Effects of prolactin on conceptus survival and uterine secretory activity in pigs*

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Summary. Hypoprolactinaemia was induced by bromocriptine (CB154; 100 mg/day) which decreased circulating prolactin by 40% ($P < 0.06$), but did not affect conceptus survival at Day 25 when administered on Days 10–16 when compared to saline:ethanol-treated control gilts. Bromocriptine or vehicle was administered to cyclic gilts on Days 10–11, oestradiol valerate was injected on Day 11 and uterine flushings were collected on Day 12. Total recoverable protein and uteroferrin in uterine flushings were not affected by treatment. However, leucine aminopeptidase activity ($P < 0.02$) and total recoverable Ca^{2+} , Na^+ , K^+ and Cl^- ($P < 0.05$) were decreased in uterine flushings of gilts that received bromocriptine, suggesting that hypoprolactinaemia decreased general secretory activity of the endometrial epithelium and modulated ionic changes, respectively, in the uterine environment of pigs.

Subcutaneous administration of pig prolactin (1 mg/12 h) increased ($P < 0.001$) serum prolactin 4.5-fold. The interaction between hyperprolactinaemia and progesterone, without oestrogen, on components of uterine flushings were determined using gilts that received progesterone (200 mg/day) and prolactin or saline on Days 4–14 after ovariectomy on Day 4. On Day 15, there were no differences ($P > 0.05$) in any of the uterine secretory components measured. Hyperprolactinaemia (1 mg pig prolactin on Days 6–11) enhanced overall uterine secretory response on Day 12 to oestradiol (5 mg) administered on Day 11 compared to gilts that received 1 ml saline on Days 6–11 of the oestrous cycle. Total recoverable protein and leucine aminopeptidase activity were greater ($P < 0.05$) for oestradiol-treated gilts, but effects of prolactin were not significant. Total recoverable glucose ($P < 0.01$), PGF-2 α ($P < 0.02$), uteroferrin ($P < 0.01$) and specific activity of uteroferrin ($P < 0.001$) were increased by prolactin and oestradiol, but not oestradiol alone. Calcium ($P < 0.05$), chloride ($P < 0.05$) and potassium ($P < 0.01$) were increased in response to oestradiol. These results indicate an interaction between oestradiol and prolactin, but not progesterone and prolactin, which enhances secretion of some products of the pig uterine endometrium.

Keywords: prolactin; endometrium; secretion; pig; pregnancy

Introduction

Prolactin, a diverse and multifunctional hormone, affects uterine function. Species in which decidualization occurs during placentation (rat, human) have a decidual source of prolactin that is structurally similar to pituitary prolactin, but is differentially regulated (Healy & Hodgen, 1983). Receptors for prolactin have been detected in the endometrium of humans (Healy, 1984), rats

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(Williams *et al.*, 1978), mink (Rose *et al.*, 1983), rabbits, sheep (Posner *et al.*, 1974) and pigs (DeHoff *et al.*, 1986; Young & Bazer, 1987). Changes in numbers of uterine receptors for prolactin are temporally associated with: (1) increases in the concentration of circulating oestrogens; (2) endometrial proliferation, induction of steroid receptors and protein secretion (Chilton & Daniels, 1985); (3) epithelial ion transport (Rabee & McCoshen, 1986); and (4) placental transport of water and fetal growth (Goldstein *et al.*, 1980). These findings suggest that prolactin may be critical to the establishment of the optimal uterine environment for conceptus development.

