Effect of oxytocin on concentration of $\text{PGF}_{2\alpha}$ in the uterine lumen and subsequent endometrial responsiveness to oxytocin in pigs

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The aim of this study was to determine the effect of oxytocin on $\text{PGF}_{2\alpha}$ secretion into the uterine lumen of pigs and subsequent endometrial responsiveness to oxytocin *in vitro*. Cyclic, pregnant and oestradiol-induced pseudopregnant gilts were injected i.v. with vehicle or 20 iu oxytocin 10 min before hysterectomy on day 16 after oestrus. Concentrations of $\text{PGF}_{2\alpha}$ and 13,14-dihydro-15-keto $\text{PGF}_{2\alpha}$ (PGFM) were significantly increased in uterine flushings collected at hysterectomy ($P < 0.05$) in pregnant oxytocin-injected gilts. Concentrations of $\text{PGF}_{2\alpha}$ and PGFM were greater ($P < 0.001$) in pregnant than in pseudopregnant and cyclic gilts, and greater ($P < 0.01$) in pseudopregnant than in cyclic gilts. The ratio of PGFM: $\text{PGF}_{2\alpha}$ tended to be greater in cyclic ($P < 0.06$) and pseudopregnant gilts ($P < 0.1$) than in pregnant gilts. At 85 ± 5 min after oxytocin injection, endometrium from each gilt was incubated for 3 h for determination of phosphoinositide hydrolysis and $\text{PGF}_{2\alpha}$ secretion in response to treatment with 0 or 100 nmol oxytocin l^{-1} . Endometrial phosphoinositide hydrolysis in response to 100 nmol oxytocin l^{-1} *in vitro* was greater ($P < 0.05$) in cyclic oxytocin-injected gilts than in cyclic vehicle-injected gilts. Treatment with oxytocin *in vitro* did not stimulate phosphoinositide hydrolysis significantly in vehicle- or oxytocin-injected pregnant gilts or pseudopregnant gilts. Endometrial $\text{PGF}_{2\alpha}$ secretion increased after treatment with 100 nmol oxytocin l^{-1} *in vitro* in cyclic vehicle-injected ($P < 0.01$), cyclic oxytocin-injected ($P < 0.01$), pregnant vehicle-injected ($P = 0.06$), pseudopregnant vehicle-injected ($P < 0.05$) and pseudopregnant oxytocin-injected ($P < 0.05$) gilts, but not in pregnant oxytocin-injected gilts. The increase in $\text{PGF}_{2\alpha}$ in pseudopregnant oxytocin-injected gilts was less ($P < 0.05$) than that in cyclic oxytocin-injected gilts. These results indicate that oxytocin increases the concentration of $\text{PGF}_{2\alpha}$ and PGFM in the uterine lumen during pregnancy and may upregulate endometrial responsiveness to oxytocin during late dioestrus in pigs, but does not have the latter effect during early pregnancy or oestradiol-induced pseudopregnancy.

Introduction

In pigs, as in other domestic ungulates, luteolysis requires pulsatile secretion of uterine $\text{PGF}_{2\alpha}$. Oxytocin is the major stimulus for pulsatile $\text{PGF}_{2\alpha}$ release in ruminants (McCracken *et al.*, 1981; Silvia *et al.*, 1991) and is implicated in

$\text{PGF}_{2\alpha}$ release in pigs (Mirando *et al.*, 1996). Pulsatile $\text{PGF}_{2\alpha}$ secretion in sheep is primarily controlled by endometrial responsiveness to oxytocin which, in turn, is limited by the population density of oxytocin receptors (McCracken *et al.*, 1981, 1984).

