Proteins secreted by Day-16 to -18 bovine conceptuses extend corpus luteum function in cows*

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Summary. Corpus luteum function, interoestrous interval and spontaneous uterine PGF-2 α (PGF) production were evaluated in 9 cyclic Holstein cows (3/group) after intrauterine injections of pooled conceptus secretory proteins, 5 β -pregnan-3 α -ol-20-one, or homologous serum proteins on Days 15.5 through 21 after oestrus. A significant extension of corpus luteum lifespan and interoestrous interval were detected in cows treated with conceptus secretory proteins compared to the other 2 groups. CL lifespan and interoestrous interval were not different ($P > 0.25$) between 5 β -pregnan-3 α -ol-20-one and control groups. Evaluation of spontaneous PGF responses suggested that proteins synthesized and secreted by the bovine conceptus accommodate luteal maintenance during early gestation via an attenuation of endometrial PGF production.

Introduction

An essential manifestation of pregnancy in large domestic species is extended corpus luteum (CL) function. During the first 15–16 days of pregnancy in cattle, progesterone production by the CL establishes a complex uterine environment essential for conceptus (embryo plus extraembryonic membranes) growth and development. Embryos may be transferred, and pregnancies established, as late as Day 16 or 17 after oestrus (Betteridge, Eaglesome, Randall & Mitchell, 1980). Consequently, the presence of a conceptus within the uterine lumen before Day 16 is not a requirement for initiation of an embryotrophic uterine environment. Beyond this point, however, a viable conceptus within the uterine lumen must play an active role in the perpetuation of its embryotrophic environment by maintenance of the CL (Northey & French, 1980; Betteridge *et al.*, 1980; Dalla Porta & Humblot, 1983).

The conceptus of the cow produces an array of potential 'signals' during early pregnancy, including steroids (Shemesh, Milaguir, Ayalon & Hansel, 1979; Chenault, 1980; Gadsby, Heap & Burton, 1980; Eley, Thatcher, Bazer & Fields, 1983), prostaglandins (Shemesh *et al.*, 1979; Lewis, Thatcher, Bazer & Curl, 1982) and proteins (Bartol *et al.*, 1984). However, conceptus factors responsible for luteal maintenance during early pregnancy and their mechanisms of action have not been demonstrated clearly in cattle. Numerous studies have evaluated the effect of prostaglandin E-2 (PGE-2) on luteal maintenance in cattle with mixed results. Administration of PGE-2 into the uterine lumen alone (Gimenez & Henricks, 1983; Chenault, 1983) or in combination with oestradiol-17 β (Reynolds, Robertson & Ford, 1983) extends luteal function only slightly beyond the cessation of intrauterine PGE-2 treatments. In addition, systemic progesterone concentrations declined within 12 h after PGE-2 treatment (Gimenez & Henricks, 1983) or during the treatment

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period (Reynolds *et al.*, 1983), suggesting that PGE-2 (plus oestradiol-17 β) will not prevent production and transfer of uterine luteolytic substances, but affects luteal function directly (Marsh, 1970; Henderson, Scaramuzzi & Baird, 1977; Reynolds *et al.*, 1981). Others have reported no effect of intrauterine PGE-2 administration on CL maintenance in cattle (Dalla Porta & Humblot, 1983; Chenault, Van Ravenswaay & Campbell, 1984).

Extension of CL maintenance and cycle length were demonstrated after intrauterine administration of conceptus homogenates to cyclic cattle (Northey & French, 1980). Likewise, extracts and homogenates of sheep conceptuses (Rowson & Moor, 1967; Ellinwood, Nett & Niswender, 1979; Martal, Lacroix, Loudes, Saunier & Wintenberger-Torres, 1979) or conceptus secretory proteins (Godkin, Bazer, Thatcher & Roberts, 1984b) have been shown to extend CL function and cycle length when administered into the uterine lumen of cyclic ewes. However, luteotrophic and/or anti-luteolytic actions or major conceptus-produced steroids and proteins have not been evaluated in cattle. The objectives of the present experiment were to examine the effects of a major steroid produced by the cow conceptus (Eley *et al.*, 1983), 5 β -pregnan-3 α -ol-20-one, and bovine conceptus secretory proteins on luteal function, cycle length and spontaneous uterine PGF production in cyclic cattle.

