

The Effect of Estrone and Estradiol Treatment on Endometrial Total Protein, Uteroferrin, and Retinol-Binding Protein Secretion During Midpregnancy or Midpseudopregnancy in Swine¹

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ABSTRACT: It has been hypothesized that conceptus estrogens influence endometrial protein secretion during pregnancy in swine. To test this hypothesis, the effect of estrone and estradiol treatment from d 30 to 60 of pregnancy or pseudopregnancy on endometrial protein secretion was investigated. Pregnant (P; n = 16) and pseudopregnant (PP) gilts (n = 18) received either sham treatment or estrone or estradiol implants (5 mg/d release rate; 60 d release) on d 30 of P or PP. Blood samples were collected on d 30, 40, 50, and 60 to measure estrone and estradiol. On d 60, gilts were hysterectomized. For P gilts, endometrium in apposition to one placenta from each uterine horn was collected. For PP gilts, each uterine horn was flushed with 40 mL of leucine-deficient minimal essential medium (MEM), and endometrial tissue was collected from each horn. Endometrial tissues were incubated in MEM in the presence of 50 μ Ci of [³H]leucine to examine protein secretion. Estrone and estradiol treatments increased both plasma and endometrial

concentrations of estrone ($P < .01$ except endometrium for P gilts) and estradiol ($P < .01$, respectively). Endometrium from P gilts secreted more nondialyzable macromolecules (NDM), acid phosphatase activity (AP, a measure of uteroferrin), and retinol-binding protein (RBP) in culture than did endometrium from PP gilts. Estrone treatment increased ($P < .01$) endometrial NDM from P gilts but not that from PP gilts; estradiol had no effect. Both estrone and estradiol increased ($P = .069$) endometrial secretion of AP of PP but not of P gilts. Endometrial secretion of RBP was not affected by either estrone or estradiol treatment. Neither estrone nor estradiol affected total protein or AP and estrone treatment decreased ($P < .05$) RBP in uterine flushings from PP gilts. These data indicate that endometrium from P pigs secretes more protein than endometrium from PP pigs but neither estrone nor estradiol completely mimics the effect of pregnancy.

Key Words: Uterus, Pigs, Estrogen, Endometrium

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Introduction

Many proteins that are required for the normal development of the pig fetus are secreted by uterine endometrium (Roberts and Bazer, 1988). Two proteins of this type are uteroferrin, which is thought to transport iron (Roberts et al., 1986), and retinol binding protein (**RBP**), which is thought to transport retinol (Adams et al., 1981; Clawitter et al., 1990). Secretion of these and other proteins by the endometrium changes during pregnancy, and these changes are likely related to the needs of the

developing fetus. Several experiments indicate that progesterone is the primary factor controlling the proteins secreted by the endometrium during pregnancy (Knight et al., 1974; Chen et al., 1975; Adams et al., 1981; Roberts et al., 1987). However, other experiments suggest that the conceptus is capable of modulating protein secretion (Basha et al., 1980; Vallet et al., 1994). Estrogen has also been reported to modulate endometrial protein secretion in pigs (Knight et al., 1974; Geisert et al., 1982; Gries et al., 1989; Fliss et al., 1991; Trout et al., 1992) and indeed has been suggested to mediate the effect of the conceptus on the endometrium (Basha et al., 1980; Roberts et al., 1987; Roberts and Bazer, 1988). However, this concept was not supported by recent results (Vallet and Christenson, 1994). Estrone was used for that study and because of its decreased half-life after interacting with the estrogen receptor (Weichman and Notides, 1980), may be less potent than steroids used in previous experiments (i.e.,

¹Mention of names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the same by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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estradiol). Also, because pregnant gilts were used, endogenous conceptus estrogens were present, possibly activating estrogen receptors and decreasing the detection of subsequent effects of estrogen. Because the response to estradiol may differ from that of estrone, and because of possible interference from conceptus estrogens, the objective of the current experiment was to further test the effect of estrogen on endometrial protein production by comparing the effects of estrone and estradiol in pregnant and pseudopregnant gilts.

