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Retinol-Binding Protein Gene Expression in Cyclic and Pregnant Endometrium of Pigs, Sheep, and Cattle[1]

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ABSTRACT

Retinol-binding protein (RBP) is a major secretory product of conceptuses and endometrium of the large domestic animals. The present study examined the abundance of RBP mRNA in cyclic and early pregnant endometrium of pigs, sheep, and cattle and confirmed the presence of RBP mRNA in periimplantation conceptuses of the large domestic animals. Northern blot analysis, using porcine liver RBP cDNA as the probe, detected a 1.0–1.1-kb transcript in conceptuses and endometrium collected during the periimplantation period of pregnancy in pigs (Day 15), sheep (Day 16), and cows (Day 18). Slot-blot analysis of RBP mRNA in endometrium of pigs, sheep, and cows indicated differential RBP gene expression across days and statuses in pigs and sheep and across days alone in cows. In pigs, RBP gene expression was low to undetectable at Days 0, 5, and 10 of the estrous cycle and Day 10 of pregnancy. Levels of RBP mRNA increased ($p < 0.06$) from Days 10 to 12 and further ($p < 0.001$) to Day 15 across both statuses. At Day 18, RBP mRNA levels decreased ($p < 0.01$) in cyclic pigs but remained elevated in endometrium of pregnant pigs. In sheep, levels of RBP mRNA increased between Days 10 and 12 and 14 in both cyclic and pregnant ewes; however, between Days 14 and 16, RBP mRNA levels remained elevated in cyclic endometrium but decreased ($p < 0.01$) in endometrium of pregnant ewes. In a second experiment, RBP mRNA levels increased ($p < 0.01$) between Days 0–5 and Days 13–15 of the estrous cycle. RBP gene expression was similar in pregnant and cyclic ewes on Days 11 and 13, then increased in cyclic ($p = 0.07$) but not pregnant ewes, supporting results from experiment 1. Levels of RBP mRNA increased in endometrium of both cyclic and pregnant cows between Days 10, 15, and 18; then cows, like sheep but unlike pigs, exhibited RBP mRNA at estrus (Day 0) and metestrus (Day 5). Results suggest differential RBP gene expression in endometrium of large domestic animals and support an important role for the vitamin A transport protein in uterine and conceptus physiology of early pregnancy.

INTRODUCTION

Periimplantation conceptuses of the domestic farm animals, i.e., sheep, cattle, and pigs, enter the uterus during early pregnancy and remain free until the second week of pregnancy when cellular interactions between conceptus and the endometrial epithelium begin [1]. Prior to implantation, conceptuses are supported solely by secretions that accumulate in the uterine lumen [1, 2]. In fact, since conceptuses of large domestic farm animals undergo noninvasive epitheliochorial placentation and the uterine epithelium is never eroded during pregnancy, uterine secretions may be required for nutritional support of the conceptus throughout gestation [1]. For this reason, uterine secretions of cattle [3–8], sheep [9–12], and pigs [13–17] have received considerable attention.

The majority of protein production by uterine endometrium and transport of serum-derived proteins into the uterine lumen occurs during the luteal phase of the estrous cycle, in pregnancy, or in response to exogenous progesterone (P) in cattle [3, 4, 6, 7], sheep [9, 11, 18], and pigs [13, 14, 19]. Although P is responsible for stimulating synthesis of proteins by the uterine endometrium, estrogen (E) stimulates rapid accumulation of uterine luminal protein in cows [5] and pigs [17, 20, 21]. Estrogen, secreted by periimplantation pig conceptuses, stimulates uterine endometrial secretory activity resulting in accumulation of histotroph in the uterine lumen for utilization by developing conceptuses [17, 20, 21].

A major component of uterine histotroph is the vitamin A transport protein, retinol-binding protein (RBP) [15, 22]. Uterine RBP, of M_r 21 000 and secreted as at least four isoforms, binds both retinol and retinoic acid (RA) in vitro, although its in vivo ligand appears to be retinol [22]. Like most proteins in uterine secretions of pigs, RBP is stimulated by P [15]. Immunoreactive RBP is present in both surface and glandular endometrial epithelium at Day 15 of pregnancy [23], and RBP mRNA in endometrium at Day 15 of pregnancy is equivalent in magnitude to adult liver [24]; this evidence suggests that RBP is a major transcript and secretory product of the periimplantation pig uterus. Amino terminal amino acid microsequencing of the individual isoforms of uterine RBP [25] suggests that it is identical to pig serum [22] and pig conceptus [23, 24, 26] RBP.