Materials and Methods

Conceptus collection and culture. Dairy and beef cows ($N = 49$) served as conceptus donors after being mated during oestrus (Day 0). Between Days 16 and 18 after mating (17.2 ± 0.6 days), the uterine horn ipsilateral to the CL-bearing ovary was nonsurgically flushed (Bartol *et al.*, 1981). A French Foley catheter (size no. 16, 18 or 20; American Hospital Supply, Jacksonville, FL) was inserted through the cervical os and positioned at the base of the uterine horn just anterior to the uterine body. Sterile phosphate-buffered saline (pH 7.4; PBS; Dulbecco & Vogt, 1954) was warmed to 37°C and injected into the uterine lumen at a volume of 60 ml per flush. Medium containing conceptuses ($n = 33$) was collected into sterile glass containers, maintained at 37°C, and transported to the laboratory within 30 min of recovery. After the last uterine flush, 20 ml of an antibiotic solution (aqueous Procaine Penicillin G; 300 000 units/ml; Pfizer Incorporated; New York, NY) were infused into the uterine lumen, and the Foley catheter was removed. Donor cows were mated and flushed up to 3 times during successive oestrous cycles.

An additional 4 cows were superovulated, mated and slaughtered at 17 days *post coitum*. The uterus was removed after exsanguination, sealed in a plastic bag, and placed on ice while being transported to the laboratory (within 60 min of slaughter). Uterine horns were trimmed of excess tissue, ovaries and oviducts were removed, and a large, curved, Rochester-Ochsner forcep was applied to the anterior cervix. The anterior tip of the uterine horn ipsilateral to the CL containing ovary was cut to provide an enlarged opening. A plastic, 50-ml syringe fitted with an 18-gauge needle was used to administer two 30-ml flushes (sterile PBS, warmed to 37°C) into the uterine lumen through the tip of the uncut uterine horn. Medium containing conceptuses was collected into sterile plastic culture dishes. About 15–20 conceptuses were obtained by culturing conceptuses from the superovulated cows. All conceptuses, collected either nonsurgically or at post-mortem flush, were washed in and transferred to sterile culture dishes containing 15 ml of warm Minimum Essential Medium (MEM; GIBCO, Grand Island, NY) supplemented with non-essential amino acids (GIBCO), antibiotic/antimycotic (GIBCO), 200 units insulin/l (Sigma Chemical Co., St Louis, MO) and 1 g glucose/l (Fischer Scientific; Orlando, FL). Conceptuses were cultured for 24 h on a rocker platform (Bellco Glass Company, Vineland, NJ) and maintained at 37°C in a gaseous environment of nitrogen:oxygen:carbon dioxide (50:45:5, by vol.). After incubation for 24 h tissues and medium were separated by centrifugation (10 000 g; 20 min) at 4°C. Medium (supernatant) from each culture was collected and frozen individually. Conceptus wet weights were recorded.

Preparation of material for intrauterine injections. Medium from individual incubations (15 ml) was dialysed (Spectrapore 6, 1000 M_r cutoff, Spectrum Medical Industries, Los Angeles, CA) extensively (4 l changed thrice daily for 5 days) against 10 mM-Tris-HCl buffer, pH 7.2 (Tris). After dialysis, an aliquant of medium from each culture ($n = 47$) was used for determination of protein concentration (Lowry, Rosebrough, Farr & Randall, 1951). All culture medium was then pooled and concentrated by ultrafiltration (1000 M_r cutoff; Amicon Corporation, Danvers, MA) to a volume of ~ 75 ml. The concentrated filtrate was processed through a sterilization filter unit (0.45 μ m pore size; Sybron/Nalgene, Rochester, NY) and dispensed into 2-ml samples designated as pooled conceptus secretory proteins (CSP) at a protein concentration of 740 μ g/2 ml.

A serum sample from each experimental animal ($N = 9$) was collected on Day 10 of the oestrous cycle. Serum samples were dialysed individually, diluted in Tris and sterilized as described for CSP. Equal masses (740 μ g) of homologous serum proteins were added to each 2 ml injection of CSP (3 cows; 12 injections/cow; Group 1).