Responsiveness to oxytocin is markedly suppressed during early pregnancy in sheep (Fairclough *et al.*, 1984; McCracken *et al.*, 1984), cattle (Lafrance and Goff, 1985; Jenner *et al.*, 1991) and swine (Carnahan *et al.*, 1996; Edgerton *et al.*, 1996; Ludwig *et al.*, 1998). However, pregnancy does not completely prevent endometrial responsiveness to oxytocin in pigs (Carnahan *et al.*, 1996). Furthermore, basal secretion of $\text{PGF}_{2\alpha}$ is not reduced on days 14–16 in pregnant gilts compared with an equivalent period during luteolysis

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in cyclic pigs (Ludwig *et al.*, 1998) and its secretion is redirected toward the uterine lumen in a non-luteolytic exocrine direction (Bazer and Thatcher, 1977; Bazer, 1992). Therefore, oxytocin may stimulate $\text{PGF}_{2\alpha}$ secretion during early pregnancy and much of this $\text{PGF}_{2\alpha}$ may be directed toward the uterine lumen, resulting in a decrease in circulating concentrations (Carnahan *et al.*, 1996; Edgerton *et al.*, 1996).

Regulation of endometrial oxytocin receptor population density and corresponding responsiveness to oxytocin appears to differ markedly between sheep and swine. For example, oestradiol-induced expression of endometrial oxytocin receptors in ewes (Hixon and Flint, 1987; Spencer *et al.*, 1995) is suppressed during recognition of pregnancy (Spencer and Bazer, 1996). In contrast, oestradiol is anti-luteolytic, rather than luteolytic, in swine (Bazer, 1992) and would not be expected to upregulate expression of oxytocin receptors. In addition, continuous oxytocin infusion in ewes downregulates oxytocin receptors to inhibit luteolysis and increase the inter-oestrous interval (Flint and Sheldrick, 1985). However, pig endometrium synthesizes and releases large quantities of oxytocin (Trout *et al.*, 1995; Boulton *et al.*, 1996), but remains responsive to exogenous oxytocin during dioestrus (Whiteaker *et al.*, 1994; Carnahan *et al.*, 1996; Edgerton *et al.*, 1996; Ludwig *et al.*, 1998). Presumably, the endometrium of dioestrous pigs is similarly responsive to circulating oxytocin that may be of neurohypophyseal (Kotwica *et al.*, 1990), luteal (Pitzel *et al.*, 1984; Einspanier *et al.*, 1986) or uterine origin (Trout *et al.*, 1995; Boulton *et al.*, 1996). Moreover, the response to oxytocin administered systemically is not completely attenuated during early pregnancy (Carnahan *et al.*, 1996). Therefore, it seems unlikely that oxytocin downregulates the population density of oxytocin receptors in pigs as it does in ewes.

The objectives of this study were to determine whether i.v. administration of oxytocin stimulates secretion of $\text{PGF}_{2\alpha}$ into the uterine lumen *in vivo* and affects endometrial responsiveness to subsequent oxytocin treatment *in vitro*. Systemic administration of oxytocin was performed to mimic circulating oxytocin to which the uterus is exposed during dioestrus and early pregnancy in pigs.

Materials and Methods

Animals

Crossbred (Yorkshire, Landrace, Chester White, Duroc and Hampshire) gilts, 5–7 months of age and weighing 85–105 kg, were housed in total confinement and exposed to a mature intact boar for 20 min each day. Thirty gilts were randomly allocated at onset of second oestrus (that is, day 0) to one of six treatment groups in a 2×3 factorial arrangement: cyclic vehicle-injected, cyclic oxytocin-injected, pregnant vehicle-injected, pregnant oxytocin-injected, pseudopregnant vehicle-injected and pseudopregnant oxytocin-injected. Gilts assigned to the pregnant status were mated to a fertile boar at onset of oestrus and again 10 and 24 h later. Gilts allocated to the pseudopregnant status were injected i.m. with 5 mg oestradiol valerate in 1 ml corn

oil once a day on days 11–15, whereas cyclic and pregnant gilts received control injections of 1 ml corn oil.

