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Establishment of Pregnancy in the Pig: III. Endometrial Secretory Response to Estradiol Valerate Administered on Day 11 of the Estrous Cycle[1,2,3]

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ABSTRACT

The administration of estradiol valerate (E_2V, 5 mg/day) to gilts from Days 11 through 15 of the estrous cycle results in maintenance of the corpora lutea. The present study evaluated the effect of E_2V, administered on Day 11 only or Days 11, 12 and 13, on uterine endometrial secretory function between Days 11 and 14 of the estrous cycle. Each uterine horn within a gilt was randomly assigned to be removed and flushed with 0.9% saline at either 1, 4, 12 or 24 h post-injection. A jugular vein plasma sample was obtained to determine concentrations of estrone (E_1) and estradiol (E_2). Uterine flushings were analyzed for total calcium (Ca), E_1, E_2, E_1 sulfate (E_1S), E_2S, prostaglandin E and F (PGE and PGF), protein and acid phosphatase activity (AP).

Plasma concentrations of E_1 were greater ($P<0.01$) in E_2V (40.7 pg/ml) versus control (5.3 pg/ml) gilts at 1 h post-injection and remained higher in E_2V gilts at 72 h. Differences in Ca, PGE, AP, protein and estrogens in uterine flushings of gilts receiving either a single or daily injection of E_2V were not detected; however, PGF was higher ($P<0.05$) at 72 h in gilts receiving daily injections of E_2V. Total E_2 and E_1S in uterine flushings were greater ($P<0.05$) in E_2V gilts. Protein and Ca increased ($P<0.05$) in E_2V gilts at 12 h and 24 hr post-treatment and then declined, but total protein and AP increased to 48 h and 72 h. Total Ca was unchanged in control gilts, but total protein increased ($P<0.01$) by 72 h post-treatment and AP increased between 1 h and 72 h in all gilts. Two-dimensional polyacrylamide gel electrophoresis of proteins in uterine flushings detected three selected basic uterine proteins (M_r 32 to 60 k) associated with the increase in Ca and protein 12 h and 24 h post-treatment from E_2V gilts. These proteins were not detectable until 48 to 72 h in control gilts. Total recoverable PGF was similar for E_2V and control gilts between 1 h and 48 h post-treatment, but increased almost 6-fold between 48 h and 72 h in gilts receiving daily E_2V. Total PGE was greater ($P<0.01$) in E_2V gilts and increased between 48 h and 72 h post-treatment. Increases in total recoverable Ca, protein, PGF and PGE in uterine flushings in response to E_2V treatment were similar to those which occur between Days 11 and 12 of pregnancy. These data indicate that estrogens of conceptus origin induce that endometrial secretory response.

INTRODUCTION

Establishment of pregnancy in the pig involves synchronization of progesterone-stimulated endometrial function, blastocyst development and steroid synthetic capability. Embryo transfer studies have demonstrated that synchronization between stage of blastocyst development and endometrial proliferation is necessary if high conception rates are achieved in the sheep, cow and pig (see review by Cook and Hunter, 1978). In the pig, maternal recognition of pregnancy signals from blastocysts begin at approximately Day 11 (Dhindsa and Dziuk, 1968). This time period coincides with initiation of estrogen synthesis by blastocysts (Perry et al., 1973; Flint et al., 1979; Gadsby et al., 1980; Heap et al., 1981a,b), blastocyst elongation (Perry and Rowlands, 1962; Anderson, 1978; Geisert et al., 1982a,b) and sequestering of histotroph, i.e., uterine gland secretions, within the uterine lumen (Bazer and Thatcher, 1977; Zavy et al., 1980; Geisert et al., 1982b). Estrogen produced by pig

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blastocysts is assumed to be the "signal" for maternal recognition of pregnancy (Bazer and Thatcher, 1977; Flint et al., 1979) and initiation of estrogen production is associated with blastocyst elongation (Heap et al., 1979; Geisert et al., 1982b).