Two milligrams of 5 β -pregnan-3 α -ol-20-one (5 β -P) were dissolved in 2 ml ethanol and mixed with 80 ml Tris. Ethanol was evaporated from the solution using N_2 gas and gentle heating. The steroid solution was sterilized as described previously and dispensed into 2 ml aliquants (50 μ g each). Homologous serum proteins (740 μ g) were added to each 2 ml injection of 5 β -P (3 cows; 12 injections/cow; Group 2). Group 3 cows ($N = 3$) received homologous serum proteins alone (1480 μ g/2 ml Tris; 12 injections/cow). All treatment mixtures were frozen (-20°C) until time of intrauterine injection.

Animal preparations. Nine cyclic Holstein cows were assigned randomly to the three previously described treatment groups. Animals were prepared for surgery (Wolfensen *et al.*, 1985) on Day 10 of the oestrous cycle. Utilizing a midventral laparotomy, the uterus and ovaries were exposed and location of the CL were recorded. A sterile polyvinyl catheter (V-6; Bolab Incorporated, Lake Havasu City, AZ) was inserted via an incision in the isthmus of the oviduct and secured 30–50 mm into the anterior portion of the uterine lumen, ipsilateral to the CL. The catheter was exteriorized via a small flank incision. An additional catheter (Silastic tubing, i.d. 1.57 mm, o.d. 3.18 mm; Dow Corning Corporation, Midland, MI) was advanced about 1.07 m into the saphenous vein to a position in the dorsal vena cava slightly anterior to the point of utero-ovarian venous drainage. Jugular catheterizations (V-9; Bolab) were performed, if necessary, at the time of vena cava catheter failure during the experiment. Antibiotics (Polyflex, 167 mg/ml; Bristol Laboratories, Syracuse, NY) were administered on the day of surgery (10 ml i.m., 10 ml i.p. and 1 ml intrauterine) and 1 day after surgery (10 ml i.m. and 1 ml intrauterine).

Function of the CL was monitored by measuring plasma progesterone concentrations from blood samples collected twice daily (08:00 and 20:00 h) beginning on Day 12 and continuing until detection of oestrus. Three acute bleedings were conducted to monitor spontaneous uterine prostaglandin (PG) production. Plasma concentrations of PGF were measured in blood samples withdrawn every 15 min from 08:00 to 14:00 h on Days 18–20. Intrauterine injections of experimental materials (2 ml; see above) were begun at 20:00 h on Day 15 and continued every 12 h until 08:00 h of Day 21. All animals were fitted with oestrus detection devices (KaMar Incorporated, Steamboat Springs, CO), maintained on pasture in the presence of an intact bull, and observed 2–4 times daily for oestrous behaviour. Corpus luteum regression was verified by rectal palpation after oestrus. At the end of the experiment, all animals were slaughtered and reproductive tracts dissected to assess utero-ovarian condition and catheter placement.

Progesterone and prostaglandin radioimmunoassay. Progesterone was measured in heparinized plasma samples utilizing a specific antiserum generated in sheep against progesterone conjugated to bovine serum albumin (BSA) at the C11-position. [1,2,6,7- ^3H (N)]progesterone (sp. act. 97 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Cross-reactivity of the progesterone antiserum was $<1\%$ with 17 α -hydroxyprogesterone, 20 α -hydroxyprogesterone, 20 β -dihydroxyprogesterone, cortisol, testosterone, androstenedione and oestradiol-17 β . Standard

curves were prepared by adding known amounts of radioinert progesterone to phosphate-buffered saline (pH 7.4) containing 1 g gelatin/l (PBSg). The final concentrations of progesterone were: 0, 15.6, 31.2, 62.4, 125, 250, 500 and 1000 pg/100 µl PBSg. Utilizing an antiserum dilution of 1:35 000, sensitivity of the assay was 15.6 pg. A plasma sample containing ~4.5 ng of immunoreactive progesterone/ml was measured in triplicate into sample volumes of 50, 100, 200 and 300 µl. Progesterone from plasma samples was extracted by vortexing for 2 min with 2 ml freshly distilled benzene-hexane (1:2 v/v). Solvent containing the extracted progesterone was dried under N₂ and brought to a 500 µl assay volume with the addition of PBSg. A quantitative linear recovery was achieved [$y = 20.965 + 4.111x$; y = concentration of progesterone (pg/100 µl) and x = plasma volumes extracted (µl)]. No significant differences were found between concentrations of progesterone (pg/100 µl) measured using volumes of 50, 100, 200 or 300 µl ($P > 0.25$). Exogenous progesterone was added to a plasma sample (mean = 1.8 ng/ml) at doses of 0, 0.5, 1.0, 5.0 and 10 ng/ml. All doses were replicated 4 times. A linear regression equation of added vs measured progesterone [$y = 1626.1 + 1.0855x$; y = amount of progesterone measured (pg/ml) and x = amount added (pg/ml); $R^2 = 0.975$] described differences among concentrations. The intra-assay coefficient of variation (CV) for the validation was 13.4%. Intra- and interassay CVs for 8 assays were: 10.4 and 7.5%, respectively, when duplicate estimates were run in the assays for a 100 pg/500 µl (94.2 ± 4.3 pg/500 µl) reference plasma sample, and 6.1 and 4.3%, respectively, when duplicate estimates were run for a 250 pg/500 µl (269.5 ± 7.0 pg/500 µl) sample of reference plasma.