Retinol-binding protein is also a secretory product of cow [8, 27] and probably sheep endometrium [28]. Thomas et al. [8] reported that immunoreactive RBP was present in uterine flushings and endometrial explant culture media of Day 17 cyclic, bred/nonpregnant, and pregnant cows. Three molecular-weight species of RBP were detected, but the

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22 000 form was predominant; this is consistent with results from pigs [15, 22]. Addition of cyclohexamide to explant cultures decreased accumulation of the 22 000 M_r form, confirming in vitro synthesis by the uterine endometrium. Similarly, Liu et al. [27] immunoprecipitated a family of low molecular weight proteins synthesized de novo by bovine uterine endometrial explant cultures. Like porcine RBP, bovine uterine RBP increased in uterine flushings of ovariectomized cows in response to exogenous P treatment [8] and in intact cows during the late luteal phase of the estrous cycle [27]. Bovine uterine RBP [8] is probably analogous to the polypeptides in class II (M_r = 19 000–24 000; pI = 5.4–6.3) of endometrial explants [6]. Endometrial explant cultures from sheep at Days 10 [29] and 12 [30, 31] of pregnancy have radiolabeled proteins present with electrophoretic mobilities similar to bovine [6] and porcine [22] uterine RBP.

Retinol-binding protein is also a major secretory product of conceptus tissues from pigs [23], cattle [26, 32], and sheep [26, 33, 34]. Secretion of RBP by uterine endometrium and conceptus tissues during the periimplantation period of pregnancy may be important for local transport of retinoids to and within developing conceptuses. Retinoids have numerous effects on cell and tissue biology during embryonic development and throughout pregnancy [35–37] and may influence morphological changes during blastocyst expansion, conceptus elongation, placentation, and subsequent development. RBP expression may be essential for sequestration and transport of retinoids for mediating these effects. The objectives of the present study were to 1) evaluate levels of RBP mRNA in cyclic and early pregnant endometrium of pigs, sheep, and cattle and 2) confirm the presence of RBP mRNA in periimplantation conceptuses of the large domestic animals.

MATERIALS AND METHODS

Animals

Sexually mature crossbred gilts (n = 30), Rambouillet ewes (n = 54), and Hereford beef cows (n = 24) that had exhibited two estrous cycles of normal duration (18–22, 16–17, and 18–22 days, respectively) were assigned at random to either cyclic or pregnant status. Gilts and ewes were observed for estrous behavior daily and were mated to fertile boars and rams, respectively, on the morning of the onset of estrus (Day 0) and 12 and 24 h later. Cows were mated to fertile bulls upon detection of estrus.

Tissue Collection

Endometrium was obtained from gilts at hysterectomy on Days 0, 5, 10, 12, 15, and 18 (n = 3/day) of the estrous cycle and Days 10, 12, 15, and 18 (n = 3/day) of pregnancy. Gilts were assigned randomly within status to be unilaterally hysterectomized on either Days 10 and 12 or Days 15 and 18. An additional group of cyclic gilts were unilaterally hysterectomized on Days 0 and 5. Endometrium was obtained from ewes at hysterectomy on Days 10, 12, 14, and 16 (n = 4/day; experiment 1) or Days 0, 5, 11, 13, and 15 (n = 3/day; experiment 2) of the estrous cycle and Days 10, 12, 14, and 16 (n = 4/day; experiment 1) or Days 11, 13, and 15 (n = 3/day; experiment 2) of pregnancy. Care was taken to include equal amounts of caruncular and intercaruncular endometrium from both uterine horns. Endometrium was obtained from cows at slaughter (within 10 min of exsanguination) on Days 0, 5, 10, 15, and 18 (n = 3/day) of the estrous cycle and Days 10, 15, and 18 (n = 3/day) of pregnancy. Equal amounts of caruncular and intercaruncular endometrium from the uterine horn ipsilateral to the CL were collected.

Reproductive tracts were flushed with 20 ml sterile saline (0.9%) and the flushings examined for infection or presence of conceptuses to confirm pregnancy in mated animals. Conceptuses from Days 15, 16, and 18 of pregnancy in pigs, sheep, and cows, respectively, were isolated and immediately frozen in liquid nitrogen. Endometrium was excised carefully from underlying myometrium, placed in bags, frozen in liquid nitrogen, and stored at −80°C until processed for RNA.

RNA Isolation and Analyses

Total RNA was isolated from endometrium and conceptuses by the acid guanidinium thiocyanate-chloroform extraction method [38]. Total RNA extracted was quantitated spectrophotometrically by absorbance at 260 nm.

