer containing 1% (w/v) SDS and 5% (v/v) 2-mercaptoethanol, and then boiled prior to being loaded onto 1-D gels. Two-dimensional (2-D) SDS-PAGE was performed using a modification of the method described by Roberts et al. (1984). Samples containing 100,000 cpm were lyophilized and then dissolved in 0.1 ml of 5 mM K₂CO₃ containing 9.2 M urea, 2% (v/v) Nonidet P-40 and 0.5% (w/v) dithiothreitol. Proteins were resolved in the first dimension by isoelectric focusing in 4% (w/v) polyacrylamide tube gels containing N, N'-diallyltartardiamide, 8.0 M urea, 2% (v/v) Nonidet P-40 and 5.1%
(v/v) ampholines (pH 3–10, pH 5–7, and pH 9–11; 50:36:16 by volume respectively). Tube gels were equilibrated in 0.07 M Tris-HCl buffer (pH 6.8) containing 1% (w/v) SDS and 1% (v/v) 2-mercapto-ethanol and subjected to electrophoresis in the second dimension using 12.5% (w/v) polyacrylamide slab gels and the buffer system of Laemmli (1970). Proteins were localized by staining with Coomassie Brilliant Blue R-250 and fluorography as described by Roberts et al. (1984). Fluorographs were prepared with Kodak XAR film and sodium salicylate as a fluor (Chamberlain, 1979).

**Assay for Inhibition of Prostaglandin Synthesis**

Cotyledonary microsomes, endometrial microsomes and endometrial cytosolic supernatant (100,000 X g) were prepared as described previously (Gross et al., 1988a). Cotyledonary microsomes from parturient cows served as the prostaglandin-generating system. The generating system (0.5 ml; 500 mg tissue equivalent) was incubated with 0.1 mg arachidonic acid (0.1 ml) and with either 0.1 M potassium phosphate buffer (0.5 ml, pH 7.5), medium from endometrial explants (0.5 ml), endometrial microsomes (0.5 ml; 250 mg tissue equivalent), or endometrial cytosolic supernatant (0.5 ml; 250 mg tissue equivalent). Final incubation volume was brought to 2 ml with 0.1 M potassium phosphate buffer. Incubations were conducted at 39° C on a rocker platform for 1 h and then terminated with the addition of 0.25 ml ethanol. Each sample was centrifuged (1500 X g) for 20 min at 4° C to pellet the ethanol-precipitated material. Supernatants were analyzed directly for PGF by radioimmunoassay (RIA) (Gross et al., 1988a).

**RIA Procedures**

Samples of medium were analyzed for PGF using a direct RIA procedure (Knickerbocker et al., 1986c) modified to use an antibody characterized by Kennedy et al. (1985). Standard curves were prepared in MEM (0.05 ml) with known amounts of radioinert PGF (10–5000 pg). An antiserum dilution of 1:5000 was used, and the minimum concentration per tube that was distinguishable from zero was 10 pg. Cross-reactivities of the PGF antiserum were reported previously (Gross et al. 1988a). Minimal cross-reactivity of the antiserum with arachidonic acid, determined in this experiment, was <0.1%. Unextracted samples of medium (MEM from endometrial incubations) were assayed for PGF in duplicate with 0.05-ml aliquots. A pooled medium sample from endometrial explant cultures (approximately 4 ng PGF/ml) was assayed serially in 0.01, 0.02, 0.03, 0.04, and 0.05-ml volumes (final volume of 0.05 ml with MEM). This inhibition curve was parallel to the standard curve, with the test for homogeneity of regression indicating that the curves did not differ in slope. Further characterization of the assay involved measurement of known amounts of PGF in a pooled medium sample (Y = -3.4 + 1.1X; Y = amount of PGF measured [pg/0.05 ml], and X = amount of PGF added [pg/0.05 ml], R² = 0.90). Inter- and intraassay coefficients of variation were 12.2% and 14.1%, respectively.