The role(s) of prolactin and its interaction with ovarian steroids on uterine function during the critical period of establishment of pregnancy is of great interest. The agent primarily responsible for maternal recognition of pregnancy in pigs is oestrogen of conceptus origin (Bazer & Thatcher, 1977). Secreted initially on Days 11 and 12 of pregnancy, conceptus oestrogens are luteostatic and stimulate events leading to the release of histotroph from endometrial epithelium into the uterine lumen (Geisert *et al.*, 1982a) to nourish the conceptus until extensive placentation begins on Day 18. Intramuscular injection of oestradiol valerate on Day 11 of the oestrous cycle mimics the effects of the conceptus (Geisert *et al.*, 1982b). Additionally, intravenous administration of oestradiol stimulates both rapid (0.5–1 h) and protracted (12–24 h) uterine secretory responses (Young *et al.*, 1987). Oestrogen may induce this biphasic uterine secretory response indirectly through stimulation of, interaction with or synergism with other mechanisms or factors not associated with the classical receptor-mediated effects of oestrogen (Jensen *et al.*, 1968).

The pig has an epitheliochorial placenta which is non-invasive, hence there is no uterine decidual reaction or local prolactin production, and the placenta does not produce placental lactogen. Therefore, effects of prolactin from the pituitary on modulation of uterine function in pigs must be mediated through other mechanisms. The numbers of prolactin receptors increase between Days 15 and 30, remain constant from Days 30 to 45, decrease slightly by Day 60, reach maximal levels at Day 75 and then decrease dramatically to Day 90 of gestation in pigs (DeHoff *et al.*, 1984). Endometrial receptors for prolactin were greatest when there were the greatest increases in allantoic fluid volume (Goldstein *et al.*, 1980), maximal secretion of endometrial proteins (Basha *et al.*, 1979) and rates of fetal growth (Knight *et al.*, 1973). Despite chronically low circulating concentrations of prolactin, these results suggest roles for prolactin in pigs throughout pregnancy and fetal development and that changes in numbers of receptors for prolactin may be regulated by oestrogens.

The present studies determined whether prolactin affects uterine secretory activity during the period of establishment of pregnancy and examined interactions between prolactin and oestrogen and progesterone on endometrial secretory activity.

Materials and Methods

Animals. Crossbred gilts of similar age (7–9 months) and weight (110–120 kg) were used in all studies after experiencing at least 2 oestrous cycles of normal lengths (18–22 days). Using intact boars, gilts were observed for oestrus daily and the first day of behavioural oestrus was designated Day 0. Gilts were mated when detected in oestrus (Day 0) and 12 and 24 h later in studies using pregnant pigs.

Surgical procedures. Uterine flushings were collected in 20 ml double distilled water per uterine horn as described previously (Bazer *et al.*, 1978). Flushing volumes were recorded and flushings were centrifuged at 10 000 *g* for 15 min at 4°C. Supernatants were collected and stored at –20°C until analysed. Gilts used in studies of prolactin effects on progesterone-induced uterine secretory components in Exp. 4 were ovariectomized on Day 4 of the oestrous cycle. Gilts were anaesthetized, subjected to midventral laparotomy and the ovaries were exteriorized; all vessels were tied and the ovaries removed with minimal trauma to the uterus.

Catheterizations. On the assigned day, gilts were anaesthetized and fitted with indwelling jugular catheters (Ford & Maurer, 1978) which were maintained patent with 200 i.u. heparin/ml saline solution. After surgery, gilts were housed individually until collection of the final blood sample and removal of the catheters.

Exogenous hormone administration. For chronic steroid treatment, gilts received 200 mg progesterone (Sigma, St Louis, MO, USA) in 4 ml corn oil:ethanol solution (90:10, v/v). Control gilts received 4 ml vehicle only. Acute steroid treatment consisted of 0.5 ml (10 mg/ml) oestradiol valerate (Squibb, Raritan, NJ, USA) or 0.5 ml corn oil. Exogenous pig prolactin (USDA/B-1; gift from Dr Douglas Bolt, National Animal Hormone Program Director) was diluted

in phosphate-buffered saline (PBS; 1 mg/ml; pH 7.2), apportioned into 1.2 ml volumes and stored at 4°C until injected subcutaneously.

Bromocriptine. Bromocriptine (a gift of Sandoz Pharmaceutical, East Hanover, NJ, USA), a dopamine agonist, was solubilized in absolute ethanol and mixed with saline (1:1, v/v) to a concentration of 25 mg/ml. Treated gilts received 100 mg bromocriptine/day (4 ml) subcutaneously while control gilts received 4 ml vehicle solution on the basis of results of Kraeling *et al.* (1982).