Administration of exogenous estrogens between Days 11 and 15 of the estrous cycle prolongs luteal function in pigs to an average of about 120 days (Gardner et al., 1963; Frank et al., 1977). Bazer and Thatcher (1977) proposed that blastocyst estrogens are luteostatic in the pig and the theory suggests that estrogen acts locally on the uterine epithelium. Geisert et al. (1982b) reported that increases in uterine luminal content of calcium, protein and prostaglandins occurred concurrently with an increase in estradiol content which was associated with development of pig blastocysts to the tubular stage (10 mm diameter). A synchronized release of secretory vesicles from the glandular epithelium occurred coincidentally with the increase in estrogen. Although these observations suggested that increases in calcium, protein and prostaglandins were estrogen induced, direct evidence was lacking. Therefore, the present study was completed to determine the effect of exogenous estrogen on the secretory activity of endometrium of nonpregnant gilts on Day 11 of the estrous cycle. Exogenous estrogen was used to mimic blastocyst estrogen synthesis during early pregnancy but without other contributions from blastocysts.

MATERIALS AND METHODS

General

Eighteen sexually mature crossbred gilts of similar age, weight and genetic background were utilized after having expressed at least two estrous cycles of normal duration (18 to 22 days). Gilts were checked for estrus twice daily (0700 h and 1900 h) by boars. The first day of estrus was designated Day 0. Twelve gilts were assigned randomly to receive either estradiol valerate (E_2V, 5 mg, i.m., N=6) or sesame oil (control, 0.5 ml, i.m., N=6) on Day 11. Following injection on Day 11, each uterine horn within a gilt was assigned randomly to be removed and flushed with 20 ml of sterile 0.9% saline at either 1, 4, 12 or 24 h post-treatment. All samples were frozen and stored at −20°C until analyzed. Surgical and uterine flushing procedures have been described previously by Murray et al. (1972) and Bazer et al. (1978), respectively.

An additional group of six gilts were assigned randomly to receive one of three treatments: *1)* E_2V injected on Day 11 (N=2); *2)* E_2V injected on Days 11, 12 and 13 (N=2); or *3)* sesame oil injected on Days 11, 12 and 13 (N=2). Gilts were then unilaterally hysterectomized 48 h post-treatment on Day 11 followed by removal of the remaining uterine horn 72 h post-treatment. Each uterine horn was flushed with physiological saline as previously described.

Plasma samples were obtained from the jugular vein of all gilts at surgery. Plasma was frozen and stored at −20°C until assayed for concentration of estrone (E_1) and estradiol (E_2).

Analyses of Calcium

The concentration of calcium (Ca) in uterine flushings was measured with the Calcette, manufactured by Precision Systems, Inc. (Sudbury, MA). This system utilized ethylene glycol tetraacetic acid for fluorometric titration of calcium in aqueous solutions (Alexander, 1971).

Analyses of Protein and Acid Phosphatase Acitivity

Protein concentration in uterine flushings was determined by the method of Lowry et al. (1951). Standard curves were obtained from serial dilutions of bovine serum albumin (Sigma Chemical Co., St. Louis, MO).

Acid phosphatase activity was determined using p-nitrophenyl phosphate as a substrate as described by Schlosnagle et al. (1976) and Basha et al. (1979). One unit of activity was defined as the capacity to release 1 μmol nitrophenol per minute at pH 4.9 in 0.1 M acetate buffer.

Two-Dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE)

An aliquot of estradiol and vehicle-treated gilt uterine flushings containing about 2 mg protein were dialyzed (molecular weight cut off ≅3500) against 1 mM tris-HCl buffer (pH 8.2) and then lyophilized. Lyophilized uterine protein was prepared for 2D-PAGE analysis by dissolving in 1 ml of 5 mM K_2CO_3 containing 9.4 M urea, 2% (v/v) Nonidet P-40 and 0.5% (w/v) dithiothreitol (Horst and Roberts, 1979). The 2D-PAGE was performed for acidic and basic uterine proteins according to the method of Horst and Roberts (1979) and Basha et al. (1980) using 300 μg protein for each gel.