Materials and Methods

Thirty six mature (~200 d of age) white crossbred (1/4 Landrace, 1/4 Chester White, 1/4 Yorkshire, 1/4 Large White) gilts were used in this experiment. Eighteen gilts were mated at estrus after at least one estrous cycle of normal length. Eighteen other gilts received estradiol valerate (5 mg/d, d 11 to 15 of the estrous cycle) to induce pseudopregnancy. On d 30 of pregnancy or pseudopregnancy, gilts in each group were anesthetized and given either 1) sham implant (control), 2) estrone implants, or 3) estradiol-17 β implants. For each gilt, two implants designed to release steroid constantly for 60 d and containing 150 mg each (Innovative Research of America, Toledo, OH) were surgically placed subcutaneously behind the ear. Blood samples for measurement of estrone and estradiol by RIA were collected by jugular venipuncture into heparinized tubes just before implants were installed and on d 40, 50, and 60. On d 60, pigs were hysterectomized under general anesthesia. For pregnant gilts, placentas were dissected from the endometrium and then endometrial tissue in apposition to one placenta per uterine horn was collected and placed into 20 mL of cold leucine-deficient (one-tenth the normal concentration) minimum essential medium (MEM). For pseudopregnant gilts, each uterine horn was flushed with 40 mL of MEM and the flushings were collected and centrifuged ($10,000 \times g$) and the supernatant was retained for measurement of total protein, acid phosphatase (AP, a measure of uteroferrin), and RBP. The uterus was then opened and an area of endometrium in the middle of each uterine horn was gently wiped clear of debris with sterile gauze. Endometrium was then collected from each horn and placed into 20 mL of leucine-deficient MEM. For both pregnant and pseudopregnant gilts, an additional sample (1 g) of endometrium from the same site as the previous sample was collected from each uterine horn to measure tissue concentrations of free estrone and estradiol.

Endometrial tissue collected into MEM was blotted on sterile gauze and cut into small pieces (1 to 2 mm²) and then 500 mg of tissue was incubated in the presence of 50 μ Ci of [4,5-³H]leucine (specific activity 151 Ci/mmol, Amersham, Arlington Heights, IL) in 15

mL of leucine-deficient MEM using conditions described by Vallet and Christenson (1993). Endometrial culture medium was dialyzed (M_r 3,500 cutoff) against three changes of 8 L of 10 mM Tris, pH 7.6, and then an aliquot was subjected to scintillation counting to measure the amount of radioactivity incorporated into nondialyzable macromolecules (NDM), a measure of total protein synthesis. Acid phosphatase was measured in dialyzed endometrial culture medium and in uterine flushings as described by Vallet and Christenson (1994). Retinol-binding protein in endometrial culture medium and in uterine flushings was measured by RIA (Vallet, 1994). The inter- and intraassay coefficients of variation were 8.1 and 12.9%. Unconjugated estrone in plasma and endometrial homogenates was measured as described by Vallet and Christenson (1994). Unconjugated estradiol was measured in plasma as described by Redmer et al. (1984). Endometrial homogenates were extracted twice with diethyl ether, the extract was evaporated to dryness under a stream of nitrogen, redissolved in 90:10 benzene/methanol, and subjected to Sephadex LH-20 chromatography as described by Carr et al. (1971). The estradiol fraction was evaporated to dryness, redissolved in assay buffer, and then these samples were assayed as for plasma samples. Using this procedure, dilutions of sample were parallel to the standard curve, and the slope of the regression of estradiol added to samples on estradiol measured in the assay was .85. Recovery of estradiol was routinely greater than 90%, so no adjustment was made for recovery. Intra- and interassay coefficients of variation for endometrial estradiol assays were 27 and 23%, respectively. Endometrial estradiol was determined in triplicate to improve accuracy of the determinations. Plasma estrone, estradiol, and endometrial estrone were each determined in a single assay; intraassay coefficients of variation were 4.9, 10.1, and 6.2%, respectively.

Plasma estrone and estradiol concentrations were log-transformed to alleviate a scale effect and were then analyzed using ANOVA with a model that included effects of status (pregnant or pseudopregnant), treatment (sham, estrone, or estradiol), status \times treatment interaction, pig within status \times treatment interaction, day of pregnancy or pseudopregnancy (30, 40, 50, 60), day \times status interaction, day \times treatment interaction, and day \times status \times treatment interaction. Treatment \times day interactions were examined further using orthogonal contrasts.

Uterine weight, endometrial estrone and estradiol concentrations, and endometrial secretion of NDM, AP, and RBP were analyzed using ANOVA with a model that included effects of status, treatment, and status \times treatment interaction. Pig within status \times treatment interaction was used as error term. Contrasts were used to further examine main effects of treatment and the treatment \times status interaction.

Fetal weights, placental weights, number of CL, number of fetuses, and uterine flush total protein, AP, and RBP were analyzed using ANOVA with the effect of treatment as the model. Treatment effects were further examined using orthogonal contrasts.

