

Retinol and estradiol regulation of retinol binding protein and prostaglandin production by porcine uterine epithelial cells in vitro¹

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ABSTRACT: Secretion into the uterine lumen follows a precise pattern during early pregnancy. Near the end of the second week of pregnancy and coincident with elongation of conceptuses, retinol, retinol binding protein (RBP), estradiol (E2), and prostaglandins E (PGE) and F (PGF) increase in the uterine lumen, and RBP mRNA increases in the endometrium. In the present studies the potential for E2 (0.1 μ M) and retinol (10 μ M) to regulate RBP and PG production by cultured luminal (LEC) and glandular (GEC) epithelial cells collected from postpubertal females and LEC from prepubertal gilts was examined. Endometrial tissue was collected surgically from cyclic and pregnant females (n = 8) on d 10 and 13 postestrus (first day of estrus = d 0) and from 120- and 150-d-old prepubertal gilts that were treated with progesterone (P4) (2.2 mg·kg⁻¹·d⁻¹, n = 6) or corn oil (n = 6) for 14 d prior to tissue collection. The

LEC from postpubertal females responded to retinol with increased ($P < 0.05$) RBP, PGE, and PGF in culture medium and increased ($P < 0.07$) RBP mRNA but E2 decreased ($P < 0.05$) RBP and RBP mRNA and had no effect on prostaglandins. No E2 or retinol effects on secretions of GEC occurred in vitro, but a day × pregnancy status interaction ($P < 0.06$) affected PGE output by the GEC. Secretion of PGE was greater when GEC were collected on d 10 of pregnancy than from d-10 cyclic or d-13 pregnant or cyclic females. Both E2 and retinol stimulated ($P < 0.05$) secretion of RBP by LEC isolated from prepubertal gilts, but their effects were not additive. In vivo treatment of prepubertal gilts with P4 increased ($P < 0.05$) RBP and decreased ($P < 0.05$) PG production by LEC in vitro. Therefore responses to E2 and retinol differ between pre- and post-pubertal females, and retinol may function in the regulation of endometrial RBP and PG secretion.

Key Words: Endometrium, Estradiol, Pigs, Prostaglandins, Retinol Binding Protein

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Introduction

Uterine histotroph appears to be necessary for pregnancy survival throughout gestation in pigs, and secretion of specific components appears to be related to conceptus development (Geisert et al., 1982a; Trout et al., 1992). In particular, dramatic changes in the composition of uterine secretions occur during early pregnancy (Geisert et al., 1982a). During the immediate preattachment stage (between d 9 and 13 of gestation), conceptus estrogen secretion surges (Perry et al., 1973; Mondschein et al., 1985), and the regression of the cor-

pora lutea (CL) is prevented (Dhinsda and Dziuk, 1968). Coincident with these changes, increases in histotrophic constituents appear in the uterine lumen (Geisert et al., 1982a).

Regulation of the secretion of histotroph into the uterine lumen is incompletely understood. One experimental strategy to address the regulation of secretion is to isolate uterine luminal (LEC) and glandular (GEC) epithelial cells from the porcine uterus and to determine the effects of factors present in vivo on gene activity and secretory activity in vitro.

Retinol and its binding protein (RBP) increase in the uterine lumen parallel to estrogen during the immediate preattachment stage (Trout et al., 1992), and pregnancy status might influence cell responses due to embryonic signals prior to cell harvest. Therefore responses to estradiol (E2) and retinol were tested using uterine epithelial cells from pregnant and cyclic pigs. To investigate the prepubertal maturation of the regulation of endometrial secretions, the responses of cells harvested from prepubertal (120- and 150-d-old) gilts that received either progesterone (P4) or vehicle were

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evaluated. Gilts are just acquiring the ability to maintain pregnancies at these ages (Ellicott et al., 1973; Segal and Baker, 1973), and comparisons of the regulation of histotroph during this maturation may be informative.