On day 16, all gilts were anaesthetized (Whiteaker *et al.*, 1994; Carnahan *et al.*, 1996) and received 1 ml injections of vehicle (0.25% (v/v) acetic acid and 5 mg chlorbutanol ml^{-1}) or oxytocin (20 iu ml^{-1} vehicle). Gilts were hysterectomized while under general anaesthesia (Whiteaker *et al.*, 1994; Carnahan *et al.*, 1996) 10 min after injection of vehicle or oxytocin because i.v. injection of oxytocin resulted in peak plasma concentrations of the $\text{PGF}_{2\alpha}$ metabolite, 13,14-dihydro-15-keto- $\text{PGF}_{2\alpha}$ (PGFM), 10 min after injection of cyclic gilts and 10–20 min after injection of pregnant gilts in an earlier study (Carnahan *et al.*, 1996). One uterine horn of each gilt was randomly selected and flushed with 20 ml 0.9% saline for quantification of $\text{PGF}_{2\alpha}$ and PGFM concentrations. Endometrium was collected from the remaining uterine horn and placed immediately in ice-cold Krebs–Ringer bicarbonate (KRB) buffer containing 10 mmol glucose l^{-1} and 10 μmol myo-inositol l^{-1} for determination of phosphoinositide hydrolysis and $\text{PGF}_{2\alpha}$ secretion.

Determination of endometrial phosphoinositide hydrolysis

For determination of phosphoinositide hydrolysis, 100 mg endometrium was incubated in 1.0 ml KRB containing 10 μCi [^3H]inositol (specific activity 18.8 Ci mmol^{-1} ; Amersham, Arlington Heights, IL) as described by Whiteaker *et al.* (1994). Incubation was initiated 85 ± 5 min after injection of vehicle or oxytocin and continued for 2.5 h before addition of LiCl. At 10 min after addition of LiCl, 0 or 100 nmol oxytocin l^{-1} (Sigma Chemical Co., St Louis, MO) treatments were added in duplicate for 20 min. Formation of total [^3H]inositol phosphates was determined after Dowex (1 $\times$ 8-200, chloride form; Sigma) anion exchange chromatography.

Incubation of endometrium for $\text{PGF}_{2\alpha}$ secretion

For determination of $\text{PGF}_{2\alpha}$ secretion, 200 mg endometrium was incubated as described by Whiteaker *et al.* (1994) and Ludwig *et al.* (1998). Tissue was rinsed with 2 ml KRB and the tissue was incubated for 3 h, during which time the KRB was replaced at 30 min intervals. After 2.5 h, the KRB was collected and replaced with 2 ml fresh KRB containing 0 or 100 nmol oxytocin l^{-1} (Sigma) in duplicate. The KRB samples collected during the final two 30 min incubation periods (before and after treatment) were assayed for concentrations of $\text{PGF}_{2\alpha}$.

Radioimmunoassay of $\text{PGF}_{2\alpha}$ in uterine flushings and endometrial incubation medium

The concentrations of $\text{PGF}_{2\alpha}$ in 5–200 μl uterine flushings or 25 μl KRB incubation medium were quantified by radioimmunoassay using [5,6,8,9,11,12,14,15 ^3H (N)] $\text{PGF}_{2\alpha}$ (specific activity 195 Ci mmol^{-1} ; New England Nuclear, Boston, MA), anti- $\text{PGF}_{2\alpha}$ (1:25 000 final dilution) and Lutalyse (Upjohn Co., Kalamazoo, MI) as standards

(Whiteaker *et al.*, 1994). The sensitivity of the assay was 2.5 pg per tube ($P < 0.01$). Uterine flushings were diluted with 0.9% saline and then quantified in one assay with an intra-assay coefficient of variation of 8.6%. For the incubation medium, intra- and interassay coefficients of variation were 9.7% and 14.1%, respectively.

The radioimmunoassay for $\text{PGF}_{2\alpha}$ in uterine flushings was validated by quantifying concentrations of $\text{PGF}_{2\alpha}$ in 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 μl pooled uterine flushings. The slope of the inhibition curve was parallel to that of the standard curve. The mass of $\text{PGF}_{2\alpha}$ measured after addition of 5, 10, 20, 40, 80, 120, 160 and 200 pg to 100 μl pooled uterine flushings averaged 88.6% of the amount added. Regression of the amount of $\text{PGF}_{2\alpha}$ recovered (Y) on the amount of $\text{PGF}_{2\alpha}$ added (X) was $Y = 0.80 + 0.87X$ ($r^2 = 0.997$, $P < 0.001$).