Protein. Total recoverable protein concentrations in uterine flushings were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Uteroferrin. Concentrations of uteroferrin in uterine flushings were determined by measurement of acid phosphatase activity (Scholsnagle *et al.*, 1974; Basha *et al.*, 1979). Values are expressed as μmol *p*-nitrophenol phosphate (pNP) released per ml per 10 min at pH 4.9 and 37°C.

Calcium. The Calcette 4009 (Precision Systems, Inc., Sudbury, MA, USA) was used to determine calcium concentrations using ethylene glycol-bis-*N,N'*-tetraacetic acid (EGTA) for fluorometric titration of calcium in an aqueous solution (Alexander, 1971).

Chloride. Concentrations of chloride in uterine flushings were determined by a colorimetric assay, sensitive to 60 mequiv./l, adapted from Hamilton (1966).

Sodium and potassium. A flame photometer (Perkin Elmer 51Ca; Coleman Instruments Division, Oak Brook, IL, USA) was used to determine concentrations of sodium and potassium as described previously (Young & Bazer, 1987).

Glucose. The Beckman Glucose Analyzer 2 (Beckman Instruments, Columbia, MD, USA) was used to determine glucose concentrations as a direct proportion to oxygen consumption (Bazer *et al.*, 1984).

Leucine-acyl aminopeptidase. This membrane marker protein was used as an index of secretory activity and its concentration was determined using a colorimetric assay (Zavy *et al.*, 1984). One Sigma Unit (SU) will release 1 μmol (143 μg) of β -naphthylamine from L-leucine- β -naphthylamine per hour at 37°C and pH 7.1.

Prostaglandin (PG) F. Uterine flushings were analysed for PGF by a radioimmunoassay (RIA) procedure described by Knickerbocker *et al.* (1986), using the antibody characterized by Kennedy (1985) and tritiated PGF-2 α ([5,6,8,9,11,12,14,15-³H]PGF-2 α ; sp. act. 160–180 Ci/mmol; Amersham Corporation, Arlington Heights, IL, USA). Standard curves were prepared in charcoal-stripped uterine flushings with known amounts of radioinert PGF-2 α . A 1:5000 dilution of antiserum enabled detection of 10 pg PGF/tube. Cross-reactivities of PGF-2 α antiserum with other prostaglandins were: 94% for PGF-1 α ; 2.4% for PGE-2; and <0.1% for 13,14-dihydro-15-keto-PGF-2 α , PGE and arachidonic acid. Inter- and intra-assay coefficients of variation were 14.1% and 15.7%, respectively.

Prostaglandin E. Concentrations of PGE-2 in uterine flushings were determined using an assay similar to that described for PGF with a modification (Lewis *et al.*, 1978) using tritiated PGE-2 ([5,6,8,11,12,14,15-³H]PGE; sp. act. 140–170 Ci/mmol; Amersham). A 1:6000 dilution of antiserum (Eli Lilly, Indianapolis, IN, USA) enabled detection of 5 pg PGE-2/tube as different from zero. Cross-reactivities of PGE antiserum with other prostaglandins were: 24% for PGE; 1.7% for PGF-2 α ; 0.1% for 13,14-dihydro-15-keto-PGF-2 α , PGF-1 α and arachidonic acid. Inter- and intra-assay coefficients of variation were 9.7 and 12.4%, respectively.

Prolactin. Concentrations of prolactin in serum were measured by a radioimmunoassay (RIA) sensitive to 1 ng/ml (Kraeling *et al.*, 1982). The inter- and intra-assay coefficients of variation were 15.2 and 16.3%, respectively.

