Effect of Estrone Treatment from Day 30 to 45 of Pregnancy on Endometrial Protein Secretion and Uterine Capacity[1]

J. L. Vallet and R. K. Christenson

Roman L. Hruska U.S. Meat Animal Research Center, USDA, ARS, Clay Center, NE 68933-0166

ABSTRACT: Secretion of proteins by the endometrium of pigs during pregnancy is reported to be influenced by progesterone and estrogen. The effect of estrone treatment from d 30 to 45 of pregnancy on endometrial protein secretion and uterine capacity was tested in unilaterally hysterectomized-ovariectomized (UHO) gilts. Ten intact and 16 UHO crossbred gilts were mated and assigned to the following treatments: 1) intact controls, n = 10; 2) UHO, n = 8; and 3) UHO + estrone (E; two 150-mg implants, 60-d release, implanted on d 30 of gestation), n = 8. Gilts were killed on d 45 of pregnancy. A sample of allantoic fluid was collected from each conceptus. Blood and endometrial tissue samples were collected to evaluate estrone concentrations, and placental and endometrial tissues were incubated to evaluate protein secretion. Estrone treatment increased ($P < .01$) plasma and endometrial tissue estrone concentrations. Unilateral hysterectomy-ovariectomy decreased ($P < .01$) fetal survival. No effect of estrone was detected on the number of live fetuses, protein secretion by endometrial tissue in culture, or allantoic fluid protein, acid phosphatase, retinol binding protein, or retinol. Estrone treatment decreased uterine weight ($P = .05$) and allantoic fluid fructose concentrations ($P < .05$). After correction for allantoic fluid volume, allantoic fluid protein, retinol, and acid phosphatase activity were decreased ($P < .05$) in UHO-treated pigs. The UHO treatment did not affect allantoic fluid fructose concentrations. These results indicate that 1) estrone treatment from d 30 to 45 of pregnancy and at the dosage used did not alter endometrial protein secretion or fetal survival, and 2) UHO decreased the amount of endometrial proteins available to conceptuses.

Key Words: Pregnancy, Pigs, Uterus, Estrone, Endometrium

J. Anim. Sci. 1994. 72:3188–3195

Introduction

Litter size in swine is influenced by uterine capacity (Bennett and Leymaster, 1989). The endometrium provides nutrients in the form of proteins required by the conceptus for growth (Roberts and Bazer, 1988). Therefore, secretion of these nutrients may influence uterine capacity. Examples of these proteins are uteroferrin, which is hypothesized to transfer iron (Ducsay et al., 1982), and retinol binding protein, which is hypothesized to transfer retinol (Adams et al., 1981). Secretion of these proteins and others is increased by progesterone (Roberts et al., 1987), and estrogen can synergize with progesterone to further stimulate endometrial protein secretion (Knight et al., 1973; Roberts et al., 1987; Fliss et al., 1991; Trout et al., 1992). Thus, estrogen may be effective in stimulating endometrial protein secretion and may therefore be useful in studying the role of proteins secreted by the endometrium in processes that influence uterine capacity.

Litter size in unilaterally hysterectomized-ovariectomized (**UHO**) swine is a measure of uterine capacity (Christenson et al., 1987). Significant fetal loss occurs between d 30 and 40 to 45 of pregnancy when fetuses are crowded by either UHO treatment (Knight et al., 1977) or by ligation of the uterine horn (Chen and Dzuik, 1993). Thus, d 30 to 45 of pregnancy is a useful period for testing the effect of putative treatments on uterine capacity as measured using UHO-treated pigs.

The objectives of this experiment were to determine whether exogenous treatment of pregnant pigs with estrone from d 30 to 45 of pregnancy would stimulate endometrial protein secretion and affect uterine capacity, as measured by litter size, in unilaterally-hysterectomized ovariectomized pigs.

[1]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 the USDA implies no approval of the product to the exclusion of others that may also be suitable.

Received March 3, 1994.
Accepted July 29, 1994.

Materials and Methods

Sixteen gilts (1/4 Yorkshire, 1/4 Large White, 1/4 Chester White, and 1/4 Landrace) were unilaterally hysterectomized and ovariectomized (UHO) at approximately 160 d of age. The UHO gilts and 10 intact control gilts were observed for estrus and mated after at least one estrous cycle of normal duration. The treatments were as follows: Treatment 1, intact control; Treatment 2, UHO control; Treatment 3, UHO + estrone. On d 30 of pregnancy, eight of the UHO gilts (Treatment 3) received two estrone implants (150-mg estrone implants, 60-d release, Innovative Research of America, Toledo, OH). These implants are designed to release 5 mg of estrone per day. Implants were placed subcutaneously in the neck behind the ear using aseptic procedures. The other eight UHO gilts (Treatment 2) and the intact control gilts (Treatment 1) received sham operations on d 30 of pregnancy. Blood samples (5 mL) were collected by jugular venipuncture before surgery on d 30 and on d 31, 38, and just before slaughter for measurement of plasma estrone. On d 44 to 46 of pregnancy, gilts were killed and their reproductive tracts were removed.