Radioimmunoassay of PGFM in uterine flushings

The concentration of PGFM in 25–200 μl uterine flushings was quantified by radioimmunoassay using rabbit anti-PGFM J57 (1:40 000 final dilution), [5,6,8,9,11,12,14(n)- ^3H]PGFM (specific activity 186 Ci mmol^{-1} ; Amersham), and 3.125–800.0 pg radioinert PGFM (Sigma) as standards as described by Carnahan *et al.* (1996). The sensitivity of the assay was 2.5 pg per tube ($P < 0.01$). Uterine flushings were diluted with 0.9% saline and then quantified in one assay with an intra-assay coefficient of variation of 5.4%.

The radioimmunoassay for PGFM was validated by quantifying concentrations of PGFM in 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 μl pooled uterine flushings. The slope of the inhibition curve was parallel to that of the standard curve. The mass of PGFM measured after addition of 5, 10, 20, 40, 80, 120, 160 and 200 pg to 100 μl pooled uterine flushings averaged 87.6% of the amount added. Regression of the amount of $\text{PGF}_{2\alpha}$ recovered (Y) on the amount of $\text{PGF}_{2\alpha}$ added (X) was $Y = 1.16 + 0.87X$ ($r^2 = 0.988$, $P < 0.001$).

Statistical analyses

Data were subjected to least squares ANOVA or analysis of covariance using the general linear models (GLM) procedure of the Statistical Analysis System (SAS System for Windows 6.12, SAS Institute Inc., Cary, NC). Data for $\text{PGF}_{2\alpha}$ and PGFM in uterine flushings were analysed for a completely randomized design with a two-way treatment structure. The statistical model included main effects of reproductive status and treatment, all appropriate interactions, and gilt nested within reproductive status $\times$ treatment group as the residual error. For the ratio of $\text{PGFM}:\text{PGF}_{2\alpha}$, data were converted to molar concentrations and analysed using the same model described for $\text{PGF}_{2\alpha}$ and PGFM in uterine flushings. Data for endometrial explant phosphoinositide hydrolysis were analysed using a split-plot ANOVA for a two-way treatment structure of main effects. The statistical model for the whole-plot included main effects of reproductive status and treatment, all appropriate interactions, and gilt nested within reproductive status $\times$ treatment as the whole-plot error. The

statistical model for the subplot included effect of *in vitro* treatment, all appropriate interactions, and residual error. Data for endometrial explant $\text{PGF}_{2\alpha}$ secretion were log-transformed to alleviate statistical problems associated with heterogeneity of variance. A split-plot analysis of covariance was performed with $\text{PGF}_{2\alpha}$ secretion during the 30 min after treatment as the response variable and $\text{PGF}_{2\alpha}$ during the 30 min immediately before treatment as the covariate. The model for $\text{PGF}_{2\alpha}$ included main effects of reproductive status and treatment, gilt nested within treatment $\times$ reproductive status as the whole-plot error, all appropriate interactions, and residual error. Least-squares means and appropriate standard errors, along with specific pre-planned comparisons of treatment means, were generated using the LSMEANS statement of SAS.

Results

Concentration of $\text{PGF}_{2\alpha}$ and PGFM in uterine flushings

Across all treatment $\times$ reproductive status groups, concentrations of PGFM in uterine flushings (results not shown) were very similar to the pattern displayed for $\text{PGF}_{2\alpha}$ (Fig. 1). $\text{PGF}_{2\alpha}$ and PGFM were increased ($P < 0.05$) after

Fig. 1. Mean concentration of (a) $\text{PGF}_{2\alpha}$ (pooled SEM = 1.09) and (b) ratio of PGFM:PGF_{2α} (pooled SEM = 1.49) in uterine flushings of cyclic, pregnant and pseudopregnant gilts after i.v. injection of vehicle (□) or oxytocin (■). The concentration of $\text{PGF}_{2\alpha}$ was increased ($P < 0.05$) after injection only in pregnant oxytocin-treated gilts and was greater ($P < 0.001$) in pregnant than in cyclic and pseudopregnant gilts. The ratio of PGFM:PGF_{2α} tended to be greater in cyclic gilts ($P < 0.06$) and pseudopregnant gilts ($P < 0.1$) than in pregnant gilts, but was not influenced by oxytocin treatment or the interaction of treatment and reproductive status.