Northern and slot-blot analyses were performed with 40 and 5 μg total RNA, respectively. For Northern blots, RNA was denatured in loading buffer (24 mM HEPES, 6 mM sodium acetate, 1.2 mM EDTA, 50% formamide, and 2.2 M formaldehyde) at 65°C for 20 min, electrophoresed through a 1.5% agarose-formaldehyde gel, and transferred to a 0.45-μm nylon membrane (Nytran; Schleicher and Schuell, Keene, NH). Intensity of ethidium bromide staining of 28S and 18S ribosomal RNAs was used to verify equivalent loading of total RNA. For slot blots, RNA was denatured as described and immobilized on nylon membrane by use of a microsample filtration unit (Schleicher and Schuell, Keene, NH). Yeast RNA was loaded onto the membrane as a background control. Filters were air dried and then baked at 80°C for 2 h in a vacuum oven.

Filters were prehybridized at 42°C overnight in 5-strength sodium citrate solution (SSC), single-strength Denhardt's, 50 mM $NaPO_4$ (pH 6.5), 50% formamide, 0.1% SDS, and 100 μg/ml yeast RNA. Hybridization was performed at 42°C for 16–20 h in the same buffer (fresh) containing purified random primer α-^{32}P-labeled (Prime-a-Gene Labeling System; Promega, Madison, WI) porcine liver RBP cDNA insert [24]. The porcine liver RBP cDNA is a 714-bp partial clone, isolated from an adult pig liver cDNA library, that shares greater than 98% sequence homology with porcine con-

ceptus RBP cDNA [26]. After hybridization, membranes were washed sequentially in double-strength SSC/0.1% SDS, single-strength SSC/0.1% SDS, and 0.1-strength SSC/0.1% SDS at room temperature, 37°C, and 42–50°C, respectively, until background counts were undetectable.

To demonstrate that differences in quantity of RBP mRNA were not related to inaccuracies in RNA quantitation and subsequent loading, a duplicate membrane was hybridized to a human 28S ribosomal RNA gene. A single pooled sample tube for each animal was utilized to prepare denatured RNA for loading of the membranes, thereby avoiding sample variation between membranes. The ribosomal gene utilized as a counterprobe was the entire 5025-bp fragment of the cloned human 28S ribosomal RNA gene [39].

Hybridization signal was detected by exposing washed filters to Kodak XRP film (Eastman Kodak, Rochester, NY) at −70°C. Autoradiograms of slot blots (exposed for 12 h or 24–72 h for 28S ribosomal and RBP membranes, respectively) were quantified with an LKB laser densitometer (Model 2202 Ultroscan; LKB Bromma, Bromma, Sweden) connected to a reporting integrator (Model 3390A; Hewlett Packard Co., Avondale, PA) that calculates area under the curve. Relative hybridization signal intensities for RBP and 28S ribosomal RNAs were expressed as unit values above background. The size of the RBP mRNA transcript on Northern blots was calculated using 28S (3.7 kb) and 18S (1.9 kb) ribosomal RNA as standards.

Statistical Analyses

Abundance of 28S ribosomal RNA was included in all statistical analyses as a covariate to correct for differences in levels of RBP mRNA due to differences in quantity of total RNA loaded. Relative levels of RBP mRNA per equivalent amount of total RNA in cyclic and pregnant endometrium, determined by densitometric analysis of slot blots, were analyzed for effects of status, day, and status-by-day interactions by least-squares analysis of variance using the General Linear Models procedures of the Statistical Analysis System [40]. Orthogonal contrasts were performed to detect differences between days across and within statuses. Regression analysis was performed to examine the pattern of change of RBP mRNA levels across days of the estrous cycle. Data were analyzed both untransformed and transformed (Log_{10}). Since no heterogeneity of error was detected, results presented are from analysis of untransformed data.