The number of corpora lutea (**CL**), number of live fetuses, sex of each fetus in the litter, allantoic fluid volume, weight of each placenta and fetus, and empty uterine weight for each gilt were recorded. Where possible, a sample (up to 10 mL) of allantoic fluid was collected from each conceptus using a syringe and needle. The uterine wall was then ruptured, and the volume of the allantoic fluid was measured in a graduated cylinder. The volume was recorded as zero when no sample and no allantoic fluid was obtainable. Dead (i.e., necrotic) fetuses were excluded from all measurements. Samples of placenta and the adjacent endometrium for tissue culture were collected from two conceptuses, taken randomly, from each gilt. Tissues were placed into sterile minimum essential medium (**MEM**) containing .1× leucine and other modifications described previously (Vallet and Christenson, 1993). Adjacent to these same fetuses, additional endometrial tissues were collected, frozen in liquid nitrogen, and maintained at −70°C until they were measured for endometrial estrone concentrations. Allantoic fluid was assayed for protein, acid phosphatase (a measure of uteroferrin), retinol, retinol binding protein, and fructose concentrations.

Placental and Endometrial Tissue Culture and Electrophoresis

Placental and adjacent endometrial tissues (500 mg) were incubated separately in MEM containing .1× leucine and 50 μCi of [^{3}H]leucine for 24 h using conditions described by Vallet and Christenson (1993). Tissues were blotted on sterile gauze and then cut into small (1- to 2-mm) pieces before they were placed in culture. The resultant media were dialyzed against 10 m*M* Tris, pH 8.2, (three changes of 4 L each), and 100 μL was subjected to scintillation counting to determine the amount of [^{3}H]leucine incorporated into nondialyzable macromolecules. Aliquots of endometrial culture medium (2 mL) from five gilts chosen at random within each treatment group were lyophilized and subjected to two-dimensional PAGE and fluorography as described by Roberts et al. (1984). Endometrial culture medium was also assayed for acid phosphatase and retinol binding protein concentrations.

Estrone Radioimmunoassay

Plasma and endometrial estrone concentrations were measured by RIA. Cross-reactivities of the antiserum used were described by Guthrie and Deaver (1979). To prepare endometrial extracts for estrone RIA, endometrial samples (1 g) were thawed, 1 mL of PBS-.2% gelatin (**PBSG**), pH 7.5, was added, and samples were homogenized using a polytron (Brinkman Instruments, Luzern, Switzerland). Samples were then centrifuged at 85,000 × *g* to remove cell debris and membranes. The supernatant was collected and estrone content was determined. Estrone in endometrium per gram of tissue was then calculated assuming 1 g of tissue equaled 2 mL of homogenate. Samples (100 μL of serum or endometrial homogenate) were extracted twice with 2.5 mL of diethyl ether. The two extracts were combined and dried under a stream of nitrogen. Preliminary trials using [^{3}H]estrone indicated that extraction efficiency was routinely 90 to 100%, so no correction was made for extraction efficiency. The dried extracts were redissolved in 500 μL of PBSG. For RIA, samples or standards were combined with 100 μL of a 1:50,000 dilution of antiserum and 50 μL of [^{3}H]estrone ([2,4,6,7-^{3}H(N)]estrone, 92.8 Ci/mmol, NEN Dupont Co., Wilmington, DE; 20,000 cpm) and were incubated overnight at 4°C. Bound estrone was separated from free estrone using 100 μL of 1.25% charcoal, .125% dextran in PBSG. Displacement of [^{3}H]estrone resulting from dilutions of samples of both plasma and endometrial extract was parallel to the standard curve. Accuracy of recovery of nonradioactive estrone was evaluated by adding estrone to samples of plasma and endometrial extract and assaying the amount of estrone in the samples. The regression of added estrone to assayed estrone was $y = 1.08\ x + 12.2$ for plasma and $y = 1.12\ x + 6.9$ for endometrial extract. The limit of detection of the assay was 1.8 pg. Inter- and intraassay CV were 27 and 13%, respectively.

Protein, Acid Phosphatase, Retinol, Retinol Binding Protein, and Fructose Assays

Protein in allantoic fluid was measured using the method of Lowry et al. (1951) using BSA as the standard. Fructose in allantoic fluid was measured as described by Zavy et al. (1982).