oxytocin injection only in pregnant gilts compared with vehicle-treated gilts. When the cyclic and pseudopregnant groups were analysed separately (to remove the high standard error term associated with the greater mean of pregnant pigs), gilts treated with oxytocin did not have significantly lower ($P > 0.10$) uterine $\text{PGF}_{2\alpha}$ or PGFM concentrations than vehicle-treated gilts. Mean concentrations of $\text{PGF}_{2\alpha}$ and PGFM were greater ($P < 0.001$) for pregnant than cyclic and pseudopregnant gilts. Concentrations of PGFM were greater ($P < 0.01$) for pseudopregnant than cyclic gilts. When pregnant gilts were excluded from the analysis (to remove the high standard error term), $\text{PGF}_{2\alpha}$ also was greater ($P < 0.001$) for pseudopregnant than cyclic gilts. The ratio of PGFM: $\text{PGF}_{2\alpha}$ tended to be greater for cyclic gilts ($P < 0.06$) and pseudopregnant gilts ($P < 0.1$) than for pregnant gilts (Fig. 1), but was not influenced by oxytocin treatment or the interaction of treatment and reproductive status.

Endometrial phosphoinositide hydrolysis and $\text{PGF}_{2\alpha}$ secretion

Endometrial phosphoinositide hydrolysis in cyclic gilts was increased ($P < 0.05$) by 100 nmol oxytocin l^{-1} *in vitro*, but the response was greater ($P < 0.05$) in cyclic oxytocin-treated gilts than in cyclic vehicle-treated gilts (Fig. 2). In pregnant and pseudopregnant gilts, treatment with 100 nmol oxytocin l^{-1} *in vitro* did not significantly increase phosphoinositide hydrolysis regardless of prior injection of vehicle or oxytocin (Fig. 2).

Endometrial secretion of $\text{PGF}_{2\alpha}$ did not differ between cyclic vehicle-treated and cyclic oxytocin-treated gilts after 0 nmol oxytocin l^{-1} *in vitro*, and was similarly increased ($P < 0.01$) by 100 nmol oxytocin l^{-1} *in vitro* in both groups of cyclic gilts (Fig. 3). After treatment with 0 nmol oxytocin l^{-1} *in vitro*, $\text{PGF}_{2\alpha}$ did not differ between pregnant vehicle-treated and pregnant oxytocin-treated gilts or between pseudopregnant vehicle-treated and pseudopregnant oxytocin-treated gilts (Fig. 3), and was increased by 100 nmol oxytocin l^{-1} *in vitro* in both groups of pseudopregnant gilts ($P < 0.05$) and in pregnant vehicle-treated gilts ($P = 0.06$). However, 100 nmol oxytocin l^{-1} *in vitro* did not stimulate $\text{PGF}_{2\alpha}$ secretion in pregnant oxytocin-treated gilts, and the response to oxytocin *in vitro* was lower for pregnant oxytocin-treated gilts ($P < 0.01$) and pseudopregnant oxytocin-treated gilts ($P < 0.05$) than for cyclic oxytocin-treated gilts.

Discussion

Bazer and Thatcher (1977) proposed that conceptus-secreted oestradiol prevents luteolysis during maternal recognition of pregnancy by reorienting endometrial $\text{PGF}_{2\alpha}$ secretion from an endocrine direction (that is, toward the uterine vasculature) to an exocrine direction (that is, toward the uterine lumen), thereby preventing the luteolysis from reaching the corpus luteum and promoting regression. Consistent with this theory, in the present study, $\text{PGF}_{2\alpha}$ and PGFM concentrations in uterine flushings of pregnant gilts increased in response to injection of oxytocin. Furthermore,

Fig. 2. Mean phosphoinositide hydrolysis (total $[^3\text{H}]$ inositol phosphates) *in vitro* from endometrial explants of (a) cyclic (pooled SEM = 4.80), (b) pregnant (pooled SEM = 4.80) and (c) pseudopregnant gilts (pooled SEM = 4.80) after i.v. injection of vehicle or oxytocin. Endometrial explants were treated with 0 ($\square$) or 100 ($\blacksquare$) nmol oxytocin l^{-1} *in vitro* 4 h after injection with vehicle or oxytocin *in vivo*. Endometrial phosphoinositide hydrolysis in cyclic gilts was increased ($P < 0.05$) by 100 nmol oxytocin l^{-1} *in vitro*, but the response was greater ($P < 0.05$) in oxytocin- than in vehicle-injected cyclic gilts. In pregnant and pseudopregnant gilts, treatment with oxytocin *in vitro* did not significantly increase phosphoinositide hydrolysis.