Materials and Methods

Animals

Crossbred females (Hampshire \times Duroc \times Yorkshire) provided endometrium. In Exp. 1, endometrium was collected at surgery on d 10 or 13 (d 0 = onset of estrus) of pregnancy or the estrous cycle. Two sows and two postpubertal gilts were included in each pregnancy status \times day subgroup). Surgical procedures were described by Zhang et al. (1991). Pregnancy was established by artificial insemination on d 0 and 1 and confirmed by the presence of blastocysts at hysterectomy. In Exp. 2, endometrial tissue was collected from 120- (n = 6) and 150- (n = 6) d-old prepubertal gilts that had received P4 (2.2 mg \cdot kg⁻¹ \cdot d⁻¹; Steraloids, Wilton, NH) or corn oil for 14 consecutive days, and the uterus was removed by hysterectomy the following day. Blood was collected to monitor peripheral P4 concentrations after treatment. Animal procedures were approved by the Institutional Animal Care and Use Committee.

Cell Separation and Culture

Populations of epithelial cells were prepared under sterile conditions as previously described (Zhang et al., 1991) and modified by Zhang and Davis (2000) to improve proliferation of LEC. Both LEC and GEC cultures were prepared with tissue from postpubertal females, but only LEC were obtained from prepubertal gilts because the endometrium from corn oil-treated prepubertal gilts was poorly developed and it was not possible to harvest adequate glands for plating. After isolation, cells were resuspended in RPMI 1640 (Gibco BRL, Grand Island, NY) supplemented with 20% fetal calf serum (FCS, Gibco BRL) and antibiotics and antimycotic (ABAM; 100 units penicillin, 100 μ g streptomycin and 0.25 μ g amphotericin B/mL all from Gibco BRL). The LEC plaques from adult and prepubertal pigs and GEC fragments from the adults were plated in 12-well (surface area 3.8 cm²) and 6-well (surfaces area 9.6 cm²) plates, respectively, at densities that covered 50 to 60% of the well. The cells were allowed to attach and proliferate under 5% CO₂ and 95% air in a closed chamber at 37°C. Cultures produced a monolayer covering about 80% of the well after 2 to 4 d at which time the culture medium was replaced by RPMI 1640 supplemented with 10% dextran-coated charcoal-stripped FCS and ABAM. Cultures were incubated for 6 h to deplete the cells of E2 and retinol possibly taken up from FCS.

Culture medium was replaced by RPMI 1640 (supplemented with insulin, 10 μ g/mL, and ABAM). All-*trans* retinol (Sigma Chemical Co., St. Louis, MO) and E2

(Steraloids Inc., Wilton, NH) were added in a 2 \times 2 factorial structure with final concentrations of 10 μ M, and 0.1 μ M, respectively. Based on amounts that were recovered by uterine flushing (Geisert et al., 1982a; Trout et al., 1992) and assuming 0.5 mL of free fluid per uterine horn, these concentrations are in the physiological range for the uterine lumen on d 12 to 13 of pregnancy. Control wells received the vehicle (ethanol) used for administration of E2 and retinol (final concentration 0.1%). After 24 h, the culture medium was collected from the 12-well plates, centrifuged at 1000 \times g for 10 min at 4°C, and stored at -20°C until analysis. After collection of culture medium, 1 mL of incomplete Hank's buffered salt solution (IHBSS; Gibco BRL) was added to the wells, and the cells were removed by scraping with a plastic policeman, rinsed with IHBSS, pelleted by centrifugation, and then frozen. Cellular protein was estimated using Folin-phenol reagent (Lowry et al., 1951).

Cultures in 6-well plates were assigned for mRNA quantification. Medium was removed and replaced by 1 mL 4 M guanidium thiocyanate with 25 mM sodium citrate (pH = 7), 0.5% sarcosyl, and 2-mercaptoethanol (7 μ L/mL). Culture plates were rocked for 1 min, and the cell extract was collected and stored at -80°C.

Radioimmunoassays (RIA)

Serum samples were assayed for P4 using Coat-a-Count kits (Diagnostic Products Corporation, Los Angeles, CA) previously validated for pig serum (Blair et al., 1993). The intraassay CV was 2.8%, and the sensitivity was 5 pg/mL.