Acid phosphatase in allantoic fluid and in incubation medium was measured using the assay described by Basha et al. (1979), but it was modified so it could be performed in microtiter plates. Briefly, samples were incubated in sodium phosphate-buffered saline, .1 *M* 2-mercaptoethanol (pH 7.0) for 20 min at 37°C. Then, sufficient 1 *M* sodium acetate (pH 4.9) was added to each sample to make a .1 *M* solution of sodium acetate. Fifty microliters of this dilution was added to microtiter wells in quadruplicate, and 50 μL of 40 m*M* *p*-nitrophenylphosphate was added to each well. Enzyme activity in two of the four replicates was inhibited immediately by adding 100 μL of 1 *M* NaOH to provide background absorbance. Plates were then incubated for 30 min at 37°C, and then the other two replicates received 100 μL of 1 *M* NaOH to stop the reaction. Absorbance was measured with a plate reader (MR 600, Dynatech Laboratories, Alexandria, VA) at a wavelength of 410 nm. Background absorbance was subtracted from total absorbance to obtain enzyme specific absorbance. The specific absorbance was compared to a standard curve ranging from 0 to 40 μg/mL of *p*-nitrophenol in .1 *M* sodium acetate buffer. Linearity with time (10 to 30 min) and with dilution of sample were demonstrated (not shown).

Retinol in allantoic fluid was measured using a modification of the method of Selvaraj and Sushella (1970). Briefly, 1-mL samples were combined with 1 mL of 1 *M* KOH in 95% ethanol. Samples were incubated at 60°C for 2 h, allowed to cool, and extracted with 3 mL of xylene. Retinol fluorescence in the xylene extracts was measured using a fluorometer (Perkin Elmer LS50, 330 nm excitation wavelength, 470 nm emission wavelength; Perkin Elmer, Beaconsfield, Buckinghams, U.K.) and was then converted to retinol concentrations by comparison with retinol standards in xylene. Samples were corrected for extraction efficiency, which was approximately 70%.

Retinol binding protein (**RBP**) in allantoic fluid and endometrial culture medium was measured using a validated RIA specific for porcine RBP (Vallet, 1994). Intra- and interassay CV were 11.0 and 10.4%, respectively. The limit of detection of the assay was 4.6 ng/mL.

Statistical Analysis

Plasma estrone data were analyzed with analysis of variance (SAS, 1990) using a model that included the effects of treatment, gilt within treatment, day of pregnancy, and the treatment × day of pregnancy interaction. The following set of orthogonal contrasts was used to determine the effect of treatment on plasma estrone concentrations: 1) Treatment 2 vs 3 (effect of estrone treatment) and 2) Treatment 1 vs 2 and 3 combined (effect of UHO).

The number of CL, live fetuses, fetal survival, sex ratio, and empty uterine weight data were analyzed with analyses of variance using a model that included the effect of treatment. The same orthogonal contrasts as above were used to determine the effects of specific treatments.

Fetal and placental weights, allantoic fluid volume, allantoic fluid protein, acid phosphatase, retinol, retinol binding protein, and fructose concentrations; total allantoic fluid protein, acid phosphatase, retinol, retinol binding protein, and fructose; endometrial nondialyzable radioactivity, acid phosphatase, retinol binding protein, and estrone; and placental nondialyzable radioactivity data were analyzed with analyses of variance using a model that included the effect of treatment and pig within treatment. Pig within treatment was used as the error term for examining treatment effects. Preliminary analysis with analysis of variance using a model that included day of slaughter (d 44 to 46) indicated that fetal and placental weights were affected by day. Because each day of slaughter was not represented within each treatment group, least squares means using all days could not be estimated. Therefore, only data collected on d 45 of pregnancy were used in the above analysis of fetal and placental weights. The orthogonal contrasts described above were used to further define specific treatment effects. Allantoic fluid protein, retinol, RBP, and acid phosphatase concentrations were negatively correlated with allantoic fluid volume, and regression analysis indicated that the linear and quadratic parameters of the relationship between each component and allantoic fluid volume were significant (see results). Therefore, to adjust the data for differences in allantoic fluid volume between fetuses, additional analyses of variance were performed for allantoic fluid protein, retinol, retinol binding protein, and acid phosphatase concentrations using a model that included the linear and quadratic effects of allantoic fluid volume as covariates along with the effects of treatment and pig within treatment. To determine whether the RBP present in allantoic fluid differed in percentage of retinol saturation across treatment, an additional analysis of variance was performed for allantoic fluid retinol concentrations using a model that included retinol binding protein concentrations as a covariate along with effects of treatment and pig within treatment.

Results

Plasma and endometrial estrone concentrations for the three treatment groups are illustrated in Figure 1. The UHO treatment had no detectable effect on either plasma or endometrial estrone concentrations. Estrone treatment increased ($P < .01$) plasma and endometrial estrone concentrations during the experimental period.