Results

Mean plasma concentrations of estrone and estradiol for the different groups are illustrated in Figure 1. For plasma estrone, both status \times day ($P = .01$) and treatment \times day ($P < .01$) interactions were observed. Contrasts indicated that the status \times day interaction resulted from a greater increase ($P = .01$) in estrone concentrations in pseudopregnant pigs than in pregnant pigs. Because the main effects and interactions with treatment are composed of 2 degrees of freedom (i.e., there are three treatments), orthogonal contrasts were performed to subdivide the main effects and interactions into single degree of freedom comparisons to further define which specific treatments were responsible for the overall main effects and interactions. No sham vs estradiol treatment \times day interaction was detected for plasma estrone, indicating that estradiol treatment did not affect plasma estrone concentrations during the treatment period. The overall treatment \times day interaction was due solely to an interaction between sham and estradiol treatment combined vs estrone treatment \times day ($P < .01$). This indicates that only estrone treatment increased plasma estrone concentrations during the treatment period.

For plasma estradiol, no status or status \times day interaction was detected, indicating that changes in concentrations of estradiol that occurred in response to treatments were unaffected by status. A treatment \times day interaction was detected ($P < .01$). This interaction was further subdivided to examine specific treatment \times day interactions. A sham vs estrone treatment \times day interaction ($P < .01$) was present, indicating that estrone treatment increased plasma estradiol concentrations. A sham and estrone treatments combined vs estradiol treatment \times day interaction ($P < .01$) was also present, indicating that estradiol treatment also increased plasma estradiol concentrations.

Endometrial estrone, estradiol, NDM, AP, and RBP are summarized in Table 1. A treatment \times status interaction ($P < .01$) was observed for endometrial estrone concentrations. Contrasts indicated that this interaction was caused by the fact that estrone treatment did not result in a detectable increase in endometrial estrone concentrations in pregnant gilts but did increase ($P < .01$) estrone concentrations in pseudopregnant gilts. No status \times treatment interaction was detected for endometrial estradiol concentrations, indicating that treatment effects were uniform for both statuses. Effects of both status ($P < .05$) and

estradiol treatment compared to sham and estrone treatments combined ($P < .01$) were detected. This indicates that estradiol was greater in pregnant pigs than in pseudopregnant pigs (status main effect) and that for both pregnant and pseudopregnant pigs, estradiol treatment was successful in increasing estradiol concentrations in the endometrium (estradiol treatment main effect).

Only a main effect of status ($P < .01$) was detected for endometrial secretion of RBP in culture. This indicates that increased RBP was associated with the presence of the conceptus. The lack of significant treatment effects suggests that RBP secretion was not influenced by estrone or estradiol treatment. A status \times treatment interaction ($P < .05$) was detected for endometrial secretion of both NDM and AP. Contrasts indicated that for NDM, the status \times treatment interaction resulted from the fact that estrone treatment of pregnant gilts significantly increased ($P < .01$) NDM in culture compared to sham- and estradiol-treated gilts combined, but estrone treatment had no effect in pseudopregnant pigs. For both pregnant and pseudopregnant pigs, estradiol had no effect on NDM. Contrasts indicated that for AP, the status \times treatment interaction resulted from the fact that both estrone and estradiol tended to increase endometrial secretion of AP in pseudopregnant gilts ($P = .069$) and neither estrone nor estradiol had any effect in pregnant gilts. Finally, endometrial secretion of both NDM and AP was greater ($P < .01$) in pregnant sham-treated gilts than in pseudopregnant sham-treated gilts, indicating that both NDM and AP secretion were greater in the presence of the conceptus.

Uterine flush content of protein and AP was unaffected by estrogen treatments. Uterine flush RBP content was decreased ($P < .05$) by estrone compared to sham- and estradiol-treated groups combined.

Empty uterine weights were lower in pseudopregnant than in pregnant pigs (Table 2). Fetal weights, placental weights, number of corpora lutea, and number of fetuses were unaffected by estrogen treatment.

Discussion

These results confirm our previous report that estrogen treatment of gilts after d 30 of pregnancy does not mimic the effect of the conceptus on endometrial protein secretion (Vallet and Christenson, 1994) and confirm that the presence of the conceptus is associated with increased protein secretion (Basha et al., 1980; Vallet et al., 1994). Collectively, these data suggest that conceptus estrogen alone does not cause the increase in endometrial protein secretion associated with the conceptus despite previous reports that estrogen modulates endometrial protein secretion (Knight et al., 1974; Geisert et al., 1982; Trout et al., 1992). Thus, it is likely that other