RESULTS

Northern Blot Analyses

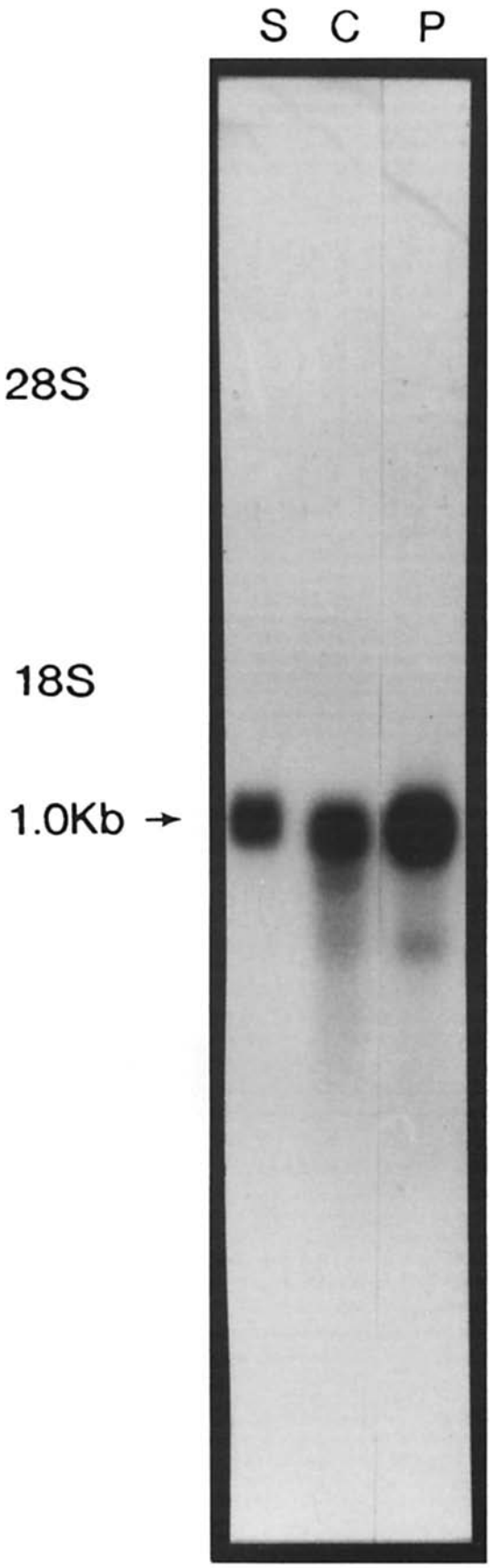
Porcine liver RBP cDNA [24] was α-^{32}P-labeled and hybridized with a Northern blot containing total RNA (40 μg) isolated from conceptuses collected at Days 16, 18, and 15 from sheep (S), cows (C), and pigs (P), respectively. The resultant autoradiograph (Fig. 1; exposed for 24 h) revealed the presence of a single major hybridizing transcript (1.0–1.1 kb) corresponding in size to RBP mRNA reported previously [24, 26]. Identical results were obtained when total RNA (40 μg) from Day 14, 18, and 15 endometrium of pregnant sheep, cows, and pigs, respectively, was examined by Northern blot (data not shown).

Levels of RBP mRNA in Cyclic and Pregnant Endometrium of Pigs, Sheep, and Cattle

Least-squares means for relative levels of RBP mRNA in cyclic and pregnant porcine endometrium (Fig. 2; n = 3/day/status) indicate effects of day ($p < 0.001$) and status by day ($p = 0.05$). RBP mRNA was low to undetectable at Days 0, 5, and 10 of the estrous cycle and Day 10 of pregnancy and increased ($p < 0.06$) in both statuses at Day 12. Levels of RBP mRNA increased further ($p < 0.001$) to Day 15 in both cyclic and pregnant endometrium. At Day 18, RBP mRNA levels decreased ($p < 0.01$) in cyclic pigs, but remained elevated in endometrium of pregnant pigs. Results suggest that expression of the RBP gene in porcine endometrium requires circulating P, which is elevated during the luteal phase of the estrous cycle and early pregnancy (Days 12–15) and remains elevated in pregnant, but decreases in cyclic, gilts from Day 15 to the end of diestrus (Day 18).

Least-squares means for relative levels of RBP mRNA in endometrium of cyclic and pregnant ewes are presented in Figures 3 and 4. Experiment 1 (Fig. 3; fall 1990) measured RBP mRNA at Days 10, 12, 14, and 16 (n = 4/day/status) of the estrous cycle and pregnancy in ewes. Effects of day ($p < 0.01$) and status by day ($p = 0.01$) were detected. Levels of RBP mRNA in endometrium increased ($p = 0.01$) between Days 10 and 14 in both cyclic and pregnant ewes; however, between Days 14 and 16, RBP mRNA levels in endometrium remained elevated in cyclic ewes but decreased ($p < 0.01$) in pregnant ewes.

In experiment 2 (Fig. 4; fall 1991), RBP mRNA was measured in endometrium from Days 0, 5, 11, 13, and 15 (n = 3/day) of the estrous cycle and Days 11, 13, and 15 (n = 3/day) of pregnancy. Effects of status ($p = 0.05$) and day ($p = 0.02$) were detected. Levels of RBP mRNA increased ($p < 0.01$) from Days 0 and 5 (which were not different from each other) to Day 15 of the estrous cycle. Regression analysis detected a distinct curvilinear (quadratic) relationship between levels of RBP mRNA and day of the estrous cycle (adjusted regression line, $Y = 0.2086 - 0.0026x + 0.00022x^2$; $r^2 = 0.74, p < 0.01$). RBP mRNA levels were similar for pregnant and cyclic ewes at Days 11 and 13, then remained unchanged for Day 15 pregnant ewes and increased for cyclic ewes ($p = 0.07$). This suggests that the decrease ($p < 0.01$) in RBP mRNA detected on Day 16 of pregnancy in experiment 1 may have begun on Day 15. Collectively, results from experiments in sheep suggest that RBP mRNA in endometrium of ewes increases during the



luteal phase of the estrous cycle but that in ewes, in contrast to pigs, RBP mRNA is also present at estrus and metestrus (Day 0–5) and in pregnant ewes decreases at Days 15–16 of pregnancy.