A similar assay was developed for PGE₂ with a modification of an earlier RIA procedure and an antibody characterized by Lewis et al. (1978). An antiserum dilution of 1:6000 was used, and the minimum concentration per tube that was distinguishable from zero was 5 pg. Cross-reactivities of the PGE₂ antiserum were reported previously (Gross et al., 1988a). There was minimal cross-reactivity of the antiserum with arachidonic acid (<0.1%). A pooled sample of medium from endometrial explant cultures (approximately 3 ng PGE₂/ml) was assayed serially as noted for the PGF RIA. This inhibition curve was parallel to the standard curve, with the test for homogeneity of regression indicating that the curves did not differ. Further characterization of the assay involved measurement of known amounts of PGE₂ as noted for the PGF RIA (Y = 2.5 + 1.04X; Y = amount of PGE₂ measured [pg/0.05 ml]; and X = amount of PGE₂ added [pg/0.05 ml]; R² = 0.90). Inter- and intraassay coefficients of variation were 10.3% and 13.2%, respectively.

**Statistical Analyses**

Data were analyzed statistically via least squares analysis of variance using the General Linear Models procedure of the Statistical Analysis System (1985). Incorporation of radiolabel into proteins and prostaglandin secretion were analyzed using the model components of status (pregnant and cyclic), cow (status), treatment (0%, 10%, 100% bCSP), status x treatment and residual. Data for inhibition of prostaglandin synthesis by endometrial intracellular preparations were analyzed using the model components of status (pregnant and cyclic), cow (status), treatment, status x treatment, preparation (explant medium,
endometrial microsomes and endometrial high-speed supernatant), status X preparation, treatment X preparation, status X treatment X preparation and residual. Treatments were compared within each status using orthogonal contrasts: 0% bCSP versus 10% and 100% bCSP, and 10% bCSP versus 100% bCSP. Effects of time on incorporation of radiolabel into proteins, prostaglandin synthesis, and prostaglandin inhibition by cytosol from endometrium were determined using the model components of treatment (BSA and 10% bCSP), cow, treatment X cow, replicate (treatment X cow), time, cow X time, treatment X time, treatment X cow X time, time X replicate (treatment X cow), and residual. For data on inhibition of PGF synthesis by the prostaglandin-generating system, Dunnett's t-test for comparison of multiple means to a single mean (Steele and Torrie, 1960) was used to compare each endometrial intracellular preparation to the cotyledonary prostaglandin-generating system.

RESULTS

Incorporation of Radio-label into Proteins

Treatment of endometrium from cyclic cows with 10% and 100% bCSP reduced incorporation of [3H]leucine into secreted proteins, whereas treatment of endometrium from pregnant cows with bCSP (10% and 100%) did not alter incorporation into secreted proteins (Fig. 1). This resulted in a significant (p<0.01) status X treatment interaction. In the second experiment, incorporation of [3H]leucine into secreted proteins by endometrium from cyclic cows increased with time (p<0.01) for both BSA- and bCSP-treated tissue. The overall secretion of radiolabeled protein by bCSP-treated tissue was reduced from 6 h of incubation through 24 h of incubation compared to tissue treated with BSA (Fig. 2; treatment X time interaction [p<0.01]).

In the first experiment, treatment of endometrium from cyclic cows with 10% or 100% bCSP increased (p<0.05) incorporation of [3H]leucine into intracellular proteins (13,363 ± 1152 dpm/mg tissue and 15,975 ± 933 dpm/mg tissue, respectively) compared to tissue treated with 0% bCSP (9402 ± 1589 dpm/mg tissue). In the second experiment, however, 10% bCSP treatment reduced (p<0.05) incorporation into intracellular proteins (18,715 ± 1699 dpm/mg tissue) compared to tissue treated with 0% bCSP (9402 ± 1589 dpm/mg tissue). In the second experiment, however, 10% bCSP treatment reduced (p<0.05) incorporation into intracellular proteins (18,715 ± 1699 dpm/mg tissue) compared to tissue treated with BSA (37,180 ± 2665 dpm/mg tissue). Treatment of endometrium from pregnant cows with bCSP (10% and 100%) did not alter incorporation of [3H]leucine into intracellular (tissue) proteins (23,462 ± 642 dpm/mg tissue), although incorporation was greater than that for endometrium from cyclic cows treated with 0% bCSP (9402 ± 1589 dpm/mg tissue) (p<0.01).