Experiment 1. This experiment determined effects of hypoprolactinaemia on conceptus survival. Gilts were mated on Days 0 and 1 of the oestrous cycle and assigned randomly, 5 per treatment group, to receive either bromocriptine (100 mg/day) or vehicle (4 ml/day) once daily on Days 10–16 of gestation. Jugular vein blood samples were collected on Days 10 (preinjection), 15 (during injection) and 20 (4 days postinjection) using a vacutainer single-sample collection method to avoid prolonged stress. Serum was assayed for concentrations of prolactin. On Day 25 of gestation, gilts were injected with sodium thiamylal (1 g, i.v.) to induce anaesthesia, which was then maintained with halothane using a closed-circuit unit, and subjected to midventral laparotomy. The uterus was exposed and examined for evidence of normal pregnancy. Each gilt received 15 mg PGF-2 α (Lutalyse, Upjohn Company, Kalamazoo, MI, USA) to terminate the pregnancy.

Experiment 2. This experiment was to determine effects of hypoprolactinaemia on uterine secretory activity. The protocol was to mimic effects of oestrogens from conceptuses on endometrial secretory activity during the time of maternal recognition of pregnancy without interactions with other conceptus products (Geisert *et al.*, 1982a). After two normal oestrous cycles, the 10 gilts used in Exp. 1 were assigned randomly, 5 per treatment group, to receive either bromocriptine (100 mg/day) or vehicle (4 ml/day) on Days 10 and 11 of the oestrous cycle. All gilts then received 5 mg oestradiol valerate (i.m.) on Day 11. On Day 12, uterine flushings and a jugular vein blood sample were collected.

Experiment 3. This study was conducted to establish hyperprolactinaemia and avoid compromising results in Exps 4 and 5 due to confinement and chronic bleeding of the gilts. Non-pregnant gilts, 3 per treatment group, were assigned randomly to receive pig prolactin (1 mg) or vehicle (1 ml saline). On Day 7, gilts were anaesthetized and fitted with

indwelling jugular vein catheters. Jugular vein blood (7 ml) samples were collected on Days 10 through 13 of the oestrous cycle at 07:30, 10:00, 12:00, 19:30 and 24:00 h. Prolactin or saline was administered subcutaneously at 08:00 and 20:00 h, 30 min after the morning and evening blood samples, on Days 10 through 14. Blood samples were assayed for concentration of prolactin in serum.

Experiment 4. This experiment investigated the interaction between prolactin and progesterone in the absence of oestrogens of ovarian origin. Ovariectomized pigs secrete uterine proteins as do ovary-intact gilts on Day 15 of the oestrous cycle or pregnancy when treated with an optimal dose (200 mg/ml) of progesterone alone (Knight *et al.*, 1974). Non-pregnant gilts were ovariectomized on Day 4 of the oestrous cycle and injected with 200 mg progesterone once daily from Days 4 to 14. Gilts were assigned randomly to receive prolactin (N = 4; 1 mg) or vehicle (N = 3; 1 ml saline) daily at 08:00 and 20:00 h on Days 4–14. Uterine flushings were collected on Day 15.

Experiment 5. This experiment determined effects of the interaction between oestrogen and prolactin using the protocol designed to mimic the effect of oestrogens of conceptus origin without confounding effects of other conceptus secretory products (Geisert *et al.*, 1982a). Non-pregnant gilts, 4 per treatment group, were assigned randomly to receive one of three treatments. Gilts in the negative control group (corn oil only on Day 11) and positive control group (5 mg oestradiol valerate in corn oil on Day 11) received 1 ml saline twice daily at 07:30 and 19:30 h on Days 6 through 11. Gilts in the treatment group received 1 mg pig prolactin in saline twice daily at 07:30 and 19:30 h and 5 mg oestradiol valerate on Day 11. Uterine flushings were collected on Day 12, approximately 24 h after treatment with corn oil or oestradiol. A prolactin and corn oil treatment group was not included because results of Exp. 4 indicated no interaction between prolactin and progesterone on uterine secretory activity.

Statistics. Data were analysed by least squares analysis of variance using the General Linear Models procedures of the Statistical Analysis System (SAS) (Barr *et al.*, 1979) to detect effects of treatment. When appropriate, orthogonal contrasts were used to detect differences between means.