Least-squares means for relative levels of RBP mRNA in bovine endometrium at Days 0, 5, 10, 15, and 18 (n = 3/day) of the estrous cycle and Days 10, 15, and 18 (n = 3/day) of pregnancy are presented in Figure 5. An effect of day ($p = 0.06$) was detected across statuses, and orthogonal contrasts indicated that RBP mRNA levels increased ($p < 0.05$) between Days 10 and 18. Due to large variation between cows within and across days (SEM), regression analysis detected only a slight curvilinear (quadratic) relationship between levels of RBP mRNA and day of the estrous cycle (adjusted regression curve, $Y = 0.801 - 0.0042x + 0.00026x^2$; $r^2 = 0.29$, $p = 0.09$). In addition, no differences were detected between days of the estrous cycle analyzed as discrete variables. Levels of RBP mRNA in bovine endometrium, therefore, increased in both cyclic and pregnant cows between Days 10 and 18; and cows, like sheep but unlike pigs, exhibited RBP mRNA at estrus and metestrus (Days 0 and 5).

DISCUSSION

The results indicate that RBP mRNA is present in conceptuses and endometrium of pigs, sheep, and cattle during the periimplantation period of pregnancy. The presence of RBP mRNA in conceptuses supports recent reports that RBP is a major secretory product of pig [23, 26], sheep [26, 33, 34] and cattle [26, 32] conceptuses. Pig conceptuses secrete RBP as early as Day 10 of gestation and through the periimplantation period (Days 10–16 [23]). RBP mRNA is present in both trophectoderm and yolk sac endoderm at Day 20 of gestation [24]. Furthermore, the presence of mRNA transcripts encoding cellular RBP and retinoic acid receptors—both intracellular proteins required for the metabolism and mechanism of action of retinoids—in conceptuses and endometrium of pigs during the periimplantation period [41] suggests that local actions of retinoids in those tissues may be required for the establishment and maintenance of pregnancy.

Adams et al. [15] reported that RBP accumulates in uterine secretions of pigs in response to P during diestrus or when administered to ovariectomized gilts. Results from the present study indicate that RBP gene expression in endometrium of pigs also requires P. Levels of RBP mRNA in-

FIG. 1. Autoradiograph from Northern blot hybridization of porcine liver RBP cDNA with total RNA (40 μg) isolated from conceptuses on Days 16, 18, and 15 of gestation from sheep (S), cows (C), and pigs (P), respectively. α-^{32}P-Labeled porcine liver RBP cDNA was hybridized with the Northern blot overnight and washed as described in *Materials and Methods*. The washed blot was exposed to x-ray film for 12 h at −70°C. The 28S (3.7 kb) and 18S (1.9 kb) ribosomal RNA bands were used to estimate size of the RBP transcript (1.0–1.1 kb).

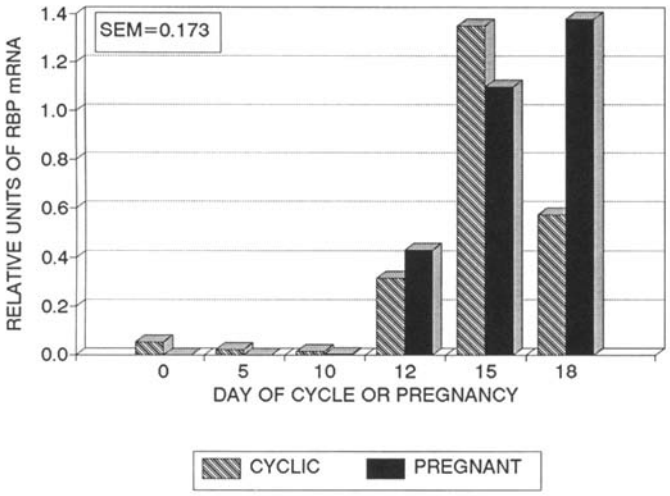


FIG. 2. Relative levels (ls mean ± SEM) of RBP mRNA in endometrium of gilts at Days 0, 5, 10, 12, 15, and 18 (n = 3/day) of the estrous cycle and Days 10, 12, 15, and 18 (n = 3/day) of pregnancy. Units of RNA were generated from densitometric analysis of autoradiographs of slot blots of total RNA (5 μg) hybridized with porcine liver RBP cDNA and 28S human ribosomal gene (see *Materials and Methods*). The washed RBP and 28S ribosomal slot blots were exposed to x-ray film for 12 h at −70°C. Relative units of RBP mRNA presented were corrected for total RNA by using abundance of 28S ribosomal RNA as a covariate in the statistical analysis. Effects of day ($p < 0.001$) and status by day ($p = 0.01$) were detected. The SEM is given in the upper left corner of the graph.