Culture medium was collected from the 12-well plates after a 24-h incubation with E2 and retinol treatments and radioimmunoassayed for RBP (Vallet et al., 1994), PGE (Rosenkrans et al., 1990), and PGF (Groothuis et al., 1997). Intra- and interassay CV were 12.6 and 14.6% for RBP, 11.0 and 13.6% for PGE, and 12.1 and 7.7% for PGF assays. The sensitivities were 10 ng/mL for the RBP assay, 7 pg/mL for the PGE assay, and 10 pg/mL for the PGF assay, respectively.

RNA Isolation and Evaluation

Total RNA was separated from the cell extracts by guanidium isothiocyanate-phenol-chloroform RNA extraction (Chomzynski and Sacchi, 1987) with some modifications. Water-saturated phenol, 3 M sodium acetate, and chloroform/isoamyl alcohol (24:1) were added, and the mixture was vortexed and centrifuged (14,000 μ g at 40°C). The aqueous phase was precipitated twice with 95% ethanol and the RNA resuspended with 20 μ L of diethylpyrocarbonate-treated water.

Total RNA concentrations for each sample were determined with the GeneQuant spectrophotometer (Amersham Pharmacia Biotech, Piscataway, NJ). Total cellular RNA was separated by formaldehyde-agarose gel electrophoresis and transferred to Hybond nylon filters

(Amersham Pharmacia Biotech) for northern analysis of RBP mRNA to confirm the integrity of the RNA.

Hybridization

The nylon membranes were hybridized overnight in 5× Denhart's solution, 50% deionized formamide, 5× SSC, 50 mM sodium phosphate, and denatured salmon sperm DNA (100 µg/mL) at 42°C in a hybridization incubator (Model 310, Robins Scientific; Sunnyvale, CA). The following day, the RBP probe (described below) was added (10⁶ cpm/mL) and allowed to hybridize overnight. Membranes next were washed with 2× SSC, 0.1% SDS for 30 min at 42°C and then 0.5× SSC, 0.1% SDS for 30 min at 65°C and exposed to Kodak X-Omat film (Eastman Kodak, Rochester, NY) for 24 and 48 h. Resulting autoradiograms were analyzed on a Pharmacia Ultrascan-XL densitometer (Piscataway, NJ).

Slot Blot Analysis

Relative changes in RBP mRNA in response to culture treatments were measured by quantitative slot blot analysis modified from Nett et al. (1990). For slot blot analysis, 5 µg of total RNA was loaded for each sample. Samples were run in duplicate using a PCR-generated probe that was produced by using an upstream primer corresponding to base pairs 251 to 270 and a downstream primer complementary to base pairs 423 to 442 of the sequence reported by Trout et al. (1991). The template for this PCR reaction was a 700-base pair RBP cDNA sequence that was isolated from the plasmid PBS-2KS (18; Stratagene, LaJolla, CA) by Eco RI digestion followed by electroelution. The PCR consisted of 35 cycles of 94°C (1 min), 60°C (1 min), and 74°C (2 min) that resulted in a 191-base pair amplification product. Incorporation of ³²P-labeled dCTP was approximately 65%.

To quantify relative changes in RBP mRNA abundance, a standard curve cRNA was produced by *in vitro* transcription of the 191-base pair RBP cDNA fragment that had been subcloned into PBS-2KS. Identity and orientation of the subcloned fragments were verified by sequencing. Sense strand cRNA production was driven by T7 RNA polymerase (Promega, Madison, WI), and transcription products were electrophoresed on a 1.5% agarose gel to ensure that transcription resulted in a uniform product of the correct size. A cRNA standard curve, in amounts ranging from 25 pg to 4 ng, was loaded in duplicate on each slot blot.

Statistical Analyses

Prostaglandins and RBP were expressed as ng/µg cellular protein to account for differences in cell number between culture wells. Retinol binding protein mRNA was expressed as pg/µg of total RNA as a measure of relative abundance of the message. Duplicate culture wells (12-well plates) for each pig were included in each treatment combination for measuring secretory prod-

ucts, and one well (6-well plates) for mRNA determination. Statistical analyses were conducted using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC).