previous results (Carnahan *et al.*, 1996; Edgerton *et al.*, 1996) indicated that endocrine secretion of uterine $\text{PGF}_{2\alpha}$ in response to oxytocin, as determined by plasma PGFM concentrations, was attenuated during pregnancy. However, small but significant increases in plasma PGFM were detected after oxytocin injection on days 10–16 of pregnancy (Carnahan *et al.*, 1996). This finding indicates that the endometrium remains responsive to oxytocin during early pregnancy, and $\text{PGF}_{2\alpha}$ secretion induced by oxytocin is not as

Fig. 3. Mean $\text{PGF}_{2\alpha}$ secretion *in vitro* from endometrial explants of (a) cyclic (pooled SEM = 0.17), (b) pregnant (pooled SEM = 0.17) and (c) pseudopregnant gilts (pooled SEM = 0.17) after i.v. injection of vehicle or oxytocin. Endometrial explants were treated with 0 (□) or 100 (■) nmol oxytocin l^{-1} *in vitro* 4 h after injection with vehicle or oxytocin *in vivo*. Endometrial secretion of $\text{PGF}_{2\alpha}$ was increased by 100 nmol oxytocin l^{-1} *in vitro* in cyclic vehicle-injected ($P < 0.01$), cyclic oxytocin-injected ($P < 0.01$), pregnant oxytocin-injected ($P = 0.06$), pseudopregnant vehicle-injected ($P < 0.05$) and pseudopregnant oxytocin-injected gilts ($P < 0.05$). Treatment with 100 nmol oxytocin l^{-1} *in vitro* did not stimulate $\text{PGF}_{2\alpha}$ secretion in pregnant oxytocin-injected gilts, and the response was lower in pregnant oxytocin-injected ($P < 0.01$) and pseudopregnant oxytocin-injected gilts ($P < 0.05$) than in cyclic oxytocin-injected gilts ($P < 0.001$).

readily detectable in the systemic circulation as it is for cyclic pigs (Carnahan *et al.*, 1996; Edgerton *et al.*, 1996) partly because it is redirected into the uterine lumen.

The period of early pregnancy during which reduced endocrine secretion of $\text{PGF}_{2\alpha}$ occurs (Bazer and Thatcher, 1977) also coincides with increased release of oxytocin into the uterine lumen (Trout *et al.*, 1995). Increased release of

oxytocin during recognition of pregnancy may contribute to reorienting $\text{PGF}_{2\alpha}$ secretion because endocrine secretion of $\text{PGF}_{2\alpha}$ in response to oxytocin was reduced during early pregnancy (Carnahan *et al.*, 1996; Edgerton *et al.*, 1996), whereas exocrine secretion of $\text{PGF}_{2\alpha}$ was increased in the present study. However, increased secretion of $\text{PGF}_{2\alpha}$ into the uterine lumen of pregnant gilts occurred in response to systemic administration of oxytocin, and the response to intrauterine application of oxytocin has not been examined. Alternatively, the rate of metabolism of $\text{PGF}_{2\alpha}$ to PGFM may be similar in the uterus of cyclic, pseudopregnant and pregnant gilts, but the tendency toward the decreased ratio of PGFM: $\text{PGF}_{2\alpha}$ in pregnant pigs may have resulted from increased luminal $\text{PGF}_{2\alpha}$ contributed by the conceptus. It is unclear why the ratio of PGFM: $\text{PGF}_{2\alpha}$ is greater in cyclic than in pregnant gilts, although $\text{PGF}_{2\alpha}$ may be metabolized at a slower rate and used during blastocyst elongation or attachment (for review, see Psychoyos *et al.*, 1995). Increased systemic and intrauterine oxytocin concentrations concomitant with some continued endometrial sensitivity to oxytocin and redirection of $\text{PGF}_{2\alpha}$ secretion toward the uterine lumen may provide a mechanism to increase the availability of $\text{PGF}_{2\alpha}$ to pig conceptuses during the establishment of pregnancy.