The UHO treatment decreased the number of fetuses ($P < .01$), fetal survival ($P < .01$), fetal weight ($P < .01$), placental weight ($P < .05$), and uterine

weight ($P < .01$), but it did not affect the number of CL or sex ratio of the fetuses (Table 1). Estrone treatment had no effect on number of fetuses, number of CL, fetal survival, fetal weight, placental weight, or sex ratio of the fetuses, but it decreased uterine weight ($P = .05$).

Allantoic Fluid Volume, Protein, Retinol, Acid Phosphatase, Fructose, and Retinol Binding Protein

No effect of UHO treatment on allantoic fluid volume, protein, retinol, acid phosphatase, retinol binding protein, or fructose was detected using analyses of variance (Table 2). Furthermore, no effect of estrone treatment was detected for allantoic fluid volume, protein, retinol, acid phosphatase, or retinol-binding protein. Estrone treatment decreased ($P < .05$) allantoic fluid fructose concentrations. Allantoic fluid volume was highly variable, ranging from 0 to 300 mL. Regression analysis indicated that there was a negative relationship between allantoic fluid volume and allantoic fluid protein ($y = 12.3 - .099x + .00022x^2$; $r^2 = .46$), retinol ($y = 389 - 2.22x + .0042x^2$; $r^2 = .35$), retinol binding protein ($y = 116.4 - .79x + .0016x^2$; $r^2 = .40$), and acid phosphatase ($y = 442 - 4.26x + .0095x^2$; $r^2 = .27$) concentrations and that the relationships were quadratic ($P < .01$). To correct for the variability, which was a consequence of variability in allantoic fluid volume, in allantoic fluid protein, retinol, retinol-binding protein, and acid phosphatase concentrations, data were reanalyzed using linear and quadratic effects of allantoic fluid volume as covariates. Results of this analysis indicated that UHO treatment decreased ($P < .05$) allantoic fluid protein, retinol, and acid phosphatase, but it did not affect retinol-binding protein concentrations. Estrone treatment did not affect any of these traits. The observation that retinol concentrations were decreased and retinol binding protein concentrations were unchanged by UHO treatment indicated that UHO treatment modified the percentage of retinol saturation of retinol binding protein. Therefore, a further analysis was performed on the allantoic fluid retinol concentrations with allantoic fluid retinol-binding protein concentrations as a covariate to determine whether UHO treatment decreased the saturation of retinol-binding protein. After fitting retinol-binding protein as a covariate, retinol was decreased in UHO pigs compared with intact control pigs ($P = .076$), confirming that after adjusting the data for retinol-binding protein concentrations, UHO-treated pigs had decreased allantoic fluid retinol concentrations.

Total protein, total retinol, total acid phosphatase, total fructose, and total retinol-binding protein content in the allantoic sac is summarized in Table 3. No effect of either UHO or estrone treatment was detected for these traits.