Statistical analyses for adult pigs were conducted separately for each cell type because several animals provided cells for only one cell type. Models included pig, pregnancy status (cyclic or pregnant), day of the estrous cycle or pregnancy, and culture medium treatment (estradiol and retinol added in a 2 × 2 factorial treatment structure). Pregnancy status effects, effects of the day of the cycle or pregnancy, and the interaction between these two main effects were tested using the pig (status × day) as the error variance. Models for prepubertal gilts included *in vivo* treatment (progesterone or vehicle), gilt age, and culture medium treatment. Effects of *in vivo* treatments, age and the *in vivo* treatment by age interaction were tested using pig (*in vivo* treatment × age) as the error variance. The experimental models included random (pig within status × day and pig within *in vivo* treatment × day) as well as fixed effects. Therefore fixed effects were tested using the interaction between the effect and the random variable (Snedecor and Cochran, 1971). Otherwise, the residual variance was used as the error term for calculating F-statistics. Prostaglandin concentrations were log transformed before analysis to alleviate heterogeneity of variance.

Results

Experiment 1. Response of LEC and GEC Cultures Prepared from Pregnant and Cyclic Sows

No ($P > 0.10$) interactions between pregnancy status and E2 or retinol were detected for either cell type. Therefore only least squares means of main effects and interactions of *in vitro* treatments are reported. Concentrations of RBP (ng/µg cellular protein) in culture medium from LEC were decreased 18% ($P < 0.05$) by E2 treatment (Figure 1A), but GEC did not ($P > 0.1$) respond to E2 treatment (0.27 ± 0.009 vs 0.27 ± 0.009). The RBP mRNA (pg/µg total RNA) was decreased ($P < 0.05$) by E2 treatment of LEC (Figure 1B) but not GEC (273 ± 12 vs 269 ± 12 for control and E2-treated, respectively). In contrast, retinol increased ($P < 0.06$) RBP in medium from LEC 39% (Figure 1A), and RBP mRNA in LEC (Figure 1B). However, GEC did not ($P > 0.1$) respond to retinol with changes in RBP (0.27 ± 0.02 vs 0.27 ± 0.02 ng/µg cell protein) or RBP mRNA (264 ± 22 vs 277 ± 22 pg/µg total RNA for control and retinol-treated, respectively).

Neither PGE nor PGF (pg/µg cellular protein) was affected ($P > 0.1$) by E2 for either cell type. Retinol increased PGE ($P < 0.05$) by 46% and PGF ($P < 0.10$) 72% in CM from LEC (Figure 2) but did not ($P > 0.10$) affect PG secretion by GEC.

Even though no treatment effects were observed for PG secretion by GEC, effects of day postestrus ($P < 0.05$) and pregnancy status of the donor ($P < 0.05$) at