Hormonal Analyses

Uterine flushings from E_2V and control gilts were analyzed for concentrations of E_1, E_2, estrone sulfate (E_1S), estradiol sulfate (E_2S), immunoreactive prostaglandin F (PGF) and prostaglandin E (PGE). Jugular vein plasma was assayed to determine E_1 and E_2 concentrations.

Two milliliter aliquots of uterine flushing and plasma were extracted for measurement of conjugated and unconjugated estrogens. All estimates were made in duplicate and corrected for procedural losses utilizing a radiolabeled internal standard. Plasma and uterine flushing E_1 and E_2 assays have been validated and described previously (Zavy et al., 1980). Intra- and inter-assay coefficients of variation were 28% and 11% for E_1 and 14% and 5% for E_2.

Procedures for assay and validation of E_1S and E_2S in porcine uterine flushings have been reported

(Geisert et al., 1982a). Inter- and intra-assay coefficients of variation were 12% and 10% for E_1S and 13% and 20% for E_2S. The PGF assay used in this laboratory has been validated and described previously for measurement of PGF concentration in porcine uterine flushings (Zavy et al., 1980). The values measured are reported as total immunoreactive PGF and not $PGF_2\alpha$ since the first antibody shows some cross-reactivity with $PGF_1\alpha$ (8%). All samples were analyzed in the same assay and the intraassay coefficient of variation was 10.3%.

Assay of PGE concentrations in uterine flushings was a modification of the procedure described by Geisert et al. (1982a). Prostaglandin E was measured directly by radioimmunoassay following extraction of the uterine flushing (1 ml) with etheyl acetate, but without Sephadex LH-20 column chromatography. Exogenous PGE_2 (50, 100, 250 and 500 pg) was added to 1 ml of uterine flushing from a Day 10 nonpregnant gilt and assayed. A quantitative linear recovery was achieved ($Y=-7.80 + 1.26X$; $R^2=0.98$). The antibody used in the radioimmunoassay (gift from D.N.R. Mason of Lilly Research Labs., Indianapolis, IN) has a cross-reactivity of 16.8% with PGE_1, therefore, data are expressed as total immunoreactive PGE. The sample intra- and inter-assay coefficients of variation were 14% and 8%, respectively.

Statistical Analyses

Data were analyzed by least squares analysis of variance using the General Linear Models procedures of the Statistical Analysis System (Barr et al., 1979). The model included effects of treatment (E_2V vs. control), time (1, 4, 12, 24, 48 and 72 h) and treatment by time interactions. Data were also analyzed separately for uterine flushings taken at 48 and 72. The analyses considered treatment (E_2V, 1 day; E_2V daily and control), time (48 and 72 h) and treatment by time interactions. Orthogonal contrasts were used to detect treatment differences at 72 h (Steel and Torrie, 1980).

RESULTS

Plasma Estrone and Estradiol

Concentrations of E_1 and E_2 in jugular vein plasma of control and E_2V gilts are summarized in Table 1. Estrone and E_2 were higher ($P<0.01$) in plasma of E_2V gilts throughout the sampling periods. Plasma E_2 concentrations were 8-fold higher at 1 h post-estradiol injection (40.7 pg/ml) as compared to control gilts (5.3 pg/ml). Plasma E_2 peaked at 48 h after a single injection of E_2V; however, E_2 concentrations were 6-fold greater at 48 and 72 h in gilts treated daily with E_2V. Although substantially lower, E_2 concentrations for control gilts also increased at 48 and 72 h.

Estrogens in Uterine Flushings

Least square means for conjugated and unconjugated estrogens in uterine flushings are presented in Table 2. Total recoverable E_2 ($P<0.05$) and E_1S ($P<0.01$) were higher in E_2V gilts, but no time or treatment by time interactions were detected. Flushings from E_2V gilts contained approximately 2-fold greater amounts of E_2 and E_1S than control gilts at 4 h post-injection and this difference was maintained throughout the sampling period, with the

TABLE 1. Least squares means for plasma estrone and estradiol concentrations (pg/ml) in jugular vein of control and estradiol valerate (E_2V) treated gilts.[a,b,c]