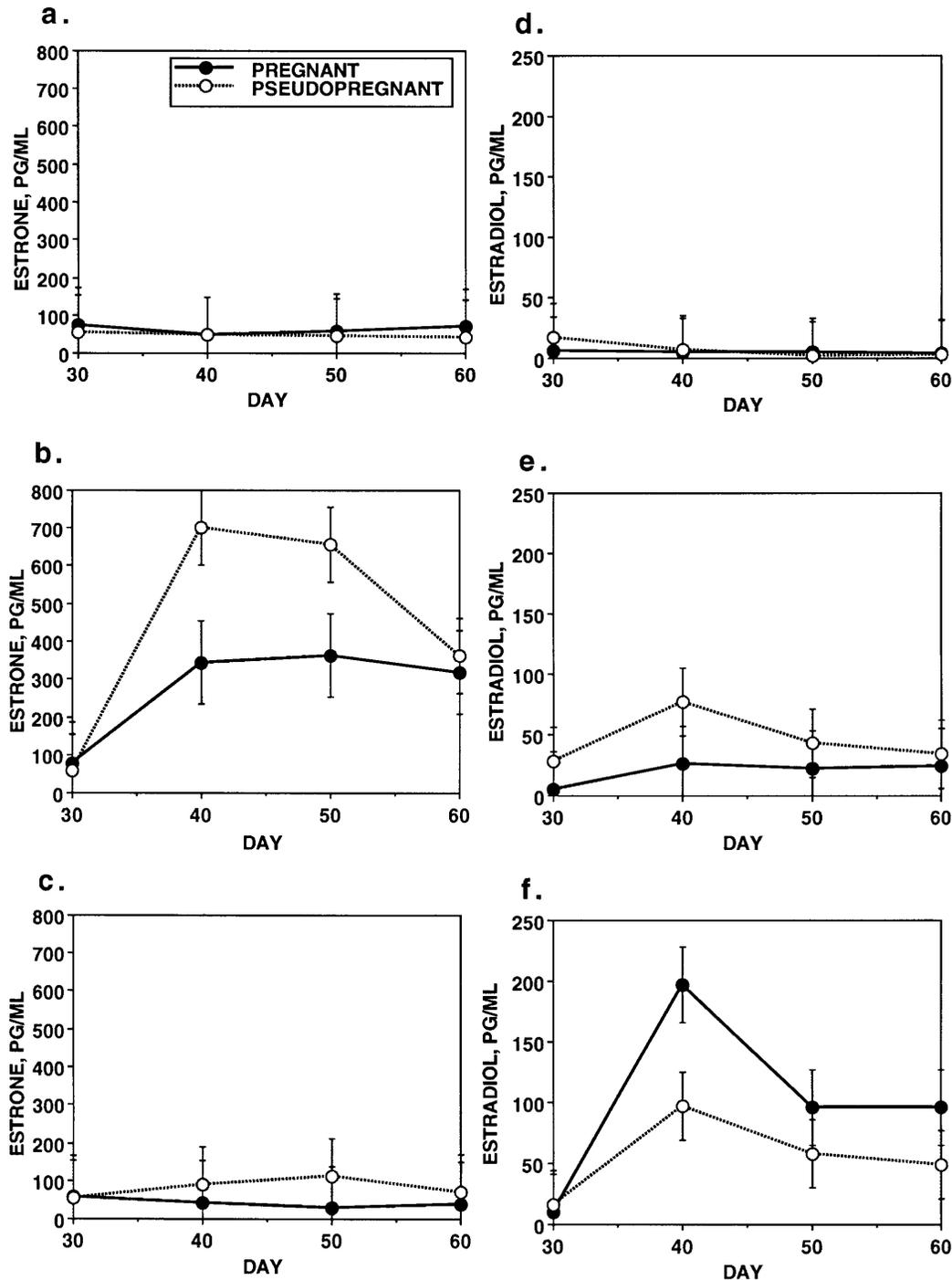


Figure 1. Least squares means (\pm SE) for plasma estrone (a–c) and estradiol (d–f) are illustrated for pregnant and pseudopregnant gilts given either sham (a, d), estrone (E_1 ; b, e), or estradiol (E_2 ; c, f) treatment. Estrone, but not estradiol, treatment increased ($P < .01$) plasma estrone concentrations, greater increases ($P = .01$) occurred in pseudopregnant than in pregnant gilts. Both estrone and estradiol treatment increased ($P < .01$) plasma estradiol concentrations, and no effect of status was detected. Note that scales for plasma estrone and estradiol are different.

conceptus factors participate in conceptus control of endometrial protein secretion.