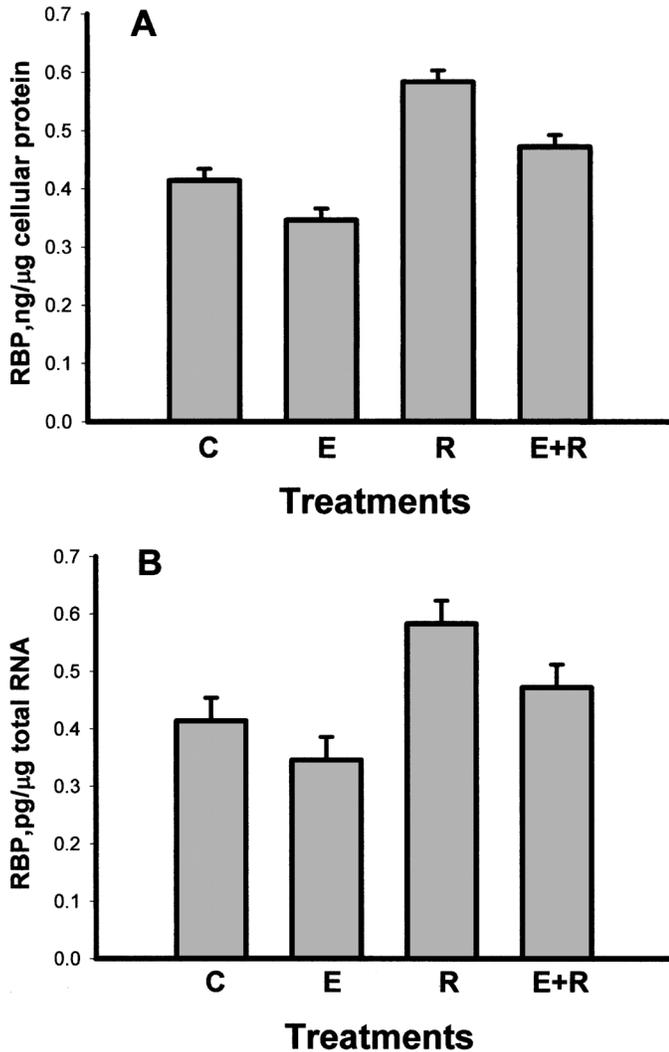


Figure 1. Effects of E2 (0.1 μ M) and retinol (10 μ M) on (A) retinol binding protein (RBP, ng/ μ g cellular protein) in medium from, and (B) RBP mRNA (pg/ μ g total RNA) in, cultured luminal epithelial cells from cyclic and pregnant sows. Bars represent least squares means \pm SEM of cultures from eight sows. C = control; E = estradiol, R = retinol and E + R = estradiol + retinol. (a) E2 decreased ($*P < 0.05$) and retinol increased ($*P < 0.06$) RBP in culture medium. (b) E2 decreased ($*P < 0.05$) RBP mRNA in LEC. Retinol increased ($*P < 0.06$) RBP mRNA in LEC.

cell harvest, as well as a day \times status interaction ($P = 0.06$) for PGE (Table 1), were observed. The PG in medium from GEC were greater on d 10 than on d 13 and also were greater in medium from cells obtained from pregnant compared to cyclic sows. The interaction affecting PGE resulted from a relatively greater increase in PGE secretion attributable to pregnancy for cells collected on d 10 vs d 13 (98 vs 41%, respectively).

Experiment 2. Response of Primary Endometrial LEC Cultures Prepared from Prepubertal Gilts

Mean peripheral P4 concentrations (ng/mL serum) after treatment with vehicle or progesterone were 0.46

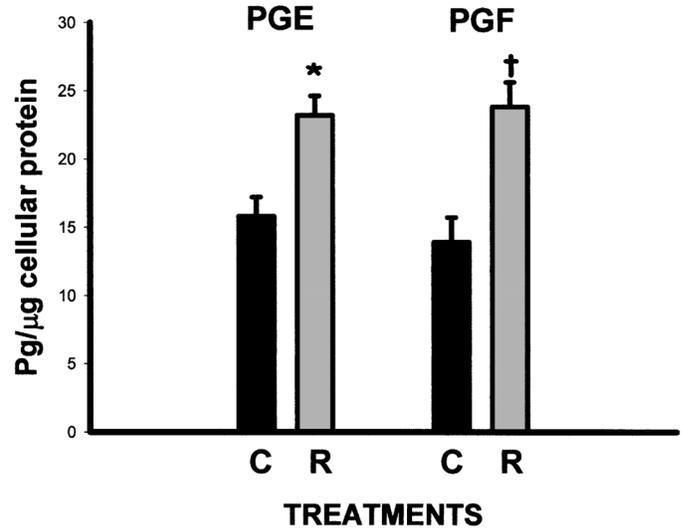


Figure 2. Prostaglandin secretion (least squares means \pm SEM) by luminal epithelial cells harvested from sows and in response to retinol (10 μ M). C = control, R = retinol. $*P < 0.05$; $\dagger P < 0.10$.

± 0.35 and 10.81 ± 4.04 in the 120-d-old gilts, and 0.29 ± 0.11 and 19.87 ± 7.89 in the 150-d-old gilts. No ($P < 0.20$) two- or three-way interactions between either gilt age, or in vivo treatment with P4, and in vitro treatment with E2 or retinol were observed. Least square means for main effects and interactions of in vitro treatments are reported.