Electrophoretic Analysis of Endometrial Proteins

Proteins secreted by endometrial explants from cyclic and pregnant cows at Day 17 post-estrus were
analyzed qualitatively by 1-D and 2-D SDS-PAGE. BSA was present as a major unlabeled protein component regardless of reproductive status. The presence of this protein probably reflects leaching of serum components. Many radiolabeled proteins were secreted by the endometrial explants regardless of reproductive status or treatment. The major radiolabeled proteins were in a 24,000–97,000 $M_r$ range, with isoelectric points of 4.5–6.5. Treatment with bCSP of endometrium from cyclic cows caused a decrease in total protein secretion (Figs. 1 and 2; TCA precipitation results). However, electrophoretic analysis (Fig. 3; equal cpm were utilized from each endometrium-conditioned medium for analyses) revealed that this overall decline in secretion was not accompanied by a decline in intensity of bands associated with specific polypeptides. Generally, all protein bands detected in medium of cultures done with 0% bCSP were also detectable in medium of cultures incubated with 10 and 100% bCSP. This result suggests that the bCSP-induced decline in endometrial protein secretion represents a general decrease in secretory activity rather than a specific inhibition of a few polypeptides.

In fact, bCSP treatment induced the secretion of a few proteins. One of these was a 13,000 $M_r$ protein consisting of one to three isoelectric variants (pI 7.8–8.4, Fig. 4) that was secreted by endometrium from pregnant cows but was not apparent in medium from cyclic cows when cultured with 0% bCSP. This polypeptide was present in culture medium of endometrium from cyclic cows cultured with 10 or 100% bCSP (Fig. 3). In addition, a protein in the 10,000 $M_r$ range was induced by treatment of endometrium from cyclic cows with 10 or 100% bCSP. However, this 10,000 $M_r$ protein was not present for endometrium from pregnant cows regardless of treatment. Secretion of a 50,000 $M_r$ protein by endometrium

![FIG. 3. Fluorograph of a one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoretogram of dialyzed medium from endometrium of a cyclic cow at Day 17 post-estrus cultured in the presence of [3H]leucine and 0, 10, or 100% bovine conceptus secretory proteins (bCSP) for 24 h. An aliquot of each sample of culture medium was lyophilized, dissolved in dissociating buffer, and separated on a 12.5% (w/v) polyacrylamide gel in the presence of a reducing agent. The axis on the side of the gels indicates molecular weight ($X 10^5$). Note the specific induction of 13,000 $M_r$ proteins by bCSP.](image1)

![FIG. 4. Fluorograph of a two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoretogram of dialyzed medium from endometrium of a cyclic cow at Day 17 post-estrus cultured in the presence of [3H]leucine and 10% bovine conceptus secretory proteins (bCSP) for 24 h. An aliquot of medium (100,000 cpm) was lyophilized, dissolved in alkaline urea buffer, separated in the first dimension by isoelectric focusing, and separated in the second dimension on a 12.5% (w/v) polyacrylamide gel in the presence of a reducing agent. The axis on the side of the gels indicates molecular weight ($X 10^5$). Note that bCSP treatment induced a 13,000 $M_r$ protein that consisted of two isoelectric variants (indicated by the arrows).](image2)
from the cyclic cow (Fig. 3) was not present after treatment with bCSP. However, this finding was not apparent for all animals. Treatment of endometrium from pregnant cows with bCSP did not alter the electrophoretic pattern of proteins secreted into medium (Fig. 3) and the 13,000 $M_r$ proteins were always present.