Proteins secreted by the endometrium deliver nutrients to the developing conceptus. Uteroferrin, which contains iron, is secreted by endometrial gland epithelial cells and is taken up by the placental

areolar epithelial cells by fluid phase pinocytosis (Roberts and Bazer, 1988). Thus, endometrial secretion rate of uteroferrin likely influences delivery of iron to the fetus during gestation. Likewise, RBP, which contains retinol, is secreted by the endometrial epithelial cells (Harney et al., 1994) and is likely

Table 1. Least squares means for endometrial estrone and estradiol, nondialyzable macromolecules (NDM), acid phosphatase (AP), and retinol binding protein (RBP) secreted by endometrium in culture, and total protein, AP, and RBP in uterine flushings from gilts given either sham, estrone or estradiol treatments from day 30 to 60 of pregnancy (P) or pseudopregnancy (PP) and then hysterectomized on day 60

Item	Status	Treatment		
		Sham	Estrone	Estradiol
Estrone, pg/g tissue ^{abc}	P	280.7 ± 41.4	241.0 ± 41.4	230 ± 46.2
	PP	129.4 ± 41.4	354.0 ± 41.4	174 ± 41.4
Estradiol, pg/g tissue ^{de}	P	168.2 ± 45.8	228.4 ± 45.8	307.6 ± 51.2
	PP	107.4 ± 45.8	121.3 ± 45.8	229.6 ± 45.8
Endometrial, RBP $\mu\text{g}/(\text{g}\cdot\text{tissue})^i$	P	66.0 ± .5	75.2 ± 6.5	69.9 ± 7.3
	PP	26.7 ± 6.5	29.0 ± 6.5	30.8 ± 6.5
NDM $\times 10^{-7}$, DPM/g tissue ^{cfg}	P	2.33 ± .17	2.84 ± .17	2.12 ± .19
	PP	1.45 ± .19	1.47 ± .17	1.62 ± .17
Endometrial AP, $\mu\text{mol Pi}/(\text{min}\cdot\text{g tissue})^{\text{cfh}}$	P	47.9 ± 4.5	44.7 ± 4.5	38.1 ± 5.1
	PP	28.7 ± 4.5	37.8 ± 4.5	38.8 ± 4.5
Uterine flush protein, g	PP	1.46 ± .37	1.23 ± .37	1.55 ± .37
Uterine flush AP, $\mu\text{mole Pi}/\text{min}$	PP	62,216 ± 20,240	64,280 ± 20,240	79,624 ± 20,240
Uterine flush RBP, mg ^j	PP	9.2 ± 1.7	4.0 ± 1.7	7.8 ± 1.7

^aTreatment \times estrogen status interaction ($P < .01$).

^bWithin pseudopregnant gilts, estrone greater than sham and estradiol treatment combined ($P < .01$).

^cPregnant, sham-treated gilts greater than pseudopregnant, sham-treated gilts ($P < .01$).

^dMain effect of estradiol treatment compared to sham and estrone-treated gilts ($P < .01$).

^eEffect of status ($P < .05$).

^fStatus \times estrogen treatment interaction ($P < .05$).

^gWithin pregnant gilts, estrone treatment was greater than estradiol and sham-treated gilts combined ($P < .01$).

^hWithin pseudopregnant gilts, estradiol and estrone-treated gilts combined were greater than sham-treated gilts ($P = .069$).

ⁱMain effect of status ($P < .01$).

^jEstrone less than estradiol and sham-treated gilts combined ($P < .05$).

taken up by the placenta in a manner similar to uteroferrin, although this has not been investigated. Uteroferrin is transferred to the fetal liver via the blood stream, where the iron in uteroferrin is used for hematopoiesis (Roberts and Bazer, 1988). Again, a similar pattern likely occurs for RBP; RBP has been immunolocalized to the fetal liver during pregnancy (Harney et al., 1994). Thus, factors that control endometrial secretion of these and other endometrial proteins likely influence the efficiency of conceptus development and uterine capacity.

Our previous study (Vallet and Christenson, 1994) employed estrone treatment from d 30 to 45 of pregnancy in an attempt to influence endometrial protein secretion, and no effect of estrone treatment was observed. Several possible mechanisms were postulated for the lack of an effect, including 1) estrone is a less active estrogen than estradiol, 2) estrone may not be metabolized to the same compounds as estradiol, thus possible active substances may not be produced when estrone is used, 3) conceptus produced estrogens may already saturate

Table 2. Least squares means for uterine, fetal, and placental weights and number of corpora lutea and fetuses for gilts given sham, estrone, or estradiol treatments from days 30 to 60 of pregnancy (P) or pseudopregnancy (PP) and then hysterectomized on day 60

Item	Status	Treatment		
		Sham	Estrone	Estradiol
Uterine wt ^a , g	P	1,984 ± 120	1,872 ± 120	1,888 ± 134
	PP	724 ± 120	753 ± 120	769 ± 120
Fetal wt, g	P	117.2 ± 8.1	122.3 ± 8.1	109.7 ± 9.0
Placental wt, g	P	144.1 ± 18.7	130.0 ± 18.7	129.4 ± 20.9
Number CL	P	12.0 ± .8	10.2 ± .8	12.8 ± .9
Number fetuses	P	10.8 ± 1.0	10.2 ± 1.0	10.6 ± 1.1