		Treatments			
		E_2V[a]		Control[a]	
Hours post-injection		E_1	E_2	E_1	E_2
1		15.7	40.7	8.6	5.3
4		29.7	45.0	15.6	7.0
12		11.0	55.3	8.6	9.0
24		10.0	34.3	8.0	11.3
48	(1 day E_2V)	16.5	92.5	4.0	24.0
	(daily E_2V)	54.0	334.0		
72	(1 day E_2V)	18.0	56.0	7.0	16.0
	(daily E_2V)	31.5	276.5		

[a]Treatment differences for E_1 ($P<0.05$) and E_2 ($P<0.01$) were detected. All values are pg/ml.

[b]The overall SEM was ± 2.7 for E_1 and ± 12.7 for E_2.

[c]Mean E_1 and E_2 plasma concentrations were different ($P<0.05$) between one E_2V injection, daily E_2V and control gilts.

TABLE 2. Least squares means for total conjugated and unconjugated estrogens (pg) in uterine flushings of control and estradiol valerate (E_2V) treated gilts[a,b]

Hours post-injection		Treatments							
		E_2V[a]				Control[a]			
		E_1	E_2	E_1S	E_2S	E_1	E_2	E_1S	E_2S
1		567	142	916	359	164	200	641	250
4		395	345	1961	404	415	211	714	209
12		333	328	1796	244	289	89	629	174
24		621	420	1589	382	308	134	543	68
48	(1 day E_2V)	620	169	1369	747	623	146	717	291
	(daily E_2V)	283	270	1102	459				
72	(1 day E_2V)	395	215	1107	342	275	107	1093	596
	(daily E_2V)	199	417	1440	306				

[a]Treatment differences were detected for estradiol ($P<0.05$) and estrone sulfate ($P<0.01$). All values are pg/ml.

[b]The overall SEM was ± 41 for E_1, ± 33 for E_2, ± 93 for E_1S and ± 37 for E_2S.

exception that E_1S was similar between treatment groups at 72 h. Although slightly higher, uterine luminal content of E_1 and E_2S was not different ($P<0.05$) between E_2V and control gilts. Total recoverable E_1S was greater than E_2S in uterine flushings of E_2V gilts at all sampling times. Treatment of gilts with a single or daily injection of E_2V appeared to maintain similar levels of estrogens in the uterine lumen.

Total Content of Calcium (Ca), Protein and Acid Phosphatase (AP)

Recoverable Ca, protein and AP in uterine flushings from E_2V and control gilts are summarized in Table 3. Calcium and protein were greater ($P<0.01$) in uterine flushings from E_2V gilts and time ($P<0.01$) and time by treatment ($P<0.01$) interactions were detected. Calcium

TABLE 3. Least squares means for total calcium, protein and acid phosphatase activity in uterine flushings of estradiol valerate (E_2V) and control gilts.[a,b,c]

Hours post-injection		Treatments					
		E_2V			Control		
		Calcium (mg)	Protein (mg)	Acid phosphatase activity[d]	Calcium (mg)	Protein (mg)	Acid phosphatase activity[d]
1		0.36	13.2	50.8	0.15	13.0	89.0
4		0.36	15.4	61.2	0.31	11.4	47.6
12		1.67	22.1	191.8	0.37	15.6	49.8
24		1.84	33.9	443.7	0.42	19.4	370.6
48	(1 day E_2V)	0.23	58.1	1022.4	0.22	25.0	875.5
	(daily E_2V)	0.12	55.6	1026.2			
72	(1 day E_2V)	0.01	68.7	2165.7	0.25	48.5	2348.9
	(daily E_2V)	0.02	78.4	1240.8			

[a]Treatment and time by treatment interaction effects were detected for Ca and protein ($P<0.01$).

[b]Time effects were detected for Ca, protein and AP ($P<0.01$).

[c]Overall SEM was ± 0.04 for Ca, ± 1.52 for protein and ± 97.7 for AP.

[d]One unit of activity is defined as the capacity to release 1 μmol nitrophenol/min.