The RBP in culture medium from LEC was not affected ($P > 0.10$) by gilt age, but was increased ($P < 0.05$) by P4 treatment in vivo (Fig. 3). In vitro secretion of RBP also was affected ($P < 0.05$) by an E2 \times retinol interaction (Figure 3). Treatment with either E2 or retinol increased RBP secretion approximately 25%, but the treatments were not additive for stimulating RBP secretion. In vivo treatment with P4 increased ($P < 0.05$) RBP secretion (Figure 3) and decreased ($P < 0.05$) PGE secretion in vitro (Table 2). Neither E2 nor retinol affected ($P > 0.10$) PGE secretion (data not shown).

Table 1. Pregnancy and day effects on prostaglandin secretion by uterine epithelial cells (Exp.1)

Cell type ^a	Prostaglandin	Cyclic		Pregnant		SEM
		D10	D13	D10	D13	
GEC	PGE ^{bc}	5.9	3.8	11.8	5.4	0.69
	PGF ^d	14.1	5.7	37.7	17.1	0.36
LEC	PGE ^e	24.0	24.1	18.9	10.9	4.1
	PGF	26.4	20.5	17.0	11.6	7.5

^aGEC, glandular epithelial cells; LEC, luminal epithelial cells.

^bLeast squares means, pg/ μ g cellular protein.

^cStatus \times day interaction ($P < 0.05$). Day 10 pregnant greater ($P < 0.05$) than other means in this line.

^dPregnant greater ($P < 0.01$) than cyclic and d 10 greater ($P < 0.05$) than d 13.

^eCyclic tends ($P < 0.10$) to be greater than pregnant.

Discussion

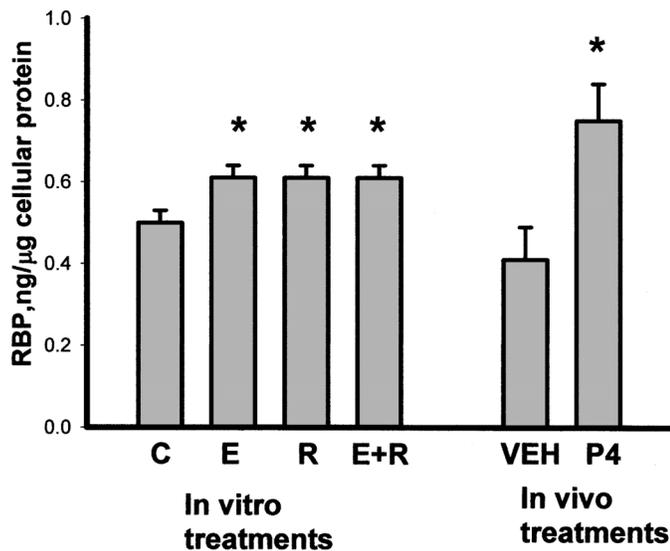


Figure 3. Effects of E2 (0.1 μ M), retinol (10 μ M) in vitro, and in vivo treatment with vehicle (VEH) or progesterone (P4) on retinol binding protein (RBP) (ng/ μ g cellular protein) secreted into CM by luminal epithelial cells from prepubertal gilts. Bars represent least squares means \pm SEM of cultures from 12 gilts. * $P < 0.05$.

Secretion of PGF by LEC in vitro was affected ($P < 0.05$) by an E2 \times retinol interaction and by a P4 treatment (in vivo) by age ($P < 0.05$) interaction (Table 2). The E2 \times retinol interaction occurred because, in the presence of both E2 and retinol, the secretion of PGF was less (46.7 ± 4 pg/ μ g cell protein) than for cultures containing E2, retinol, or neither treatment (59 to 62 ± 4 pg/ μ g cell protein). Effects of P4 and age are presented in Table 2. Cells from older gilts secreted approximately 50% less PGF than cells from 120-d-old gilts. Treatment with P4 before harvest of LEC decreased ($P < 0.01$) PGF secretion in vitro by approximately 75%.

Table 2. Least squares means for secretions of prostaglandins F (PGF) and E (PGE) by luminal epithelial cells as affected by age and progesterone (P4) treatment of the donor (Exp. 2)

Age and treatment	PGF ^{abc}	PGE ^{ac}
120 d		
Control	120.0 \pm 19	79 \pm 16
P4	31.8 \pm 18	26 \pm 16
150 d		
Control	61.6 \pm 18	57 \pm 16
P4	14.2 \pm 22	22 \pm 19

^aPg/ μ g cellular protein.