Results

Effects of hypoprolactinaemia

Administration of bromocriptine to pregnant gilts (Exp. 1) decreased circulating concentrations of prolactin by 40% ($P < 0.06$) on Day 15. Prolactin levels (± 0.33 ng/ml) were 5.0, 3.3 and 4.4 ng/ml and 5.2, 5.0 and 4.4 ng/ml on Days 10, 15 and 20 of gestation for gilts that received bromocriptine and vehicle only, respectively. The number of corpora lutea (CL) was not different on Day 25 of gestation between gilts that received bromocriptine (13.6 ± 0.9) and vehicle (14.0 ± 0.9) and litter size was similar between gilts that received bromocriptine (7.3 ± 1.75) and vehicle (8.4 ± 1.75).

Administration of bromocriptine to cyclic gilts (Exp. 2) decreased circulating concentrations of prolactin by 50% (2.7 ± 0.33 ng/ml vs 5.3 ± 0.33 ng/ml; $P < 0.06$) on Day 12 to levels that were similar to those reported for bromocriptine-treated pigs by Kraeling *et al.* (1982), Whitacre & Threlfall (1981) and Smith & Wagner (1985). The inability to suppress circulating prolactin totally in pigs may be due to its regulation by factors other than dopamine. Previously reported dosages of 480 mg/day did not affect prolactin support of corpus luteum function in mid-gestation of pigs (R. R. Kraeling, cited by Bazer & First, 1983). Total recoverable protein (mg), uteroferrin (μ mol), and glucose (mg) concentrations, respectively, were not different in uterine flushings from gilts that received bromocriptine (17.6 ± 2.4 mg; 541 ± 92 μ mol and 2.23 ± 0.13 mg) compared to those that received vehicle (19.2 ± 2.4 mg; 608 ± 92 μ mol and 2.35 ± 0.13 mg). However, concentrations of leucine aminopeptidase were lower ($P < 0.025$) in uterine flushings of bromocriptine- (140 ± 18 SU) compared to vehicle- (230 ± 18 SU) treated gilts. Concentrations of PGF (30 ± 5 vs 17 ± 5 ng/horn) and PGE (14 ± 2.2 vs 11 ± 2.2 ng/horn) in uterine flushings were not affected by treatment. Gilts that received bromocriptine had lower concentrations of calcium ($P < 0.03$), sodium ($P < 0.02$), potassium ($P < 0.01$) and chloride ($P < 0.01$) in uterine flushings (Fig. 1).

Experiment 3

Effects of administration of exogenous prolactin are presented in Fig. 2. Concentrations of prolactin in serum increased within 2 h in gilts that received exogenous pig prolactin. Over the 4

Fig. 1. Concentrations of total recoverable calcium (a), chloride (b), sodium (c) and potassium (d) in uterine flushings from Day 12 cyclic gilts (Exp. 2) treated with bromocriptine (100 mg/day; B) or vehicle (4 ml, V) on Days 10 and 11 and oestradiol valerate on Day 11. Treatment effects were detected for calcium and potassium ($P < 0.05$), as well as sodium and chloride ($P < 0.01$). The s.e.m. was ± 0.14 for calcium, ± 0.12 for chloride, ± 12 for sodium and ± 8 for potassium.

days of administration, concentrations of prolactin were 4.5-fold higher ($P < 0.001$) for gilts that received prolactin (19.6 ± 1.2 ng/ml) compared to control gilts (4.25 ± 0.13 ng/ml). Although serum concentrations of prolactin were higher than those for control gilts, they were not pharmacological since concentrations of prolactin in serum of pigs during oestrus and late luteal phase are 15–20 ng/ml (Brinkley *et al.*, 1973; Dusza & Kryzowska, 1979).