PGF was assayed in unextracted plasma samples (50, 100 and 200 µl). Concentrations of PGF were determined in a dextran-coated charcoal radioimmunoassay system previously validated for use in our laboratory (Wolfenson *et al.*, 1985). Antiserum used in the assay was generated in goats against PGF conjugated to bovine serum albumin at the C1-position. This antiserum crossreacts 53.6% with PGF-1 α and <1% with 15-keto-PGF-2 α , 15-keto-13,14-dihydro-PGF-2 α (PGFM), PGE-1, PGE-2 and arachidonic acid. Sensitivity of the assay was 10 pg/tube. The intra- and interassay CVs for 13 assays were: 20.0 and 12.7%, respectively, when duplicate estimates were run for a 50 pg/200 µl (60.9 ± 2.5 pg/200 µl) plasma reference, and 10.6 and 5.6%, respectively, when duplicate estimates were run for a 500 pg/200 µl (489.9 ± 10.1 pg/200 µl) plasma reference.

Statistical analyses. Data for progesterone and PGF concentrations in plasma, and accumulated PGF were analysed using the General Linear Models procedure of the Statistical Analysis System (SAS Institute Incorporated, 1982) for a split-plot analysis of variance with repeated measurements over time. Analysis of variance considered variability due to treatment (CSP, 5 β -P and Control), cow nested within treatment, time (for progesterone: Days 12–38.5; for PGF: samples 1–75 which consisted of 25 samples on each of Days 18–20), treatment by time interaction,

Table 1. Effects of intrauterine treatments with conceptus secretory proteins (CSP), 5 β -pregnan-3 α -ol-20-one (5 β -P) or homologous serum proteins (Control) on cycle length, luteal function and PGF production

Treatment	Cycle length (days)	Days with progesterone > 1 ng/ml	Mean accumulated PGF (pg/cow/day)
CSP	33.4 $\pm$ 2.5**	30.3 $\pm$ 1.9**	39 $\pm$ 35** (6)†
5 β -P	24.7 $\pm$ 0.8	22.7 $\pm$ 1.0	1322 $\pm$ 544** (7)
Control	23.5 $\pm$ 0.5	22.3 $\pm$ 0.6	496 $\pm$ 210 (8)

Values are mean $\pm$ s.e.m.

** $P < 0.01$ (orthogonal comparisons: CSP vs 5 β -P and Control; 5 β -P vs Control).

†Values are number of acute 6-h bleedings.

and cow nested within treatment by time interaction. Estimates for PGF produced over the 6-h sampling periods on Days 18–20 were generated by sequential summation of PGF concentrations measured in plasma on Days 18–20. Using this method, a series of PGF values were generated over days for each sample within cow. Each PGF value corresponded to the PGF concentration in the respective sample plus a summation of PGF concentrations in all prior samples (accumulated PGF). To provide estimates of temporal changes, progesterone concentrations and accumulated PGF values were analysed by least squares regression analyses, and differences in treatment means were evaluated by orthogonal comparisons (CSP *versus* 5 β -P and Control; 5 β -P *versus* Control). Differences in polynomial regressions were tested by examining for homogeneity of regression between treatment response curves. These data were analysed with time as a continuous, independent variable. Data pertaining to cycle length, days with progesterone concentrations > 1 ng/ml, and mean accumulated PGF (Table 1) as well as effects of age of conceptus on its wet weight, and μ g protein produced/mg conceptus wet weight (Table 2) were analysed by a one-way analysis of variance. Treatment differences were evaluated by orthogonal comparisons.