^bCells from younger (120-d) gilts secreted more ($P < 0.05$) PGF than cells from 150-d gilts.

^cTreatment with P4 decreased ($P < 0.05$) PG secretion.

The uterine environment in pigs includes estrogens, retinol, PG, and several proteins that appear to have a variety of roles in supporting the conceptuses. The histotrophic secretions increase after d 10 of pregnancy (Geisert et al., 1982a). Both P4 from the CL and conceptus estrogen have been implicated as regulators of secretory activity. However the regulation of uterine secretory activity is incompletely understood, and in vitro models may be useful for understanding this system. In Exp. 1, retinol stimulated RBP and PG secretion in vitro. However, there was no evidence for E2 upregulation of secretion of the RBP and PG components of the histotroph.

The hypothesis that conceptus estrogen stimulates secretion of histotroph is based on a correlation between conceptus development and uterine secretions (Geisert et al., 1982a) and the observation that administration of exogenous estrogen on d 11 to nonpregnant gilts increases luminal RBP (Trout et al., 1992), uteroferrin (Geisert et al., 1982b), and plasmin inhibitor (Fazleabas et al., 1983) 12 to 24 h later. Other evidence (Geisert et al., 1982a; Vallet et al., 1996, 1997) indicates that amounts of histotrophic constituents, including RBP, are not increased in uteri of pregnant compared to nonpregnant pigs. In Exp. 1, the LEC from postpubertal females responded with only a small decrease in RBP protein and mRNA when treated with E2, and this may be consistent with reports indicating no effects of pregnancy on histotroph secretions.

It is possible that lack of E2 effects in the present experiments resulted from limitations in the culture system employed and the disruption of interaction with stromal cells and/or the extracellular matrix. The cell isolation procedures used harvest sheets of the luminal epithelium and fragments of uterine glands, thus preserving some extracellular matrix in the cultures. Furthermore using the culture conditions employed here, E2 stimulates antileukoproteinase expression in pig GEC (Reed et al., 1996), and LEC from d-10 pregnant pigs respond to E2 with decreased expression of ornithine decarboxylase (ODC) and spermidine/spermine N^1 -acetyltransferase while LEC from d-12 pregnant pigs had increased ODC message after E2 treatment (R. Simmen, personal communication). Therefore the results of the present studies and the reports cited above indicate that primary cultures of LEC and GEC may be appropriate models for studying the regulation of histotrophic secretions.

Increased secretion of RBP by LEC collected from prepubertal gilts treated with P4 (Exp. 2) is consistent with previous work (Groothuis et al., 1997) showing that treating similar gilts with P4 for 14 d increased RBP secretion in vivo and other reports indicating that P4 stimulates RBP secretion by the uterus of postpubertal pigs (Adams et al., 1981; Trout et al., 1991). The present results indicate that P4-induced secretion of

RBP is maintained by the LEC from prepubertal gilts during culture.

The finding that RBP mRNA (Exp. 1) and protein (Exp. 1 and 2) were increased by treatment with retinol *in vitro* is also consistent with the report of Dore et al. (1995), who obtained a similar result with epithelial cells from the bovine endometrium. In other tissues (rat liver and visceral yolk sac) it has been reported that retinol did not affect the amount of message for RBP (Soprano et al., 1986, 1988). However studies utilizing human hepatoma cells (Mourey et al., 1994) demonstrated that both retinol, and its biologically active metabolite all-*trans* retinoic acid, increased expression of RBP mRNA, and a recent study utilizing murine liver (Jessen and Satre, 2000) indicated that both all-*trans* retinoic acid and 9-*cis* retinoic acid increased the abundance of mRNA for RBP. Therefore both retinol and its metabolites may regulate the secretion of RBP. Jessen and Satre (2000) suggested that this regulation could serve to minimize cell toxicity by promoting sequestration of retinol and retinoic acid by virtue of the ability of retinoic acid to both bind (Smith et al., 1985; Dixon and Goodman, 1987) and stimulate secretion of RBP. Retinol stimulation of RBP secretion could be an important protective mechanism in the gravid uterus.