Fig. 2. Concentrations of circulating immunoreactive prolactin during administration of 1 mg pig prolactin (●—●), or 1 ml saline (■—■), at 08:00 and 20:00 h (denoted by arrows) on Days 10–13 of the oestrous cycle. Blood samples were collected at 07:30, 10:00, 12:00, 19:30 and 24:00 h. The s.e.m. were ± 1.2 ng/ml for pig prolactin- and ± 0.13 ng/ml for saline-treated gilts.

Experiment 4

Exogenous administration of prolactin did not significantly affect any of the progesterone-induced uterine secretory components examined in the uterine flushings (Table 1).

Table 1. Composition of Day 15 uterine flushings from ovariectomized gilts treated with daily injections of progesterone and saline or pig prolactin from Days 4 to 14

	Saline	Prolactin
Total protein (mg)	74.3 ± 19.3	74.0 ± 16.7
Total uteroferrin* (µmol/uterine horn)	4138 ± 1903	4230 ± 1648
Uteroferrin/mg protein	45.7 ± 14.9	51.7 ± 13.0
Total calcium (mg)	0.5 ± 0.2	0.4 ± 0.2
Total chloride (mg)	1.7 ± 0.3	1.5 ± 0.2
Total sodium (µg)	125.3 ± 32.1	93.5 ± 27.9
Total potassium (µg)	170.2 ± 46.7	131.8 ± 40.4
Total glucose (mg)	2.5 ± 0.3	2.8 ± 0.3
Total L-acyl aminopeptidase†	655 ± 112	604 ± 92
Total PGF (ng/uterine horn)	235 ± 22	219 ± 19
Total PGE (ng/uterine horn)	38.6 ± 6.7	34.6 ± 5.8

Values are mean ± s.e.m.

*Activity = µmol *p*-nitrophenol released/ml/10 min at 37°C.

†Sigma Units: 1 Sigma Unit will release 1 µmol (143 µg) β-naphthylamine from L-leucine-β-naphthylamine/h at 37°C, pH 7.1.

Experiment 5

Effects of exogenous prolactin on oestrogen-induced uterine secretory activity are summarized in Table 2. Treatment effects were detected for total recoverable protein and total uteroferrin ($P < 0.001$), total glucose ($P < 0.01$), total leucine aminopeptidase ($P < 0.05$), total calcium ($P < 0.03$), total chloride ($P < 0.02$), total potassium ($P < 0.01$) and total PGF ($P < 0.02$) in uterine flushings. There was no effect of treatment on sodium or PGE in uterine flushings. Additionally, gilts treated with prolactin and oestradiol had greater amounts of uteroferrin ($P < 0.01$), glucose ($P < 0.01$) and PGF ($P < 0.01$) in uterine flushings than did gilts receiving oestradiol alone. However, values for glucose and PGF were not different for gilts treated on Day 11 with oestradiol or corn oil. The specific activity of uteroferrin (µmol/mg protein) was higher ($P < 0.01$) in uterine flushings from gilts treated with prolactin and oestradiol (106.53 ± 9.3) when compared to gilts receiving oestradiol (56.68 ± 9.3) or corn oil (8.36 ± 9.3). Gilts receiving oestradiol and prolactin had greater uterine secretory responses for total protein, potassium and aminopeptidase compared to gilts receiving oestradiol alone, but differences were not statistically significant.

Discussion

The results from the present experiments suggest that, within the pig uterus, prolactin affects the ionic environment of the developing conceptus. Prolactin influences ion and water fluxes in mammary alveolae (Falconer & Rowe, 1977) and intestine (Riddle, 1963; Mainoya *et al.*, 1974). In this study, slight hypoprolactinaemia decreased concentrations of Ca^{2+} , Na^+ , K^+ , and Cl^- ions in uterine flushings, but did not affect total protein. The inability of lowered prolactin concentrations to alter secretion of proteins may be due to lack of effects on ion ratios despite lowered individual ion levels. The effects of decreasing concentrations of ions may not have occurred in a temporal pattern necessary to result in a change in the secretion of proteins. The aminopeptidase activity in uterine flushings was decreased in response to hypoprolactinaemia suggesting a decrease in secretory activity of endometrial epithelium (Zavy *et al.*, 1984). Additionally, the decrease in circulating prolactin levels in the present study may have been compensated for physiologically through other mechanisms, e.g. an increase in prolactin receptor numbers, as has been previously