TABLE 4. Least square means for total prostaglandin F and E in uterine flushings of estradiol valerate and control gilts ($\bar{X}$ ± SEM). [a,b,c]

Hours post-injection		Treatments			
		E_2V		Control	
		PGF, ng	PGE, ng	PGF, ng	PGE, ng
1		1.6	7.6	1.4	5.5
4		1.5	7.6	1.6	5.6
12		2.5	9.8	1.5	6.8
24		1.3	8.3	2.2	6.9
48	(1 day E_2V)	2.3	13.9	2.3	12.2
	(daily E_2V)	1.7	13.4		
72	(1 day E_2V)	10.4	26.5	5.2	17.7
	(daily E_2V)	53.1	37.4		

[a]Treatment ($P<0.01$) and treatment by time ($P<0.05$) effects were detected for PGE.

[b]The overall SEM was ± 1.9 for PGF and ± 0.60 for PGE.

[c]Mean content PGF and PGE at 72 h was different ($P<0.10$) between daily E_2V vs. 1 day E_2V and control gilts.

content in uterine flushings of E_2V gilts increased 4-fold by 12 h and peaked at 24 h post-injection, followed by a decline at 48 and 72 h in both single and daily E_2V injected groups. Total Ca in uterine flushings of control gilts was similar at all time periods. A temporal association between changes in Ca and protein was observed as total protein and Ca content increased to 24 h in E_2V gilts. However, only protein content continued to increase to 48 h and 72 h post-E_2V injection. Protein content in control gilts did not increase until 72 h and was not associated with changes in Ca noted for E_2V gilts at 12 and 24 h post-injection. Acid phosphatase activity was not different ($P<0.05$) between treatment groups and no treatment by time interaction was detected, but AP activity increased from 1 h to 72 h in both E_2V and control gilts. Although the interaction was not significant, AP increased almost 3-fold at 12 h post-injection in E_2V gilts compared to controls. Daily injections of E_2V appeared to depress AP activity in uterine flushings at 72 h, but the effect was not significant.

Total PGF and PGE in Uterine Flushings

Total recoverable PGF and PGE in uterine flushings are summarized in Table 4. Uterine luminal content of PGF was similar for E_2V and control gilts between 1 h and 48 h, whereas daily treatment with E_2V resulted in 5- and 10-fold more PGF than occurred in response to a single injection and in control gilts, respectively. Although, not significant, PGF at 72 h was 2-fold higher in gilts given a single injection of E_2V compared with controls. Prostaglandin E was greater ($P<0.01$) in flushings after E_2V and a treatment by time interaction ($P<0.05$) was detected. Flushings from E_2V gilts contained slightly more PGE between 1 h and 48 h, then increased almost 2-fold at 72 h in both E_2V groups compared with control gilts.

Qualitative Changes in Uterine Protein

Positions of acidic and basic polypeptides following 2D-PAGE have been reported for porcine uterine flushings (Basha et al., 1980). Profiles of acidic proteins in flushings from E_2V and control gilts were similar (Fig. 1). Serum albumin is the dominant protein observed in all flushings along with two nonserum proteins (M_r ≅18,000–20,000; pI's=6.1 and 6.3) previously described by Basha et al. (1980). These two nonserum proteins are progesterone-induced (Basha et al., 1980) and appeared in uterine flushings on about Day 11, but their biological function is not known

Basic proteins detected in E_2V and control gilt uterine flushings are presented in Fig. 2. Uteroferrin (M_r ≅35k; pI=9.7), noted by the letter *U*, and two other nonserum proteins (M_r ≅50 to 60k; pI=9.7), located above uteroferrin and indicated by the numbers *1* and *2*, were

present in both E_2V and control uterine flushings. However, time of their appearance in uterine flushings was different between treatment groups. The three basic proteins were very evident by 12 h in gels from E_2V gilts and continued to be present at 72 h. In control gilt uterine flushings, uteroferrin was not detected until 24 h post-injection and the two nonserum proteins were only faintly visible at that time, but at 48 h and 72 h there was a marked increase in staining for uteroferrin and proteins 1 and 2. Uteroferrin and proteins 1 and 2 were selected for comparison because these basic proteins are known to be present in uterine flushings from pregnant, pseudopregnant, and ovariectomized progesterone-treated gilts (Basha et al., 1979), but not in uterine flushings from ovariectomized gilts treated with estrogen or corn oil or in serum from gilts (Basha et al., 1979; Knight et al., 1973). Data presented in Table 3 and Fig. 2B indicate that selected uterine specific proteins, as well as total protein, have increased by 24 h after E_2V treatment.