Results

Evaluation of reproductive tracts

Cows were palpated *per rectum* following detected oestrus. In all cases, a single regressing CL was detected on the same ovary bearing a functional CL at surgery. At dissection of reproductive tracts after slaughter, all catheters were intact and patency was verified. General appearance of endometrium and utero-ovarian tissues was normal for all experimental animals.

Effects of intrauterine injections on interoestrous interval and corpus luteum function

Oestrous cycle lengths immediately preceding the experimental oestrous cycle were not different ($P > 0.25$) amongst cows assigned to the three groups (20.57 ± 0.89 days). Intrauterine administration of CSP to cyclic cows resulted in extended oestrous cycle lengths ($P < 0.01$) of 30, 31.75 and 38.5 days as compared to cows which received 5 β -P (23, 25.5 and 25.5 days) and Control (22.5, 24 and 24 days) injections. No differences were detected ($P > 0.25$) in interoestrous intervals between cows of the 5 β -P and Control groups (Table 1).

Analysis of plasma progesterone concentrations verified observations associated with rectal palpations and interoestrous intervals (Fig. 1; Table 1). Function of the CL (progesterone concentrations > 1 ng/ml) was maintained for 28, 29 and 34 days after CSP treatments, whereas CL lifespans of cows in the 5 β -P (21, 22.5 and 24.5 days) and Control (21.5, 22 and 23.5 days) groups were shorter ($P < 0.01$). 5 β -Pregnan-3 α -ol-20-one did not influence CL lifespan as compared to Control cows treated with homologous serum proteins alone. Vena cava catheters were maintained to about Day 21 of the experimental cycle (21.17 ± 1.05 ; Fig. 1) when jugular catheters were installed (8 of 9 cows). After loss of vena cava catheter patency, 7 of 8 experimental cows exhibited mean $\pm$ s.e.m. jugular concentrations of progesterone of 6.1 ± 1.8 ng/ml. Concentrations of progesterone were 2–10 times higher in vena cava plasma immediately before failure of the vena cava catheter (17.5 ± 6.3 ng/ml) than in jugular plasma.

Effects of intrauterine injections on plasma prostaglandin concentrations

Analysis of PGF responses suggested that proteins secreted by bovine conceptuses reduced uterine production of PGF (Fig. 2). Circulating PGF exhibits a relatively short half-life (7–8 min; Kindahl, Edqvist, Bane & Granstrom, 1976) due to its rapid metabolism by the lung and peripheral tissues (Granstrom & Kindahl, 1982). Therefore, plasma samples from the vena cava were utilized

for PGF determinations. Higher plasma concentrations of progesterone in vena cava versus jugular vein samples were used as verification of catheter placement before PGF data analysis. The number of cows contributing to PGF responses in vena cava plasma for each group on Days 18, 19 and 20 were: CSP, 2, 2, 2; 5 β -P, 3, 3, 1; and Control, 3, 3, 2, respectively. A significant treatment by time interaction ($P < 0.05$) was detected for plasma PGF concentrations in the vena cava and supported a role for CSP in the reduction of PGF production. Pulsatile episodes of PGF concentrations in the vena cava were apparent in plasma samples collected from all cows of the 5 β -P treatment group during Days 18–20. Similarly, spontaneous elevations of PGF in vena cava plasma were detected in 2 of 3, 3 of 3, and 1 of 2 Control cows on Days 18, 19 and 20, respectively. In contrast, no measurable PGF was detected in vena cava plasma from 2 of 2 cows in the CSP treatment group on

Fig. 1. Least squares means for plasma progesterone concentrations in cyclic cattle (3 per group) after intrauterine injections of (a) homologous serum proteins (control), (b) 5 β -pregnan-3 α -ol-20-one, or (c) bovine conceptus secretory proteins from Days 15.5 to 21. The durations (days) of vena cava catheter patency for each cow were 19.5, 22.0 and 22.0 for Cows 1, 2 and 3; 19.5, 20.0 and 21.0 for Cows 4, 5 and 6; and 18.0, 20.5 and 28.0 for Cows 7, 8 and 9.