Table 2. Composition of Day 12 uterine flushings from cyclic gilts treated with twice daily injections of saline or pig prolactin from Days 6 to 11 and corn oil or oestradiol valerate on Day 11 of the oestrous cycle

Value/uterine horn	Treatment			s.e.m.
	Saline + corn oil	Prolactin + oestradiol valerate	Saline + oestradiol valerate	
Protein (mg)	14.2 ^a	36.1 ^b	31.7 ^b	±2.7
Uteroferrin* (μmol)	125.6 ^a	3709.5 ^b	1811.2 ^c	±278.3
Uteroferrin/mg protein	8.6 ^a	106.5 ^a	56.7 ^c	±9.3
Calcium (mg)	0.4 ^a	1.2 ^b	1.5 ^b	±0.3
Chloride (mg)	0.7 ^a	1.4 ^b	1.5 ^b	±0.2
Sodium (μg)	58.7 ^a	74.1 ^a	74.4 ^a	±17.4
Potassium (μg)	54.0 ^a	99.3 ^b	73.9 ^b	±9.3
Leucine aminopeptidase†	195.2 ^a	365.6 ^b	280.0 ^b	±52.7
Glucose (mg)	1.5 ^a	2.4 ^b	1.5 ^a	±0.2
PGE (ng)	26.7 ^a	27.1 ^a	26.5 ^a	±6.7
PGF (ng)	7.8 ^a	46.6 ^b	5.9 ^a	±10.2

Values are mean ± s.e.m.

^{abc}Values within rows with different letters are significantly different ($P < 0.05$ for calcium and leucine aminopeptidase; $P < 0.01$ for protein, uteroferrin, chloride, potassium, glucose and PGF).

*Activity = μmol *p*-nitrophenol released/ml/10 min at 37°C.

†One Sigma Unit will release 1 μmol (143 μg) β-naphthylamine from L-leucine β-naphthylamine/h at 37°C pH 7.1.

demonstrated for rabbit mammary tissue (Djiane *et al.*, 1977) and rat liver (Kelly *et al.*, 1979), or hormonal regulation at the central nervous system, receptor levels in the hypothalamus and target tissue (Muldoon, 1987) or by interpituitary communication (Murai *et al.*, 1988). Prolactin may stimulate the release of extracellular mediators from the liver, such as synlactin (Mick & Nicoll, 1985) or liver lactogenic factor (Hoeffler & Frawley, 1987) which act synergistically or additively with prolactin, respectively, at the target organ. Lastly, effects of prolactin may be regulated by intracellular enzymes that cleave the secreted prolactin to lower molecular weight subunits (M_r 16 000) (Nolin, 1982; Clapp, 1987; Vick *et al.*, 1987) that have specific intracellular effects. Since serum concentrations of prolactin do not change significantly over the period of maternal recognition of pregnancy in pigs, several of these factors may interact to potentiate the effect of prolactin on uterine secretory epithelium. In particular, oestrogen may regulate prolactin receptor numbers in target tissues.

Exogenous prolactin interacted with oestrogen, but not progesterone, to cause significant effects on uterine epithelial secretory activity. Shifts in concentrations of ions could account for the earlier release of several uterine secretory components; although such shifts could not be accounted for by static measurements on Day 12 in the present study. These effects, and those on PG, are probably due to prolactin and not contamination by other pituitary hormones. Pig prolactin is extensively dialysed during purification and contains <1% of the other anterior pituitary hormones; contamination by vasopressin or oxytocin is even less likely. Prolactin influences ion and water fluxes in the intestine (Mainoya *et al.*, 1974) and mammary alveolar cells (Falconer & Rowe, 1977).