DISCUSSION

Available data indicate the differential effects of estrogen and progesterone on synthesis and secretion of proteins by epithelial cells of uterine glands in only the cat and roe deer. In the cat, Bareither and Verhage (1980) demonstrated that there was a marked accumulation of secretory vesicles in uterine glandular epithelium under the influence of estrogen. Progesterone infusion intravenously was followed by release of secretory vesicle contents into the uterine lumen within 5 to 15 min (Bareither and Verhage, 1980). The rapidity of the induction of exocytosis suggested that progesterone may have induced this process by having a direct effect on the epithelial cell membrane.

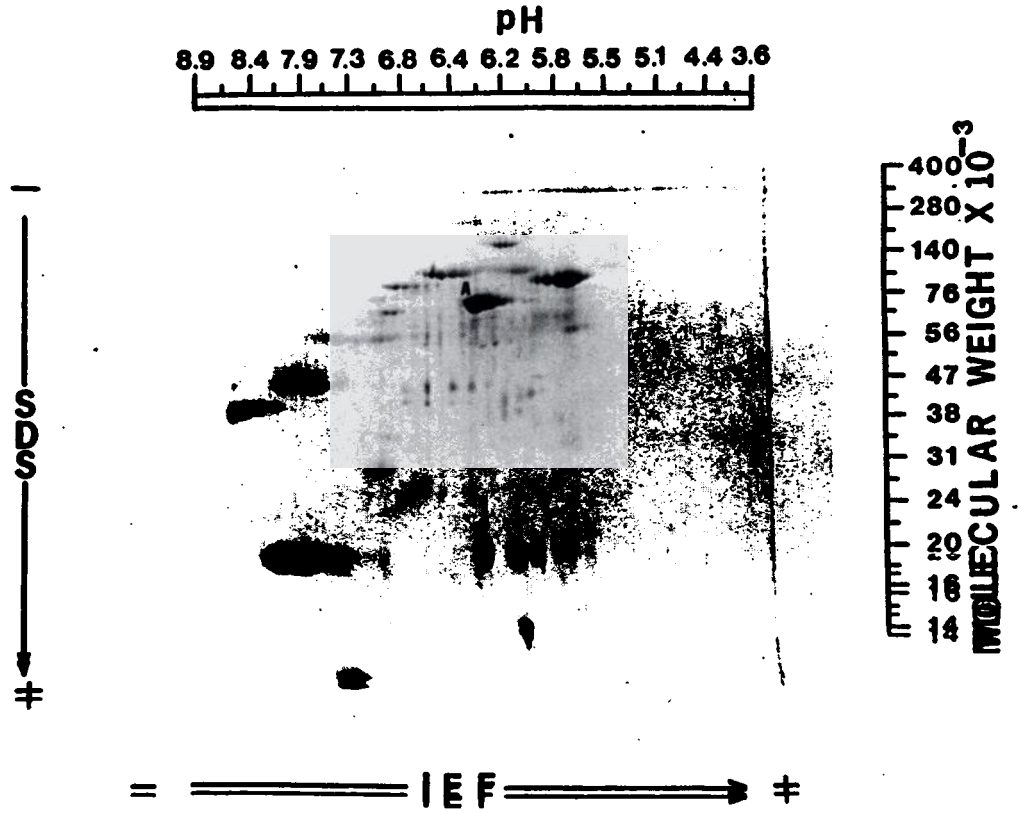

FIG. 1. Two-dimensional polyacrylamide gel electrophoresis of acidic proteins *(IEF)* characteristic of uterine flushings 24 h post-injection of either estradiol valerate or sesame oil. Two nonserum proteins with molecular weights of 18 to 20 k and pI's of 6.1 and 6.3 are evident *(arrow)* with serum albumin *(A)* being the major protein.