Fig. 2. Least squares means for concentrations of PGF in vena cava plasma collected from experimental cattle every 15 min for 6 h on Days 18, 19 and 20. Intrauterine treatments with (a) homologous serum proteins (control), (b) 5β -pregnan- 3α -ol-20-one, or (c) conceptus secretory proteins were administered twice daily from Days 15.5 to 21. Number of cows represented on each day are indicated in parentheses.

Table 2. Bovine conceptus† wet weight and protein production

Age (days)	No. of cows	Wet weight (mg)	Protein (μ g/mg wet weight)
16.5	9	31.7 ± 7.7	$4.9 \pm 2.4^\ddagger$
17.0	13	35.1 ± 3.6	15.8 ± 5.2
17.5	4	27.6 ± 8.0	12.0 ± 4.1
18.0–18.5	6	$55.8 \pm 8.6^*$	15.9 ± 5.3

Values are mean $\pm$ s.e.m.

†Data from conceptuses collected after superovulation are not included in analysis ($n = 15$ cultures; 22.3 ± 2.0 μ g protein/mg wet weight).

* $P < 0.05$ (orthogonal comparison: 18.0–18.5 vs 16.5, 17.0, 17.5).

‡ $P < 0.05$ (orthogonal comparison: 16.5 vs 17.0, 17.5, 18.0–18.5).

Days 18 and 19. One plasma sample did contain measurable amounts of PGF in 1 of 2 cows on Day 20. Profiles of PGF concentrations (least squares means) for each group are depicted in Fig. 2. Concentrations of PGF were also accumulated over the 3 days sample and analysed statistically. Orthogonal comparisons of treatment means indicated that intrauterine administration of CSP resulted in a mean accumulation per cow of less PGF than in cows after 5 β -P and Control treatments (Table 1; $P < 0.01$). Additionally, accumulation of PGF was greater in cows treated with 5 β -P compared to cows that received homologous serum proteins alone ($P < 0.01$).

Bovine conceptus observations

Thirty-two conceptuses were classified according to their age (Days 16.5, 17, 17.5 and 18–18.5) at the time of nonsurgical flushing (Table 2). Although conceptus wet weights did not increase significantly until between Day 17.5 and Day 18.0 to 18.5 after insemination, tissue secretory activity (μ g protein produced/mg conceptus wet weight) increased ~3-fold in conceptuses 17 days and older ($P < 0.05$) compared to those recovered on Day 16.5.

Discussion

Maternal recognition of pregnancy occurs by Day 16 to 17 in cattle (Northey & French, 1980; Betteridge *et al.*, 1980; Dalla Porta & Humblot, 1983). This represents a critical period during early gestation when production of conceptus signals becomes essential to luteal maintenance and continued endometrial secretory activity (histotroph). A phase of rapid conceptus elongation and differentiation is intimately associated with this period of signal transmission by the bovine conceptus (Chang, 1952; Greenstein & Foley, 1958a,b; Greenstein, Murray & Foley, 1958). Developmental stage of the conceptus may therefore determine the timing of conceptus signal production and secretion during early pregnancy (Bartol *et al.*, 1984).

In the present study, total protein production per mg of conceptus tissue increased after the initiation of conceptus elongation on Day 16. This increase in protein secretory activity was observed before any detectable increase in conceptus mass (Table 2). Conceptus mass may not be an appropriate index of conceptus expansion since Geisert, Brookbank, Roberts & Bazer (1982) concluded that the initial rapid elongation of the pig conceptus was due to cellular remodelling and not hyperplasia, whereas subsequent conceptus elongation was associated with increasing DNA and RNA content. In bovine conceptus incubations, [3 H]leucine incorporation into nondialysable secretory products (presumably proteins) supported an increase in production of labelled proteins from Days 16 to 19 and 22 (Bartol *et al.*, 1984). An increase in conceptus tissue secretory activity therefore occurs concomitantly with elongation of the cow conceptus.