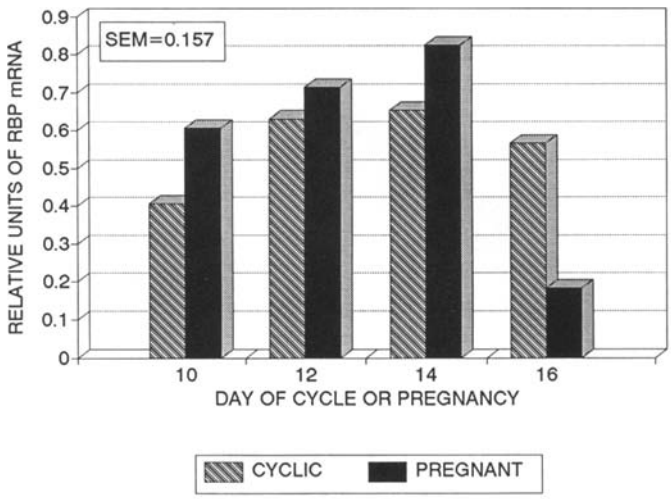


FIG. 3. Relative levels (ls mean ± SEM) of RBP mRNA in endometrium of ewes at Days 10, 12, 14, and 16 (n = 4/day/status) of the estrous cycle and pregnancy (experiment 1; fall 1990). Units of RNA were generated from densitometric analysis of autoradiographs of slot blots of total RNA (5 μg) hybridized with porcine liver RBP cDNA and 28S human ribosomal gene (see *Materials and Methods*). The washed RBP and 28S ribosomal slot blots were exposed to x-ray film for 48 and 12 h, respectively, at −70°C. Relative units of RBP mRNA presented were corrected for total RNA by using abundance of 28S ribosomal RNA as a covariate in the statistical analysis. Effects of day ($p < 0.05$) and status by day ($p < 0.01$) were detected. The SEM is given in the upper left corner of the graph.

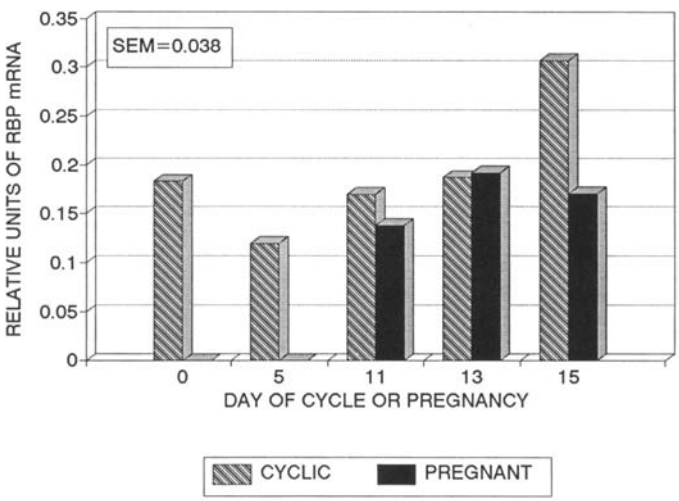


FIG. 4. Relative levels (ls mean ± SEM) of RBP mRNA in endometrium of ewes at Days 0, 5, 11, 13, and 15 (n = 3/day) of the estrous cycle and Days 11, 13, and 15 (n = 3/day) of pregnancy (experiment 2; fall 1991). Units of RNA were generated from densitometric analysis of autoradiographs of slot blots of total RNA (5 μg) hybridized with porcine liver RBP cDNA and 28S human ribosomal gene (see *Materials and Methods*). The washed RBP and 28S ribosomal slot blots were exposed to x-ray film for 48 and 12 h, respectively, at −70°C. Relative units of RBP mRNA presented were corrected for total RNA by using abundance of 28S ribosomal RNA as a covariate in the statistical analysis. A distinct quadratic relationship exists between RBP mRNA abundance and day of the estrous cycle ($p < 0.01$).