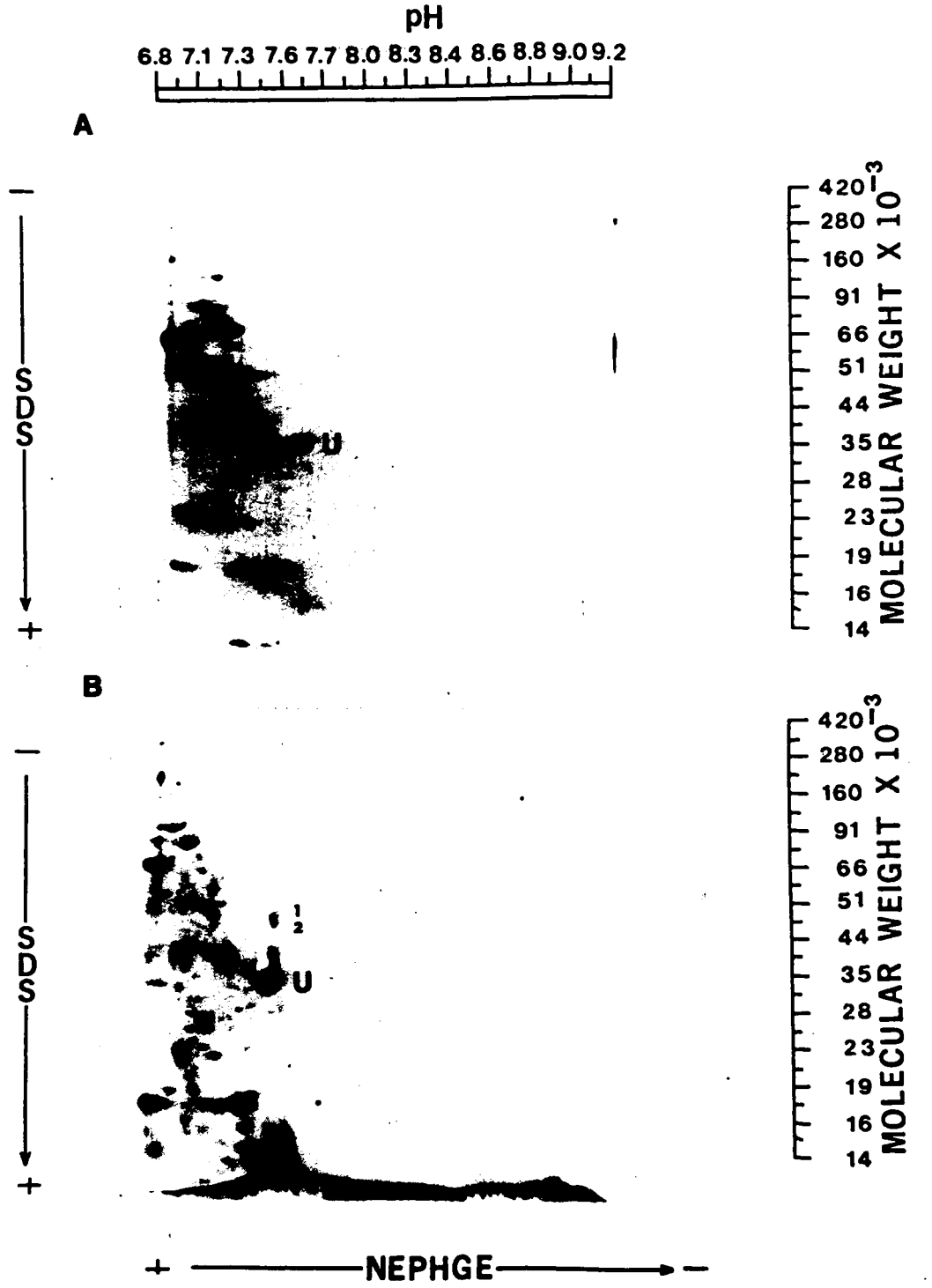

FIG. 2. Two-dimensional polyacrylamide gel electrophoresis of basic proteins *(NEPHGE)* in uterine flushings of control *(A)* and E_2V-treated *(B)* gilts 24 h post-injection. Uteroferrin *(U)* was observed in both treatments at this time. The two nonserum proteins (1 and 2) were observed at 24 h post-treatment in EV gilts, but not until 48 h in control gilts. Uteroferrin and proteins 1 and 2 were selected for comparison since they are known to be present in uterine flushings from pregnant, pseudopregnant, ovariectomized progesterone: estradiol-treated gilts and gilts during diestrous (Basha et al., 1980), but are not present in serum.

Aitken et al. (1973) reported that roe deer uterine gland epithelial cells have numerous secretory vesicles which accumulate during delayed implantation, presumably under the influence of progesterone. These secretory vesicles release their contents into the uterine lumen at the termination of delayed implantation. As conceptus development in the roe deer resumes, there is an increase in total unconjugated estrogens in maternal plasma and an increase in protein, calcium, α-amino nitrogen and total hexoses in uterine flushings (Aitken, 1979). Gadsby et al. (1980) demonstrated that roe deer blastocysts can produce estrogen and suggest that estrogen may initiate events leading to increased secretory activity of uterine gland epithelium and resumption of blastocyst growth.

Data from our laboratory (Geisert et al., 1982b) indicate that pig uterine epithelial cells accumulate secretory vesicles until about Day 11 or 12 of pregnancy and that the contents of these vesicles are released during the period of blastocyst elongation and initiation of estrogen production by the blastocysts. The accumulation of secretory vesicles in uterine gland epithelium of nonpregnant gilts to Days 12 to 14 was also reported (Geisert et al., 1982b). In the pregnant pig there was a "synchronized" release of secretory vesicle content, whereas in nonpregnant gilts this was a protracted process. The accumulation of these secretory vesicles in pigs occurs during the diestrous period and is assumed to occur in response to progesterone. Knight et al. (1973) demonstrated that progesterone was responsible for both quantitative and qualitative changes in proteins in uterine secretions of ovariectomized pigs.