Adding E2 to the cultures did not affect PG secretion, a result consistent with the findings of Zhang and Davis, (1991). However retinol increased PG secretion by LEC in Exp. 1. Prostaglandins have been implicated in many events in pregnancy, and their concentrations increase in the uterine lumen parallel to those of RBP and retinol (Trout et al., 1992; Davis and Blair, 1993). A regulatory role for retinol on PG secretion might function to control the local PG environment in the uterus to support the events of implantation. The ability of retinol to increase steady-state amounts of RBP message in LEC might further reinforce the associated increase of these components in the histotroph in early pregnancy.

Another role for retinol stimulation of PGE secretion is suggested by the report of Napoli (1993) that PGE1 inhibits the conversion of retinol to retinoic acid in cultures of Madin-Darby canine kidney cells. If this mechanism operates in the pig uterus and conceptus, then increased PGE stimulated by retinol could provide negative regulation to protect against overstimulation by retinoic acid in an environment with large amounts of retinol. The implications of retinol mediation of PG secretion in the uterus should be studied further.

In contrast to retinol's stimulatory effects on PG secretion in cells from postpubertal females, retinol alone did not stimulate PG secretion in LEC cultures from prepubertal gilts and when combined with E2, decreased PGF secretion. Reports indicate that pregnancies do not survive in very young (120 d of age or less) prepubertal gilts that are induced to ovulate even though ovulation and fertilization occur (Ellicott et al., 1973; Segal and Baker, 1973). Erices and Schnurrbusch (1979) observed that between 84 and 168 d of age, the

uterus of the prepubertal gilt acquires the histological characteristics consistent with secretory function. Therefore it may be informative to compare the response of endometrial cells from pre- and post-pubertal animals. The present results suggest an undeveloped response to retinol could be one factor in pregnancy failure in prepubertal gilts.

Although the secretion of endometrial cells harvested from post- and prepubertal females was not compared in the same experiment, PG secretion appeared to be greater for LEC from control gilts (Table 2) vs postpubertal females (Figure 2). Treating gilts with P4 reduced *in vitro* PG secretion by LEC to amounts similar to those secreted by postpubertal LEC. Guthrie and Lewis (1986) observed that endometrial explants taken from d 16 to 19 of the estrous cycle, when progesterone concentrations are low, secreted more PGF than endometrium from d 13 cyclic gilts and d 13 and 25 pregnant gilts. Circulating P4 would be high in the latter three groups. Consistent with these observations, Zhang and Davis (1991) reported that P4 treatment *in vitro* decreased PG secretion by pig GEC cells. Even though P4 treatment reduced the secretion of PG *in vitro*, the LEC from P4-treated prepubertal gilts did not respond to retinol with increased PG secretion as observed for postpubertal females. Therefore factors additional to exposure to P4 are required for the mature response, and these maturations occur during the same ages that the ability to maintain pregnancy develops. The GEC, while not affected by *in vitro* treatments, were affected by their *in vivo* environment. Increased PGE secretion was observed for GEC from pregnant sows and might reflect the stimulatory effect of conceptuses on PG secretion (Harney and Bazer, 1990; Dubois and Bazer, 1991). However, the effects of pregnancy status on PGF secretion *in vitro* are inconsistent with other work indicating an inhibitory effect of pregnancy on PGF secretion (Zhang et al., 1991).

Implications

The present observations are relevant for understanding the regulation of uterine secretions in early pregnancy. A role for progesterone, but not estrogen, in regulating retinol binding protein and prostaglandin secretions by the uterus is indicated. Furthermore retinol's ability to stimulate retinol binding protein and prostaglandin secretions could have profound effects on the establishment and maintenance of pregnancy. The markedly different responses of luminal epithelial cells from prepubertal vs mature females suggest important prepubertal maturations. Prepubertal gilts, and uterine cell cultures derived from them, may provide a model useful for studying developmental regulations leading to fertility.

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