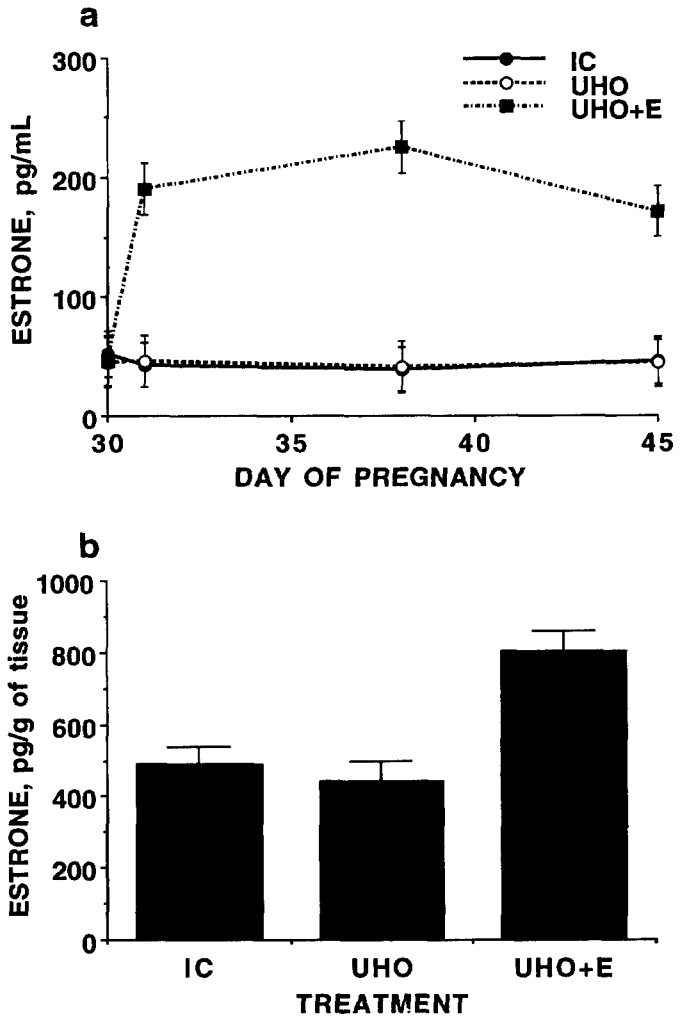

Figure 1. Least squares means for estrone in plasma (a) and endometrium (b) from pigs in the intact control (IC), unilateral hysterectomized-ovariectomized (UHO), and UHO plus estrone (UHO + E) treatment groups. Estrone implants increased ($P < .01$) estrone in plasma and endometrium.

Placental and Endometrial Tissue Culture

Neither UHO nor estrone treatment affected the incorporation of radioactivity into nondialyzable macromolecules by placental tissue during incubation or retinol-binding protein secretion or incorporation of radioactivity into nondialyzable macromolecules by incubated endometrial tissue (Table 4). Acid phosphatase secretion by incubated endometrial tissue was greater ($P = .055$) in UHO-treated gilts than in intact controls and was unaffected by estrone treatment. Neither UHO nor estrone treatment influenced consistently the array of proteins secreted by incubated endometrial tissue as determined by two-dimensional PAGE and fluorography (not shown).

Table 1. Least squares means (± SE) for number of fetuses, number of corpora lutea (CL), fetal survival, fetal weight, placental weight, empty uterine weight, and sex ratio of fetuses from day-45 pregnant pigs from the intact control (IC), unilaterally hysterectomized-ovariectomized (UHO), and UHO plus estrone (UHO + E) treatment groups

Item	IC	UHO	UHO + E
Fetuses[ab]	11.5 ± .7 (10)	7.9 ± .8 (8)	7.5 ± .8 (8)
CL	14.1 ± .8 (10)	13.3 ± .9 (8)	13.1 ± .9 (8)
Survival (fetuses/CL)[b]	.81 ± .05 (10)	.61 ± .06 (8)	.58 ± .06 (8)
Fetal wt, g[b]	21.2 ± .6 (8)	18.2 ± .8 (5)	18.6 ± .7 (6)
Placental wt, g[c]	63.5 ± 5.3 (8)	52.5 ± 6.9 (5)	46.3 ± 6.2 (6)
Uterine wt, kg[cd]	1.56 ± .09 (9)	1.14 ± .10 (8)	.88 ± .10 (8)
Sex ratio (females/fetuses)	.49 ± .05 (10)	.38 ± .05 (8)	.46 ± .05 (8)

[a]Numbers of observations per treatment group are in parentheses.
[b]Effect of UHO treatment ($P < .01$).
[c]Effect of UHO treatment ($P < .05$).
[d]Effect of estrone treatment ($P = .05$).

Discussion

Examination of plasma and endometrial estrone concentrations in each treatment group indicated that estrone treatment increased estrone concentrations within the endometrium, indicating that the dose used should have been adequate to test the effect of estrone on endometrial function. Results of this experiment indicate that estrone treatment had no effect on fetal survival, endometrial or placental protein secretion, allantoic fluid volume, protein, uteroferrin (estimated as acid phosphatase activity), retinol, or retinol-binding protein concentrations. These results indicate that the response of d-30 to -45 pregnant pigs may differ from that of ovariectomized pigs that were treated with either progesterone or progesterone plus estrogen, and estrogen was found to have a stimulatory effect on protein secretion (Knight et al., 1973; Roberts et al., 1987; Fliss et al., 1991; Trout et al., 1992). Assuming that progesterone is unaffected by estrone treatment (increases in estrogens that occur later in pregnancy do not influence plasma progesterone concentration), these results are not consistent with the hypothesis that the progesterone:estrogen ratio influences endometrial secretion of protein (Knight et al., 1974) and allantoic fluid volume (Bazer, 1989). Finally, these results are not consistent with the hypothesis suggested by Basha et al.