The present study provides direct evidence that estrogen, either directly or indirectly, initiates events leading to increases in protein, calcium, PGF and PGE within the uterine lumen within 12 to 24 h of its intramuscular injection. Since these changes also occur in pregnant pigs coincidental with initiation of estrogen production by blastocysts, it seems certain that estrogen, and not some other agent of blastocyst origin, is responsible for inducing the uterine gland epithelial cell secretory response.

The mechanism whereby progesterone, in the cat, and estrogen, in the pig and possibly roe deer, initiate exocytosis of the secretory vesicle contents is not known. Bareither and Verhage (1980) suggested that progesterone may be acting directly on the cell membrane since exocytosis occurred within 5 min of its intravenous infusion into cats. Pietras and Szego (1977) suggested that steroids may exert a direct effect on endometrial cell membranes. Calcium (Rasmussen and Goodman, 1977), as well as arachidonic acid and/or lysophospholipids (Rubin and Laychock; Pollard et al., 1981), are possible mediators of membrane fusion and exocytosis. It is possible that, in the pig, estrogens, either injected or of blastocyst origin, may stimulate uterine epithelial cell secretion through calcium mediated events and that the increase in prostaglandins represents calcium activation of phospholipase A_2 and, in turn, the arachidonic acid cascade.

In the present study, a single estradiol valerate injection resulted in values of 0.6 and 0.4 ng total recoverable E_1 and E_2, respectively, and 1.6 ng E_1S in uterine flushings at 24 h post-injection. These values are comparable to those found in uterine flushings from pregnant pigs containing Day 12 filamentous blastocysts, i.e., 2.8 ng E_1, 2.3 ng E_2 and 3.4 ng E_1S (Geisert et al., 1982b). The injected estradiol valerate allows for prolonged corpora lutea maintenance and has no detectable effect on maternal plasma concentrations of progesterone when compared to values for pregnant gilts (Frank et al., 1977; Kensinger, 1982).

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