^aMain effect of status ($P < .01$).

the available receptors on d 45 of pregnancy, and may therefore prevent any further effect of estrogen, and 4) receptors for estrogen may not have been present on d 45 of pregnancy but may develop later (i.e., by d 60 of pregnancy). The current experiment was designed to address these possible explanations for the lack of an effect of estrone on endometrial protein secretion. Estrone and estradiol treatments were compared. The lack of effect of estradiol indicates that our previous results (Vallet and Christenson, 1994) were not due to a lack of potency of estrone or an inability of estrone to be metabolized to the same products as estradiol. Pseudopregnant pigs, in which conceptus estrogens are not present, were used to address whether conceptus estrogens may prevent detection of effects of administration of exogenous estrogen. Several experiments indicate that progesterone concentrations in pregnant and pseudopregnant gilts are similar (Frank et al., 1977; DeHoff et al., 1986; Geisert et al., 1987; King and Rajamahandran, 1988; Gadsby et al., 1991). The fact that estrone and estradiol treatment of pseudopregnant pigs did not mimic the effect of pregnancy therefore indicates that the previous lack of effect of estrone in pregnant gilts (Vallet and Christenson, 1994) was not due to saturation of receptors by conceptus estrogens. Finally, we examined the effects of estrone and estradiol on d 60 of pregnancy and pseudopregnancy. Both the current and previous results (Vallet et al., 1994) indicate that the effect of the presence of the conceptus on endometrial protein secretion is detectable by this day, suggesting that if the hypothesis that estrogen mediates the conceptus effect is correct, the endometrium of pregnant gilts should possess the necessary receptors by d 60 of pregnancy and a response to either estrone or estradiol should have been detected in the current experiment.

Results of the current study were complex; the effect of estrogen treatments and their interaction with pregnancy or pseudopregnancy depended on the endometrial secretory product examined. In the simplest case, neither estrone nor estradiol treatment had an effect on endometrial secretion of RBP in culture. However, estrone treatment suppressed RBP in uterine flushings.

As previously indicated, the presence of the conceptus is associated with increased RBP secretion. Thus, these data suggest that the conceptus' effect on RBP is not mediated by estrogen. This result contrasts those of Trout et al. (1992) in which estrogen treatment stimulated RBP mRNA and intrauterine retinol concentrations. Also, Harney et al. (1994) and Christenson et al. (1995) reported that increased endometrial RBP mRNA concentrations and RBP secretion peak at d 30 of pregnancy, decrease, and then increase again in late gestation (i.e., after d 80). This is similar to the timing of conceptus estrogen production (Robertson and King, 1974) and it has been suggested that estrogen controls these changes (Harney et al., 1994).

Our results indicate that the response of endometrium from d 30 to 60 of pregnancy differs from that during early pregnancy (Trout et al., 1992) and that obtained using ovariectomized pigs treated with steroid replacement (Trout et al., 1992). Our results also suggest that the temporal association between RBP and conceptus estrogen is not caused by the influence of estrogen on endometrial RBP secretion.

Treatment of pregnant gilts with estrone resulted in increased endometrial secretion of NDM in culture; estradiol did not affect NDM in pregnant gilts, and neither estrone nor estradiol affected NDM in pseudopregnant pigs. Total protein in uterine flushings from pseudopregnant gilts was also not affected by estrogen treatments. Estrone treatment increased NDM in culture despite our inability to detect increased estrone concentrations in the endometrium of pregnant gilts. However, plasma estradiol concentrations indicated that estrone treatment resulted in increased plasma estradiol, suggesting that some of the estrone administered was metabolized to estradiol. Furthermore, despite administration of similar doses of estrone and estradiol, estradiol concentrations obtained were lower than estrone concentrations obtained. These results may reflect the fact that both estrone and estradiol are metabolized to a variety of products with varying biological activities (Perry et al., 1976; Chakraborty et al., 1989). Treatment of pseudopregnant gilts with estrone resulted in greater plasma estrone concentrations than similar treatment of pregnant gilts. The most likely explanation of this result and the failure of estrone treatment to increase endometrial estrone is that the conceptus may have metabolized the administered estrone to water-soluble products (i.e., sulfates and glucuronide conjugates; Perry et al., 1976) that would not be detected in our RIA. The conversion of estrone to catecholestrogens (Chakraborty et al., 1989), which may have biological activity (Rosenkrans et al., 1990), may provide an explanation for the effect of estrone on NDM, despite no detectable change in endometrial estrone concentrations. Because estradiol treatment did not affect NDM, it is unlikely that conversion of estrone to estradiol explains the effect of estrone. Because estrogen treatment did not influence NDM secretion in pseudopregnant gilts and a clear difference in NDM secretion was obtained between pregnant and pseudopregnant gilts, our results suggest that the effect of the presence of the conceptus on endometrial protein secretion in general is not due solely to conceptus estrogen secretion. It may be that the conceptus is required to metabolize estrone to an active metabolite.