Based on the progesterone profiles (Fig. 1; Table 1) and PGF data (Fig. 2; Table 1), conceptus secretory proteins appeared not to stimulate luteal synthesis of progesterone (luteotrophic), but allowed for extended luteal function via local suppression of spontaneous uterine PGF production (antiluteolytic). A significant reduction in oestradiol-induced uterine PGF production after intrauterine administration of bovine CSP to cyclic cows was demonstrated by Knickerbocker, Thatcher, Bazer, Barron & Roberts (1984). Collectively, these data indicate that proteins secreted by the bovine conceptus are involved in mediation of antiluteolytic effects during early pregnancy. Reduction in uterine luteolytic activity by conceptus secretory proteins may involve regulation at several levels of the arachidonic acid metabolic cascade (Thatcher *et al.*, 1984; Milvae & Hansel, 1984), control of endometrial oxytocin and steroid receptor populations (McCracken, Schramm, Barcikowski & Wilson, 1981; McCracken, 1984) or stimulation of endometrial prostaglandin inhibitors (Wlodawer, Kindahl & Hamberg, 1976; Shemesh, Hansel & Strauss, 1984).

In this study, protein signals secreted in culture by elongating cow conceptuses (Days 16.5–18.5) extended luteal function and interoestrous interval to about 30 and 33 days, respectively, when

administered into the uterine lumen of cyclic cattle on Days 15–21 after oestrus. Similar data were reported by Godkin *et al.* (1984b); intrauterine administration of the low molecular weight acidic polypeptide, termed ovine trophoblast protein-1 (oTP-1), extended luteal function in cyclic ewes. Roles of specific proteins secreted by the cow conceptus have not been examined. However, several recent reports suggest that there may be similarities in the nature and function of conceptus protein signals in cattle and sheep during early pregnancy.

Major components of the protein synthesized and released in culture by Day 16–24 cow conceptuses are low molecular weight, acidic polypeptides (Bartol *et al.*, 1984), similar in nature to those produced by the elongating sheep conceptus (Days 13–21); Godkin, Bazer, Sessions & Roberts, 1982; Martal, Charlier, Camous, Fevre & Heyman, 1984b). When cow (Day 14) and sheep (Day 11–13) trophoblastic vesicles, composed of extraembryonic trophectoderm and endoderm, were transferred and allowed to develop in cyclic cattle and sheep, respectively, a majority of the recipients exhibited prolonged CL maintenance (Heyman, Camous, Fevre, Méziou & Martal, 1984; Martal *et al.*, 1984b). These data support previous reports (Godkin *et al.*, 1982; Godkin, Bazer & Roberts, 1984a) that the extraembryonic trophectodermal layer of the conceptus secretes proteinaceous signals responsible for CL maintenance in early pregnancy. In a related study (Martal, Camous, Fevre, Charlier & Heyman, 1984a), cross-species transfers of cow and sheep trophoblastic vesicles led to extended CL function in about 20% of ewe and cow recipients. The authors suggested that nonspecific conceptus signals in the cow and ewe were sufficient for maintaining CL function, and the biologically active molecules responsible for CL maintenance in these species may be very similar. Additional support for this argument was obtained when antibodies produced against oTP-1 (Godkin *et al.*, 1984a) were found to cross-react with low molecular weight polypeptides secreted by the bovine conceptus (Helmer, Hansen, Thatcher, Roberts & Bazer, 1985). These observations indicate that luteal maintenance during early gestation may be achieved by analogous mechanisms in cattle and sheep.

The duration of CL extension in recipient cattle (range 25–37 days) after species-specific transfers of trophoblastic vesicles (Heyman *et al.*, 1984), and transfers of sheep trophoblastic vesicles (31 and 35 days; Martal *et al.*, 1984a) is similar to luteal lifespans achieved in this study (Fig. 1; Table 1) after intrauterine treatments with pooled bovine conceptus secretory proteins in cyclic cattle (range 28–34 days).

In contrast to the observed antiluteolytic activity of conceptus secretory proteins, 5 β -pregnan-3 α -ol-20-one, a major steroid produced by the conceptus, did not influence CL lifespan, cycle length or decrease uterine PGF production. In fact, 5 β -pregnan-3 α -ol-20-one apparently increased uterine PGF production. It is possible that the progestational activity of this 5 β -reduced progestagen was sufficient to stimulate endometrial accumulation of lipid stores and prostaglandin precursors, thereby resulting in a greater uterine ability to synthesize PGF.

In conclusion, intrauterine administration of bovine conceptus secretory proteins to cyclic cattle was shown to extend CL lifespan and interoestrous interval, and attenuate spontaneous uterine PGF production (PGF in vena cava plasma). These results provide strong evidence that protein signals secreted by the conceptus during the first 2–3 weeks of gestation in the cow are required for the establishment of pregnancy.

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