Shifts in ions occur before protein secretion during early pregnancy (Geisert *et al.*, 1982a; Bazer *et al.*, 1984) and in response to administration of exogenous oestradiol on Day 11. With respect to the secretory profiles of uterine components in the experiments reported here, exogenous prolactin may have advanced the rapid release and reuptake of calcium which characteristically follows

oestrogen stimulation and precedes accumulation of proteins in the uterine lumen. Luminal calcium flux may have occurred early on Day 11 in hyperprolactinaemic gilts. Therefore, calcium measured in uterine flushings on Day 12 could be influenced by advanced calcium reuptake that does not normally occur until Days 13–14 of pregnancy. This may account for the increase in glucose and uteroferrin on Day 12 for prolactin-treated pigs compared to previous reports that glucose and uteroferrin concentrations do not increase until Days 14–16 during a normal pregnancy. Hyperprolactinaemia resulted in increased concentrations and specific activity of uteroferrin in (Day 12) uterine flushings. Increases in this protein usually occur on Day 14 of gestation (Zavy *et al.*, 1982). Since there was not a concomitant increase in aminopeptidase, a membrane marker protein indicative of secretory activity, prolactin may have altered uteroferrin synthesis resulting in increased amounts stored within intracellular secretory granules and available for release after oestrogen stimulation. Additionally, hyperprolactinaemia resulted in increased concentration of glucose in uterine flushings on Day 12 which normally does not occur until Days 14–16 of pregnancy (Zavy *et al.*, 1984). Administration of prolactin appeared to advance the ability of uterine epithelium to respond to oestrogen-induced secretory activity.

Stimulation of secretion of prostaglandins of the F, but not the E, series suggests that effects of prolactin may be on the uterine epithelium since PGF and PGE secretion has been localized to the endometrial epithelium and stroma, respectively, of cows (Grasso *et al.*, 1987). Prolactin exerts luteostatic effects in several species (Murphy & Rajkumar, 1985), primarily through stimulation of ovarian steroid production. Prolactin appears to advance the ability of the uterus to respond to oestradiol since an increase in luminal PGF occurred 48–72 h after a single injection of oestradiol valerate to Day-11 cyclic gilts (Geisert *et al.*, 1982a). During establishment of pregnancy in the pig, PGF secretion must be redirected from an endocrine to an exocrine direction (Bazer & Thatcher, 1977) to protect the corpus luteum from regression. The ability of prolactin to stimulate secretion of PGF and enhance its secretion into the uterine lumen (Mirando *et al.*, 1988) is a novel finding suggesting that prolactin plays a luteostatic role, in conjunction with oestrogen, in early pregnancy of pigs.

The present findings in the pig support the hypothesis that prolactin modulates uterine secretory activity during establishment of pregnancy as previously described for the long-term ovariectomized rabbit (Chilton & Daniels, 1985). Prolactin increased uterine secretory function and caused differential changes in ions, increased secretion of proteins, PGF and glucose. Although the mechanism(s) by which prolactin influences the endometrial secretory profile is not known, the effects may occur through several mechanisms. Previously reported effects include: (1) activation of ion channels to facilitate transport and secretion of cellular components (Petersen & Maruyama, 1985) or stimulation of membrane cycling of calcium (Alkon & Rasmussen, 1988); (2) increased oestrogen binding by cells of rat liver (Chamness *et al.*, 1975) and uterus (Leung & Sasaki, 1973); (3) facilitation of the formation of gap junctions to increase intercellular communication (Sorenson *et al.*, 1987), and (4) up-regulation of prolactin receptors to increase membrane fluidity (Dave & Witorsch, 1985) and increase the availability of cryptic hormone receptors, e.g. Dave *et al.* (1983) observed an increase in prolactin binding during early pregnancy which they attributed to alterations in membrane fluidity.

During the time of maternal recognition of pregnancy in the pig, prolactin interacts with oestrogen, rather than progesterone, to influence uterine endometrial secretory activity. This selective interaction with oestrogen may allow the uterine endometrium to respond maximally to the oestrogen signal from the pig conceptus which allows establishment of pregnancy.

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