Table 2. Least squares means (± SE) for allantoic fluid volume (ALFV) and protein, retinol, acid phosphatase, fructose, and retinol-binding protein concentrations in allantoic fluid from day-45 pregnant pigs from the intact control (IC), unilateral hysterectomized-ovariectomized (UHO), and UHO plus estrone (UHO + E) treatment groups

Item	IC (10)[a]	UHO (8)	UHO + E (8)
ALFV, mL	75 ± 15	86 ± 17	73 ± 17
Protein, ng/mL	7.6 ± 1.0	6.7 ± 1.1	5.9 ± 1.1
Retinol, ng/mL	287 ± 31	246 ± 35	215 ± 35
Acid phosphatase, μmol Pi/min·mL	250 ± 49	178 ± 56	169 ± 56
Fructose, mg/mL[b]	1.15 ± .09	1.37 ± .10	1.06 ± .10
Retinol binding protein, μg/mL	76 ± 9	64 ± 11	71 ± 11
	— After fitting allantoic fluid volume as a covariate[c] —		
Protein, ng/mL[d]	7.7 ± .6	6.4 ± .6	5.6 ± .6
Retinol, ng/mL[d]	288 ± 23	243 ± 26	207 ± 26
Acid phosphatase, μmol Pi/min·mL[d]	252 ± 33	166 ± 37	163 ± 37
Retinol binding protein, μg/mL	76 ± 6	63 ± 7	69 ± 7
	— After fitting retinol binding protein as a covariate —		
Retinol, ng/mL[e]	276 ± 18	255 ± 21	214 ± 21

[a]Numbers of observations in each treatment group are in parentheses.
[b]Effect of estrone ($P < .05$).
[c]No effect of allantoic fluid volume on fructose concentrations was detected.
[d]Effect of UHO treatment ($P < .05$).
[e]Effect of UHO treatment ($P = .076$).

Table 3. Least squares means (± SE) for total protein, retinol, acid phosphatase, fructose, and retinol-binding protein content per allantoic sac in allantoic fluid from day-45 pregnant pigs from the intact control (IC), unilateral hysterectomized-ovariectomized (UHO), and UHO plus estrone (UHO + E) treatment groups

Item	IC (10)[a]	UHO (8)	UHO + E (8)
Protein, mg	376 ± 39	347 ± 44	332 ± 44
Retinol, μg	17.0 ± 2.1	17.0 ± 2.4	13.0 ± 2.4
Acid phosphatase, mmol Pi/min	9.4 ± 1.2	7.0 ± 1.4	9.0 ± 1.4
Fructose, mg	93 ± 25	126 ± 29	84 ± 29
Retinol binding protein, mg	4.1 ± .5	3.8 ± .6	4.2 ± .6

[a]No differences were detected between treatments. Numbers of observations for each treatment are in parentheses.

(1980) that estrogen may be the cause of the conceptus-induced stimulation of protein secretion on d 60 of pregnancy.

A possible criticism of this experiment is that estrone, which is less biologically active than estradiol, was used to test for effects of estrogen. The affinity of the estrogen receptor for estrone is less than that of estradiol, because the dissociation rate of estrone from the estrogen receptor is greater than that of estradiol, whereas association rates for the two steroids are similar (Weichman and Notides, 1980). There is a similar relationship between estriol and estradiol (Weichman and Notides, 1980). Studies with estriol indicate that although a single injection of estriol has less biological activity than estradiol, the biological activities of estriol and estradiol are similar when treatments are applied continuously (Anderson et al., 1975). Estrone and estradiol also have equivalent biological activity when given continuously (Fishman, 1981). This is because when estrogens are applied continuously and the so-called weak estrogens dissociate from the receptor, circulating concentrations of the estrogen are sufficient to reoccupy the receptor. The pig conceptus secretes estrone over a prolonged period; therefore, the estrone is likely to be as active as estradiol. Similarly, because we used estrone implants, which maintain constant plasma concentrations of estrone, the estrone should have been biologically active.

Possibly the amount of estrone secreted at d 45 of pregnancy is sufficient to saturate receptors for estrogen. However, previous experiments indicated that placental secretion of estrogens is low at this time (Knight and Kukoly, 1990). If estrogen concentrations are great enough to saturate receptors on d 45 of pregnancy, the peaks in estrogen production, which occur before (d 30) and after (d 70 to term) this time of pregnancy, would most likely have little influence on endometrial protein secretion, because the changes in estrogen would be unable to further stimulate protein secretion. Thus, the enhancement of protein secretion in the presence of the conceptus reported by Basha et al. (1980) and Vallet et al. (1994) could not therefore be explained by changes in placental estrogen production.

An alternative possibility is that endometrial secretion of proteins was maximal in the intact control group. However, Basha et al. (1979) reported that secretion of uteroferrin by the endometrium was submaximal at d 45 of pregnancy. Also, endometrial incorporation of radioactivity into nondialyzable macromolecules increases between d 40 and 60 of pregnancy (Vallet et al., 1994). Therefore, it would seem likely that protein secretion on d 45 of pregnancy is submaximal.