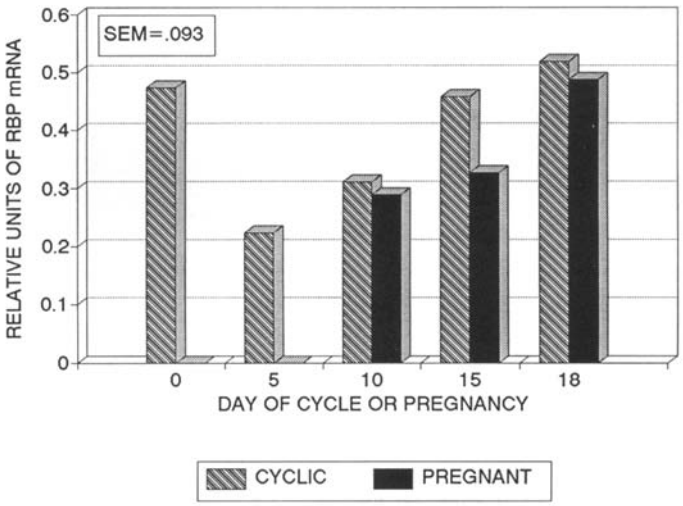


FIG. 5. Relative levels (ls mean ± SEM) of RBP mRNA in endometrium of cows at Days 0, 5, 10, 15, and 18 (n = 3/day) of the estrous cycle and Days 10, 15, and 18 (n = 3/day) of pregnancy. Units of RNA were generated from densitometric analysis of autoradiographs of slot blots of total RNA (5 μg) hybridized with porcine liver RBP cDNA and 28S human ribosomal gene (see *Materials and Methods*). The washed RBP and 28S ribosomal slot blots were exposed to x-ray film for 72 and 12 h, respectively, at −70°C. Relative units of RBP mRNA were generated by correcting for abundance of 28S ribosomal RNA in each sample using covariate analysis. A distinct quadratic relationship exists between RBP mRNA abundance and day of the estrous cycle ($p = 0.06$). The SEM is given in the upper left corner of the graph.

creased during the luteal phase of the estrous cycle of pigs only after plasma P was maximum (Days 12–14) [42, 43]. Levels of RBP mRNA increased to Day 15, when plasma P is just beginning to decline, and then decreased to Day 18 when plasma P is at its nadir. In pregnant endometrium at Day 18, RBP mRNA levels remained elevated, since functional CL ensured stimulation of the endometrium by P. Progesterone induction of RBP gene expression in pigs is protracted, since P increases in plasma after Day 3 of the estrous cycle [42]; but RBP mRNA in endometrium is not detectable until after Day 10 (this study; [24]). The decline in RBP mRNA levels in endometrium at Day 18 of the estrous cycle (when plasma E is increasing) and its absence at Days 0, 5, and 10 suggest that E alone is not stimulatory and, in fact, may be inhibitory to RBP gene expression in the absence of P. Although E alone does not stimulate RBP gene expression in pig endometrium in the absence of P, E may modulate endometrial RBP gene expression in the presence of circulating P [24]. Results reported previously [24] suggest that RBP mRNA levels in endometrium of pregnant gilts follows plasma E, high between Days 15 and 30 and Days 90 and 112, but low between Days 45 and 75 [44, 45]. In addition, Trout et al. [46] reported a significant increase in porcine endometrial RBP gene expression in pregnant versus cyclic gilts at Day 13, suggesting an acute stimulatory effect of conceptus E in the presence of circulating P. Similar results were not found in the present study, and this have been due a limited length of endometrial exposure to conceptus E (endometrium was collected at Day 12 in the present study). The effect of E may be mediated through effects on the P receptor, as levels of P receptor mRNA in endometrium of pregnant gilts follow plasma E [47]. Additionally, E may affect RBP gene expression through mechanisms yet to be determined.

Temporal changes in levels of RBP mRNA in endometrium of sheep and cows were similar during the estrous cycle, but they differed from the pattern in pigs. In sheep and cows, the levels of RBP mRNA increased during the luteal phase of the estrous cycle (sheep, Days 5–15) or during both the luteal phase of the estrous cycle and analogous days of pregnancy (cows, Days 10–18). In contrast to results in pigs, RBP mRNA was relatively high at the end of diestrus (Day 16 in sheep and Day 18 in cows) and still present at estrus (Day 0) and metestrus (Day 5). Thus more complex endocrine regulatory mechanisms may affect RBP gene expression in endometrium of sheep and cows than in that of pigs.