**Prostaglandin Secretion by Endometrial Tissue**

In the first experiment, endometrial PGF secretion was lower ($p<0.01$) and PGE$_2$ secretion higher ($p<0.01$) for pregnant cows than for cyclic cows. Secretion of PGF by endometrium from cyclic cows was decreased by bCSP treatment ($p<0.01$), whereas PGE$_2$ secretion was unaltered by bCSP (Fig. 5). In contrast, secretion of prostaglandins (PGF and PGE$_2$) by endometrial explants from pregnant cows was not altered by treatment with bCSP, resulting in a significant ($p<0.01$) status X treatment interaction. For the second experiment, endometrial prostaglandin secretion (PGF and PGE$_2$) during 48 h of incubation is presented in Figure 6 as the sequential accumulation of prostaglandin secretion throughout 48 h. Prostaglandin secretion continued throughout 48 h of incubation in all treatments. For endometrial explants treated with BSA, prostaglandins accumulated at a rate of 13.9 and 6.5 ng/h for PGF and PGE$_2$, respectively, whereas for explants treated with 10% bCSP, accumulations rates were 8.9 and 5.1 ng/h, respective-

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**FIG. 6.** Effect of 10% bovine conceptus secretory protein (bCSP) treatment on the accumulation of prostaglandins secreted by endometrial explants from cyclic cows during 48 h of incubation. Medium was collected after 24 h of incubation and fresh MEM (15 ml) of the appropriate treatment added. Prostaglandin concentrations at 24 h were added to subsequent sampling times to indicate accumulation throughout 48 h. Results are expressed as ng prostaglandin F ($PGF$) or prostaglandin E$_2$ ($PGE_2$)/0.5 g tissue (mean ± SE). Prostaglandin secretion continued throughout 48 h of incubation, regardless of treatment. However, treatment with 10% bCSP reduced ($p<0.01$) secretion of PGF from 6 h through 48 h of incubation. Secretion of PGE$_2$ was not altered by bCSP treatment.
ly. Treatment with 10% bCSP reduced PGF (p<0.01) accumulation throughout the sampling period, while not significantly (p<0.10) altering PGE₂ accumulation.

**Induction of Intracellular Inhibitor of PGF Synthesis by bCSP**

Medium and tissue from endometrium of pregnant and cyclic cows following 24 h of incubation were analyzed for levels of an inhibitor of prostaglandin synthesis. Endometrial intracellular preparations (microsomes and cytosolic supernatants) from pregnant cows treated with 0% bCSP (p<0.01) decreased PGF synthesis by the cotyledonary prostaglandin-generating system (4.1 ± 0.1 ng PGF/h), and this inhibitory activity was not altered by bCSP treatment (Table 1). For endometrial explants from cyclic cows not treated with bCSP, only the cytosolic supernatants inhibited (p<0.05) PGF synthesis by the cotyledonary prostaglandin-generating system and this inhibition was less (p<0.05) than that for cytosolic supernatant from pregnant cows. Cytosolic supernatants from endometrium of cyclic cows treated with 10 or 100% bCSP markedly reduced (p<0.01) PGF synthesis by the generating system, and this reduction did not differ between 10 and 100% bCSP-treated endometrium. Inhibition of PGF synthesis was greater (p<0.01) for cytosolic supernatant fractions than for microsomes, regardless of reproductive status. Medium from endometrial incubations did not inhibit prostaglandin (PGF) synthesis by the cotyledonary prostaglandin-generating system for explants from cyclic cows (4.2 ± 0.2, 4.0 ± 0.1, and 3.8 ± 0.2 ng/h for 0, 10, and 100% bCSP, respectively) and for 0 and 10% bCSP-treated explants from pregnant cows (3.9 ± 0.2 and 4.0 ± 0.1 ng/h, respectively). However, PGF synthesis by the generating system was slightly decreased (p<0.05) when incubated with medium from endometrial explants of pregnant cows treated with 100% bCSP (3.7 ± 0.2 ng/h). This decrease, associated with medium for 100% bCSP-treated endometrium from pregnant cows, is probably due to endogenous endometrial effects rather than direct effects of bCSP, since unincubated 100% bCSP medium did not inhibit PGF synthesis by the generating system (3.9 ± 0.2 ng/h). In addition, unincubated medium containing 0 and 10% bCSP did not inhibit PGF synthesis by the generating system (4.1 ± 0.2 and 3.9 ± 0.1, respectively).