Continuous infusion of oxytocin downregulated oxytocin receptors and extended the inter-oestrous interval in cyclic ewes (Flint and Sheldrick, 1985). Therefore, it was speculated that the continuous presence of oxytocin secreted by the endometrium, acting either locally or systemically, may similarly desensitize the endometrium of pigs, and that this response may be more pronounced in pregnant than in cyclic gilts because of the apparently greater endometrial secretion of oxytocin during pregnancy (Trout *et al.*, 1995). Two observations in the present study are consistent with this contention. Firstly, administration of oxytocin enhanced endometrial phosphoinositide hydrolysis in response to subsequent oxytocin treatment *in vitro* in cyclic gilts but not in pregnant or pseudopregnant gilts. In addition, oxytocin injection did not attenuate endometrial $\text{PGF}_{2\alpha}$ secretion in response to oxytocin treatment *in vitro* in cyclic gilts as was observed in pregnant and pseudopregnant gilts. The reason why oxytocin injection in cyclic gilts increased responsiveness to oxytocin *in vitro* for phosphoinositide hydrolysis but not $\text{PGF}_{2\alpha}$ secretion is unclear, but may be due to a more rapid upregulation of oxytocin-stimulated phosphoinositide hydrolysis than $\text{PGF}_{2\alpha}$ secretion as observed in ewes (Hixon and Flint, 1987). This explanation is consistent with the report that responsiveness to oxytocin during dioestrus is controlled primarily at G-protein (that is, G_q) coupling of oxytocin receptors to phospholipase C (Ludwig *et al.*, 1998).

Other significant differences between pseudopregnant and pregnant gilts were also observed in the present study. Uterine luminal content of $\text{PGF}_{2\alpha}$ and PGFM were greater in pregnant than pseudopregnant gilts, whereas the ratio of PGFM: $\text{PGF}_{2\alpha}$ was lower in pregnant gilts than in pseudopregnant gilts. This effect may be attributed, in part, to conceptus $\text{PGF}_{2\alpha}$ secretion (Shemesh *et al.*, 1979; Lewis and Waterman, 1985). Concentrations of $\text{PGF}_{2\alpha}$ and PGFM in the uterine lumen were also increased by oxytocin injection in pregnant but not in pseudopregnant gilts. Edgerton *et al.*

(1996) also reported that pseudopregnant gilts had lower endocrine secretion of $\text{PGF}_{2\alpha}$ and a reduced response to oxytocin compared with pregnant gilts. Although pregnancy and exogenous oestradiol promote a similar reorientation from endocrine to exocrine $\text{PGF}_{2\alpha}$ secretion (Bazer and Thatcher, 1977) and may be primarily responsible for maintenance of the corpus luteum, clearly the overall endometrial response to pregnancy is not mediated solely by conceptus-secreted oestradiol. The factors that are involved remain to be elucidated, but may include conceptus secretory proteins because intrauterine infusion of these proteins in the presence of oestradiol increased uterine secretion of $\text{PGF}_{2\alpha}$ and PGE_2 (Harney and Bazer, 1989) and influenced uterine morphology (Harney and Bazer, 1990), but did not promote luteal maintenance (Harney and Bazer, 1989).

In summary, this study demonstrated that systemic administration of oxytocin increases $\text{PGF}_{2\alpha}$ secretion into the uterine lumen of pregnant gilts and indicated that the response to oxytocin is not completely abrogated during pregnancy in pigs. These results also indicate that previous exposure to oxytocin may upregulate subsequent responsiveness to oxytocin during luteolysis and may contribute to the increased response in dioestrous gilts compared with pregnant and pseudopregnant gilts. Finally, control of $\text{PGF}_{2\alpha}$ secretion in early pregnant gilts occurs at points in the secretory pathway other than the oxytocin receptors and involves conceptus-secreted factors in addition to oestradiol. Collectively, these results indicate that control of endometrial responsiveness to oxytocin in pigs differs markedly from that in ruminants.

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