Both estrone and estradiol tended to increase endometrial secretion of AP in pseudopregnant but not pregnant gilts. This increase, which approached statistical significance, was not reflected by increased AP in uterine flushings from pseudopregnant gilts. Thus, the effect of estrogens on AP is equivocal. However, AP concentrations in flushings were highly

variable (SEM was 33% of the mean) and further factors may influence intrauterine AP concentrations (such as degradation of AP, feedback inhibition, etc.), which may not occur in culture. A possible explanation of these results is that the effect of the conceptus on AP secretion could be at least partially controlled by conceptus estrogen, and that receptors for estrogen in pregnant gilts may be saturated, preventing detection of further increases. Further experimentation will be required to confirm the role of conceptus estrogen in AP secretion.

In conclusion, our study indicates that of the proteins examined only endometrial secretion of uteroferrin, measured as AP, could be influenced by conceptus estrogen secretion, although this effect only approached statistical significance. The effect of the conceptus on total protein secretion, measured as NDM, and RBP could not be mimicked using either estrone or estradiol treatment. Thus, other conceptus factors are likely to be involved in controlling endometrial protein secretion.

Implications

Our study indicates that treatment of gilts with estrogens did not mimic the enhancement of endometrial protein secretion that occurs in the presence of the conceptus by d 60 of pregnancy in swine. Thus, other conceptus factors may be involved and could be important in controlling the flow of endometrial proteins, and therefore essential nutrients, to the developing conceptus during this period. Elucidation of the conceptus factors that influence endometrial protein secretion would be useful in enhancing uterine capacity during pregnancy in swine.