In sheep and cows, plasma P is elevated between Days 5 and 13 and Days 7 and 18, respectively [48], coincident with increasing RBP mRNA levels in endometrium of cyclic and pregnant animals in the present study. The increase in plasma E and decrease in plasma P, which coincides with rapid decline to undetectable levels of RBP mRNA in endometrium of pigs at late diestrus and estrus, is not associated with a similarly rapid decline in RBP mRNA levels in endometrium of sheep and cows. In fact, mean level of RBP mRNA in cows was similar at Days 18 and 0 of the estrous cycle, suggesting no decline in RBP mRNA between diestrus, proestrus, and estrus. These results suggest multiple mechanisms of hormonal (steroid) regulation of RBP mRNA in endometrium of sheep and cattle. First, P may stimulate RBP gene expression in endometrium of ruminants and E may modulate this effect, as in the pregnant pig [24, 46], since E increases between late diestrus and estrus [48] when RBP mRNA levels are also high for both sheep and cows. The inhibitory effect of E between late diestrus and estrus may occur in pigs, but not in ruminants, as a result of the prolonged proestrus (low P, high E) phase in pigs compared to the short proestrus phase in ruminants [48]. Second, several days of elevated P may gradually increase levels of RBP mRNA, which then remains elevated into the next estrous cycle when circulating P is low (estrus). Immunoreactive RBP increases in uterine flushings of both ovariectomized cows in response to 12 or 30 days of P treatment [8] and intact cows during the late luteal phase of the estrous cycle [27], supporting a role for P in stimulating synthesis and secretion of RBP by bovine uterine endometrium, as reported previously for pigs [15]. Similarly, the P-induced uterine milk proteins accumulate in the uterine lumen of ewes and cows in significant amounts only after prolonged P exposure [7, 11], suggesting that a similar scenario may be true for RBP. In addition to RBP, lactoferrin is another bovine endometrial transport protein that is regulated by P [4].

Finally, E and P may stimulate, but not be required for, RBP gene expression. Three peaks of E occur in plasma of both sheep and cows. Levels of RBP mRNA in endometrium of cyclic sheep and cows are elevated when E is increasing (proestrus/estrus) and during diestrus when P is elevated, suggesting that both hormones may stimulate RBP gene expression. Thomas et al. [8] did not investigate effects of E or E plus P on RBP in cow uterine flushings, so possible interactions between E and P or effects of E alone on RBP and RBP mRNA in endometrium of cows are not known.

The decline in RBP mRNA in endometrium of pregnant ewes began on Day 15 and was clearly apparent at Day 16. This result may support a stimulatory role for E, because E receptor protein is low in ewes on Day 16 of pregnancy but high on Day 16 of the estrous cycle [49]. Pregnancy, as well as intrauterine infusion of interferon tau (IFNτ) into the uterine lumen of cycling ewes (Days 11–15), significantly suppresses E receptors in endometrium at Day 16 [50]. The reduced responsiveness of uterine endometrium to circulating E may result in the decline observed in the present study in RBP mRNA in pregnant ewes at Day 16 of gestation.

IFNτ, the first and major sheep conceptus secretory protein between Days 13 and 21 of gestation [30, 31], resembles the IFNα family of antiviral proteins [51] and is responsible for maternal recognition of pregnancy in sheep

[52]. In addition to effects of IFNτ on the E receptor, IFNτ may alter RBP gene expression directly. Interferons are known to be inhibited at the transcriptional level by retinoic acid [53]. Although not previously demonstrated, a reciprocal relationship may exist whereby IFNs affect vitamin A metabolism or transport. Such a relationship would explain inhibitory effects of pregnancy and IFNτ on endometrial RBP mRNA gene expression to decrease transport of vitamin A into the uterine lumen and to prevent inhibition of IFNτ secretion by trophoblast (which would interfere with the maternal recognition of pregnancy in sheep) [2, 52]. The major conceptus secretory protein in cattle is also an IFNτ [51]; therefore, a similar scenario could occur in cows. Although no decrease in RBP mRNA levels in pregnant endometrium was detected at Day 18 in the present study, it may occur by Days 20–22, which would be comparable to Day 16 of pregnancy in ewes.

Production of RBP by conceptuses and endometrium of periimplantation pig, sheep, and cattle suggests that transport of retinol to and within developing conceptuses and endometrium is essential for establishment and maintenance of pregnancy. The major function of RBP is endocrine and paracrine transport of retinol [54]. Retinoids are required for normal function, proliferation, and differentiation of cells and may be essential for steroid metabolism, placentation, production, and transport of uterine secretions and growth and development of fetuses to term [36]. The recent establishment of retinoic acid as a morphogen [37] supports results of the present study indicating that RBP, the transport protein for vitamin A, is a major transcript produced by periimplantation conceptuses and endometrium undergoing the rapid developmental and physiological changes required for the establishment and maintenance of pregnancy.

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