In the second experiment, endometrial tissues from cyclic cows also were examined for levels of the intracellular inhibitor of prostaglandin synthesis in the cytosolic supernatant after 24 and 48 h of incubation with BSA or 10% bCSP. The cotyledonary prostaglandin-generating system produced 5.4 ± 0.2 ng PGF/h when incubated without endometrial cytosolic supernatant. Intracellular preparations from endometrium treated with BSA did not alter PGF synthesis by the cotyledonary prostaglandin-generating system (5.1 ± 0.1 ng/h at 24 h, 5.2 ± 0.1 ng/h at 48 h), whereas preparations from endometrium treated with 10% bCSP decreased (p<0.05) PGF synthesis (4.5 ± 0.1 ng/h, 17 ± 3% reduction at 24 h; 4.3 ± 0.1 ng/h, 20 ± 2% reduction at 48 h). The amount of bCSP-induced inhibitory activity did not differ between endometrial tissues incubated for 24 and 48 h.

**DISCUSSION**

The in vitro synthesis of PGF and PGFM is lower for endometrium collected from cattle during early pregnancy than for endometrium collected during comparable periods of the estrous cycle (Thatcher et al., 1984; Gross et al., 1988b). Previous reports (Basu and Kindahl, 1987; Gross et al., 1988a) and the present results indicate that endometrial PGF synthesis is reduced during early pregnancy because of

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**TABLE 1. Effect of intracellular preparations from bovine conceptus secretory proteins (bCSP)-treated endometrial explants on the synthesis of prostaglandin F (PGF) by a cotyledonary prostaglandin-generating system (4.0 ± 0.1 ng PGF/h).**

<table>
<thead>
<tr>
<th>Status</th>
<th>Cytosolic supernatant</th>
<th>Microsomes</th>
</tr>
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<tbody>
<tr>
<td>Cyclic:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% bCSP</td>
<td>3.8 ± 0.1b</td>
<td>4.1 ± 0.1bc</td>
</tr>
<tr>
<td>10% bCSP</td>
<td>3.2 ± 0.2**</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>100% bCSP</td>
<td>3.0 ± 0.1**</td>
<td>3.6 ± 0.1*</td>
</tr>
<tr>
<td>Pregnant:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% bCSP</td>
<td>2.4 ± 0.1**d</td>
<td>3.3 ± 0.1**d</td>
</tr>
<tr>
<td>10% bCSP</td>
<td>2.2 ± 0.1**</td>
<td>3.4 ± 0.2**</td>
</tr>
<tr>
<td>100% bCSP</td>
<td>2.0 ± 0.2**</td>
<td>3.1 ± 0.1**</td>
</tr>
</tbody>
</table>

*When data were analyzed across reproductive status, there was a status X treatment interaction (p<0.01).

b-dWithin each status, *0% differs from 10 + 100% bCSP (p<0.05),
10% differs from 100% bCSP (p<0.05), b no treatment differences.

*Differs from generating system (4.0 ± 0.1 ng PGF/h) by Dunnett’s t-test, p<0.05.