Table 4. Least squares means (± SE) for nondialyzable radioactivity in placental incubations (NDRP) and acid phosphatase activity, retinol-binding protein, and nondialyzable radioactivity in endometrial incubations (NDRE) prepared using placental and endometrial tissue collected from day-45 pregnant pigs from the intact control (IC), unilaterally hysterectomized-ovariectomized (UHO), and UHO plus estrone (UHO + E) treatment groups

Item	IC (10)[a]	UHO (8)	UHO + E (8)
NDRP, $\times 10^{-6}$	1.16 ± .11	1.22 ± .13	1.21 ± .13
Acid phosphatase, μmol/g tissue·min[b]	.76 ± .12	.96 ± .13	1.13 ± .13
Retinol binding protein, μg/g tissue	94 ± 9	99 ± 10	116 ± 10
NDRE, $\times 10^{-6}$	4.74 ± .53	5.79 ± .60	5.18 ± .60

[a]Numbers of observations for each treatment group are in parentheses.
[b]Effect of UHO treatment ($P = .055$).

It is possible that the effects reported by Basha et al. (1980) and Vallet et al. (1994) are caused by changes in endometrial receptor concentrations during mid-gestation. The current study was not designed to test that possibility. Instead, the design tested only whether changes in estrogen concentrations alone during pregnancy could explain the previous observations, because previous reports indicated that estrogen treatment combined with progesterone could stimulate protein secretion compared to progesterone alone. Further experimentation is required to address the possibility that receptor concentrations may change during this period.

A final possibility is that another product of the conceptus may be responsible for the enhancement of endometrial protein secretion, which was reported by Basha et al. (1980) and Vallet et al. (1994). The porcine placenta is known to produce many factors, including growth factors, prostaglandins, and steroids that may be responsible for increased protein secretion. Further experimentation will be necessary to distinguish between the above explanations.

Several experiments indicate that fetal loss due to uterine crowding occurs in early gestation. Using UHO treatment, Knight et al. (1977) demonstrated that fetal survival is unaffected by uterine space before d 30 of gestation, but fetal survival is decreased by d 45 of pregnancy. Vallet and Christenson (1993) reported that when fetuses are crowded within the uterus by ligation of the uterine horn, significant loss of conceptuses occurs between d 25 and 35 of pregnancy, and Chen and Dzuik (1993) reported that when conceptuses are given 20 cm of uterine space or less, significant fetal losses occur between d 29 and 35 of gestation. Data from this experiment are consistent with those observations. The UHO treatment caused significant conceptus loss by d 45 of pregnancy. Collectively, these experiments indicate that conceptuses are susceptible to death caused by some aspect of uterine crowding between d 30 and 45 of pregnancy. The question remains as to what process does or does not occur during this period to render the conceptuses susceptible to uterine crowding.

Deficits in oxygen, glucose, amino acids, vitamins, or minerals are all possible explanations for the effect of crowding on fetal loss. Acid phosphatase (iron), retinol-binding protein (retinol), and fructose (glucose) concentrations may indicate whether UHO treatment significantly affects nutrient transport to the developing fetus. Results of this experiment indicate that UHO treatment tended to increase endometrial secretion of uteroferrin, but it had no effect on other endometrial secreted proteins. This indicates that the endometrium may have a limited ability response to increased conceptus crowding, which agrees with a previous report (Vallet and Christenson, 1993). The UHO treatment did not affect allantoic fluid fructose concentrations or total fructose, indicating that conceptus crowding does not significantly limit the amount of glucose transported to the fetus (Huggett, 1961). After correction for allantoic fluid volume, UHO treatment significantly reduced allantoic fluid retinol, acid phosphatase, and protein concentrations. Allantoic fluid acid phosphatase, retinol, and several other allantoic fluid proteins (Buhi et al., 1983) originate from the endometrium. Concentrations of these proteins in allantoic fluid are an indication of the balance between uptake of these components by the placenta and use of the components by the fetus. The decrease in these components caused by uterine crowding may indicate that conceptuses in crowded uterine conditions used more of these components, relative to the amount taken up by the placenta, compared to conceptuses under normal uterine conditions. These results are consistent with the possibility that an endometrial secreted factor may become limiting under crowded uterine conditions.

In conclusion, estrone treatment had no effect on endometrial protein secretion or fetal survival when administered between d 30 and 45 of pregnancy. Unilateral hysterectomy-ovariectomy decreases fetal survival by d 45 of pregnancy and is a useful model with which to test effects of treatments on fetal survival in a crowded uterine environment. Further experimentation is required to determine whether the inability of estrone to stimulate endometrial protein secretion is due to the presence of saturating concentrations of estrogen contributed by the conceptus, the lack of receptors for estrogen during this period of pregnancy, or the inability of estrogens to perform this function.

Implications

Estrone treatment from d 30 to 45 of pregnancy does not influence endometrial protein secretion or litter size in unilaterally hysterectomized-ovariectomized swine. Because litter size of unilaterally hysterectomized-ovariectomized swine is a reflection of uterine capacity, one may conclude that estrone treatment during this period and at the dosage used does not affect uterine capacity of 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.