Literature Cited

- Adams, K. L., F. W. Bazer, and R. M. Roberts. 1981. Progesterone-induced secretion of a retinol-binding protein in the pig uterus. *J. Reprod. Fertil.* 62:39.
- Basha, S.M.M., F. W. Bazer, and R. M. Roberts. 1980. Effect of the conceptus on quantitative and qualitative aspects of uterine secretion in pigs. *J. Reprod. Fertil.* 60:41.
- Carr, B. R., G. Mikhail, and G. L. Flickinger. 1971. Column chromatography of steroids on Sephadex LH-20. *J. Clin. Endocrinol. Metab.* 33:358.
- Chakraborty, C., S. K. Dey, and D. L. Davis. 1989. Pattern and tissue distribution of catechol estrogen forming activity by pig conceptuses during the peri-implantation period. *J. Anim. Sci.* 67:991.
- Chen, T. T., F. W. Bazer, B. M. Gebhardt, and R. M. Roberts. 1975. Uterine secretion in mammals: Synthesis and placental transport of a purple acid phosphatase in pigs. *Biol. Reprod.* 13:304.
- Christenson, R. K., J. L. Vallet, and W. J. McGuire. 1995. Association of allantoic fluid and endometrial retinol binding protein (RBP) and uteroferrin (UF) during gestation in the pig. *J. Anim. Sci.* 73(Suppl. 1):214 (Abstr.).
- Clawitter, J., W. E. Trout, M. G. Burke, S. Arah, and R. M. Roberts. 1990. A novel family of progesterone-induced, retinol-binding proteins from uterine secretions of the pig. *J. Biol. Chem.* 265:3248.
- DeHoff, M. H., C. S. Stoner, F. W. Bazer, R. J. Collier, R. R. Kraeling, and F. C. Buonomo. 1986. Temporal changes in steroids, prolactin and growth hormone in pregnant and pseudopregnant gilts during mammogenesis and lactogenesis. *Domest. Anim. Endocrinol.* 3:95.
- Fliss, A. E., F. J. Michel, C.-L. Chen, A. Hofig, F. W. Bazer, J. Y. Chou, and R.C.M. Simmen. 1991. Regulation of the uteroferrin gene promoter in endometrial cells: Interactions among estrogen, progesterone, and prolactin. *Endocrinology* 129:697.
- Frank, M., F. W. Bazer, W. W. Thatcher, and C. J. Wilcox. 1977. A study of prostaglandin F_{2α} as the luteolysin in swine: III Effects of estradiol valerate on prostaglandin F, progestins, estrone and estradiol concentrations in the utero-ovarian vein of nonpregnant gilts. *Prostaglandins* 14:1183.
- Gadsby, J. E., C. A. Smith, and G. W. Almond. 1991. Acute stimulatory effects of prostaglandin F_{2α} on serum progesterone concentrations in pregnant and pseudopregnant pigs. *Prostaglandins* 41:419.
- Geisert, R. D., W. W. Thatcher, R. M. Roberts, and F. W. Bazer. 1982. Establishment of pregnancy in the pig: III. Endometrial secretory response to estradiol valerate administered on day 11 of the estrous cycle. *Biol. Reprod.* 27:957.
- Geisert, R. D., M. T. Zavy, R. P. Wettemann, and B. G. Biggers. 1987. Length of pseudopregnancy and pattern of uterine protein release as influenced by time and duration of oestrogen administration in the pig. *J. Reprod. Fertil.* 79:163.
- Gries, L. K., R. D. Geisert, M. T. Zavy, J. E. Garrett, and G. L. Morgan. 1989. Uterine secretory alterations coincident with embryonic mortality in the gilt after exogenous estrogen administration. *J. Anim. Sci.* 67:276.
- Harney, J. P., L. C. Smith, R.C.M. Simmen, A. E. Fliss, and F. W. Bazer. 1994. Retinol-binding protein: Immunolocalization of protein and abundance of messenger ribonucleic acid in conceptus and maternal tissues during pregnancy in pigs. *Biol. Reprod.* 50:1126.
- King, G. J., and R. Rajamahendran. 1988. Comparison of plasma progesterone profiles in cyclic, pregnant, pseudopregnant and hysterectomized pigs between 8 and 27 days after oestrus. *J. Endocrinol.* 119:111.
- Knight, J. W., F. W. Bazer, H. D. Wallace, and C. J. Wilcox. 1974. Dose-response relationships between exogenous progesterone and estradiol and porcine uterine protein secretions. *J. Anim. Sci.* 39:747.
- Perry, J. S., R. B. Heap, R. D. Burton, and J. E. Gadsby. 1976. Endocrinology of the blastocyst and its role in the establishment of pregnancy. *J. Reprod. Fertil. Suppl.* 25:85.
- Redmer, D. A., R. K. Christenson, J. J. Ford, and B. N. Day. 1984. Effect of unilateral ovariectomy on compensatory ovarian hypertrophy, peripheral concentrations of follicle-stimulating hormone and luteinizing hormone, and ovarian venous concentrations of estradiol-17β in prepubertal gilts. *Biol. Reprod.* 31:59.
- Roberts, R. M., and F. W. Bazer. 1988. The functions of uterine secretions. *J. Reprod. Fertil.* 82:875.
- Roberts, R. M., M. K. Murray, M. G. Burke, C. M. Ketcham, and F. W. Bazer. 1987. Hormonal control and function of secretory proteins. In: W. W. Leavitt (Ed.) *Cell and Molecular Biology of the Uterus*. p. 137 Plenum Publishing Corp., New York.
- Roberts, R. M., T. J. Raub, and F. W. Bazer. 1986. Role of uteroferrin in transplacental iron transport in the pig. *Fed. Proc.* 45:2513.
- Robertson, H. A., and G. J. King. 1974. Plasma concentrations of progesterone, oestrone, oestradiol-17β and of oestrone sulphate in the pig at implantation, during pregnancy and at parturition. *J. Reprod. Fertil.* 40:133.
- Rosenkrans, C. F., Jr., B. C. Paria, D. L. Davis, and G. Milliken. 1990. In vitro synthesis of prostaglandin E and F_{2α} by pig endometrium in the presence of estradiol, catechol estrogen and ascorbic acid. *J. Anim. Sci.* 68:435.

- Trout, W. E., J. A. Hall, M. L. Stallings-Mann, J. M. Galvin, R. V. Anthony, and R. M. Roberts. 1992. Steroid regulation of the synthesis and secretion of retinol-binding protein by the uterus of the pig. *Endocrinology* 130:2557.
- Vallet, J. L. 1994. Technical note: A radioimmunoassay for porcine retinol binding protein. *J. Anim. Sci.* 72:2449.
- Vallet, J. L., and R. K. Christenson. 1993. Uterine space affects placental protein secretion in swine. *Biol. Reprod.* 48:575-584.
- Vallet, J. L., and R. K. Christenson. 1994. Effect of estrone treatment from day 30 to 45 of pregnancy on endometrial protein secretion and uterine capacity. *J. Anim. Sci.* 72:3188.
- Vallet, J. L., H. G. Klemcke, and R. K. Christenson. 1994. Effect of the conceptus and lack of effect of uterine space on endometrial protein secretion during mid-gestation in swine. *Biol. Reprod.* 51:748.
- Weichman, B. M., and A. C. Notides. 1980. Estrogen receptor activation and the dissociation kinetics of estradiol, estriol and estrone. *Endocrinology* 106:434.