**Differs from generating system (4.0 ± 0.1 ng PGF/h) by Dunnett’s t-test, p<0.01.
increased levels of an intracellular endometrial inhibitor of prostaglandin synthesis (Table 1). The inhibitor is present primarily in the cytosolic (100,000 X g) supernatant fraction from endometrial tissue and is present in much higher amounts in endometrium from pregnant cows than from cyclic cows at Day 17 post-estrus. This inhibitor can be induced within a 24-h period by secretory proteins of the conceptus (bCSP) when administered in vitro. This is consistent with the antiluteolytic role of bCSP, which when infused into the uterine lumen extended CL lifespan (Knickerbocker et al., 1986a). The observation that bCSP treatment did not induce levels of inhibitor to an extent similar to levels in endometrium from pregnant cows may reflect an inability of endometrial explants to fully mimic the in vivo uterine environment during early pregnancy.

In spite of the presence of an intracellular inhibitor of PGF and PGE2 synthesis (Gross et al., 1988a), endometrial secretion of PGE2 was greater for pregnant than cyclic cows. This implies that there is a differential regulation of prostaglandin synthesis or that different cell types are influenced by bCSP via induction of an inhibitor of prostaglandin synthesis during early pregnancy. Grasso et al. (1987) demonstrated that PGF is secreted primarily by epithelial cells of bovine endometrium, whereas PGE2 is secreted primarily by stromal cells. Therefore, it is possible that the action of bCSP is primarily on the epithelial cells of the bovine endometrium since PGF secretion was inhibited—probably via the induction of an intracellular inhibitor of prostaglandin synthesis. Since PGE2 production by the epithelium is lower than that from the stroma, it is likely that a net increase or no change in PGE2 synthesis may be observed for endometrium from pregnant cows. Collectively, these results suggest that the intracellular endometrial inhibitor of prostaglandin synthesis is present in the epithelial rather than the stromal cells.

The present study demonstrates that bCSP have a quantitative and qualitative effect on the secretion of radiolabeled endometrial proteins in vitro. Endometrial explants from cyclic cows at Day 17 post-estrus had reduced secretion of radiolabeled proteins into medium when treated with 10 or 100% bCSP regardless of whether 0 or 5 μg BSA/ml were utilized as the control treatments. In addition, secretion of radio-
Laster (1977) did not detect any difference in endometrial protein content between pregnant and nonpregnant cows from Day 2 to Day 15 post-breeding.

Although bCSP caused a consistent decrease in overall protein secretion, there was no repeatable effect on amounts of de novo synthesized intra-cellular proteins. In Experiment 1, bCSP increased radiolabel incorporation into tissue proteins compared to control cultures that did not contain exogenous BSA, whereas in Experiment 2, bCSP decreased radiolabel incorporation as compared to control cultures treated with 5 μg BSA/ml. The discrepancy between the two experiments may be due to the stimulatory effect of BSA on the incorporation of radiolabel into tissue and secreted proteins. The current results do not indicate whether the effect of BSA on protein synthesis is a specific effect of BSA or a nonspecific effect of protein.

In sheep, it has been reported that ovine trophoblast protein-1 (oTP-1) induces the secretion of an acidic protein (70,000 M_r, pl 4.0) by endometrial explants from cyclic ewes at Days 12–16 post-estrus (Godkin et al. 1984). However, a similar protein was not induced in the present study by bCSP even though bCSP contain a protein similar to oTP-1 (Helmer et al., 1987). These data suggest that functional differences for endometrium may exist between cattle and sheep during early pregnancy. Indeed, endometrial prostaglandin secretion is not altered or increases slightly during early pregnancy in sheep (Lacroix and Kann, 1983), whereas in cattle, endometrial PGF secretion is greatly reduced during early pregnancy (Gross et al., 1988a). These differences suggest a different mode of action for conceptus products between these species as well as differing maternal responses for pregnancy recognition.

In conclusion, the present experiment suggests that secretory products of the perimplantation conceptus could play at least two roles in early pregnancy. The first is a selective induction of intracellular factors that act to inhibit endometrial prostaglandin synthesis and therefore prevent luteolysis. The second may involve the regulation of secretion of endometrial proteins that provide an environment suitable for embryonic development.

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