Anderson, J. N., E. J. Peck, Jr., and J. H. Clark. 1975. Estrogen-induced uterine responses and growth: Relationship to receptor estrogen binding by uterine nuclei. Endocrinology 96:160.

Basha, S.M.M., F. W. Bazer, and R. M. Roberts. 1979. The secretion of a uterine specific, purple phosphatase by cultured explants of porcine endometrium dependency upon the state of pregnancy of the donor animal. Biol. Reprod. 20:431.

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.

Bazer, F. W. 1989. Allantoic fluid: Regulation of volume and composition. In: R. A. Brace and J. E. Robillard (Ed.) Reproductive and Perinatal Medicine, Vol. XI, Fetal and Neonatal Body Fluids. p 135. Perinatology Press, Ithaca, NY.

Bennett, G. L., and K. A. Leymaster. 1989. Integration of ovulation rate, potential embryonic viability and uterine capacity into a model of litter size in swine. J. Anim. Sci. 67:1230.

Buhi, W. C., C. A. Ducsay, F. F. Bartol, F. W. Bazer, and R. M. Roberts. 1983. A function of the allantoic sac in the metabolism of uteroferrin and maternal iron by the fetal pig. Placenta 4: 455.

Chen, Z.-Y., and P. J. Dziuk. 1993. Influence of initial length of uterus per embryo and gestation stage on prenatal survival, development, and sex ratio in the pig. J. Anim. Sci. 71:1895.

Christenson, R. K., K. A. Leymaster, and L. D. Young. 1987. Justification of unilateral hysterectomy-ovariectomy as a model to evaluate uterine capacity in swine. J. Anim. Sci. 65:738.

Ducsay, C. A., W. C. Buhi, F. W. Bazer, and R. M. Roberts. 1982. Role of uteroferrin in iron transport and macromolecular uptake by allantoic epithelium of the porcine conceptus. Biol. Reprod. 26:729.

Fishman, J. 1981. Biological action of catechol oestrogens. J. Endocrinol. 89:59P.

Fliss, A. E., F. J. Michel, C.-L. Chen, A. Hofig, F. W. Bazer, Y. Y. Chou, and R.L.M. Simmen. 1991. Regulation of the uteroferrin gene promoter in endometrial cells: Interactions among estrogen, progesterone, and prolactin. Endocrinology 129:697.

Guthrie, H. D., and D. R. Deaver. 1979. Estrone concentration in the peripheral plasma of pregnant and non-pregnant gilts. Theriogenology 11:321.

Huggett, A.S.G. 1961. Carbohydrate metabolism in the placenta and foetus. Br. Med. Bull. 17:122.

Knight, J. W., F. W. Bazer, W. W. Thatcher, D. E. Franke, and H. D. Wallace. 1977. Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: Interrelations among hormonal status, placental development, fetal fluids and fetal growth. J. Anim. Sci. 44:620.

Knight, J. W., F. W. Bazer, and H. D. Wallace. 1973. Hormonal regulation of porcine uterine protein secretion. J. Anim. Sci. 36: 546.

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.

Knight, J. W., and C. A. Kukoly. 1990. In vitro release of progesterone and estrone by the porcine placenta throughout gestation. Domest. Anim. Endocrinol. 7:497.

Lowry, D. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265.

Roberts, R. M., G. A. Baumbach, W. C. Buhi, J. B. Denny, L. A. Fitzgerald, S. F. Babelyn, and M. N. Horst. 1984. Analysis of membrane polypeptides by two-dimensional polyacrylamide gel electrophoresis. In: J. C. Venter and L. C. Harrison (Ed.) Molecular and Chemical Characterization of Membrane Receptors. Vol. 3. p 61. Alan R. Liss, New York.

Roberts, R. M., and F. W. Bazer. 1988. The function of uterine secretions. J. Reprod. Fertil. 82:875.

Roberts, R. M., M. K. Murray, M. G. Burke, C. M. Keetcham, 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, New York.

SAS. 1990. SAS/STAT® User's Guide (Version 6, 4th Ed.). SAS Inst. Inc., Cary, NC.

Selvaraj, R. J., and T. P. Sushella. 1970. Estimation of serum vitamin A by a microfluorometric procedure. Clin. Chim. Acta 27:165.

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. 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.

Vallet, J. L., H. G. Klemcke, and R. K. Christenson. 1994. The effect of the conceptus and 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.

Zavy, M. T., W. R. Clark, D. C. Sharp, R. M. Roberts, and F. W. Bazer. 1982. Comparison of glucose, fructose, ascorbic acid and glucose phosphate isomerase enzymatic activity in uterine flushings from nonpregnant and pregnant gilts and pony mares. Biol. Reprod. 27:1147.