Progesterone secretion and prostaglandin F release *in vitro* by endometrial and luteal tissue of cyclic pigs

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Summary. Blood and tissue were collected from 30 pigs on various days of the oestrous cycle to compare progesterone in peripheral plasma and progesterone release *in vitro* with prostaglandin (PG) F release *in vitro* by endometrial and luteal tissue (~100 mg in 5 ml Krebs–Ringer–bicarbonate buffer, pH 7.4, for 2 h with three different incubation treatments). Plasma progesterone increased from Days 8 to 12 of the oestrous cycle (25.1 to 42.8 ng/ml, $P < 0.05$) and decreased from Day 14 to 16 (36.3 to 5.9 ng/ml, $P < 0.05$). Progesterone release *in vitro* at 37°C decreased from 18.0 to 2.7 ng/mg tissue from Days 8 to 16 ($P < 0.05$). Endometrial PGF release *in vitro* at 37°C increased slightly from Days 8 to 14, then increased significantly from 43.7 to 103.5 ng/100 mg tissue between Days 14 and 16 ($P < 0.05$). The rapid increase in endometrial PGF release coincided with the decrease in plasma progesterone. A small, non-significant increase in mean luteal PGF release was detected between Days 14 and 16. The results of this experiment did not suggest a critical role for luteal PGF-2 α in the process of luteolysis in the pig, but do provide indirect evidence for the involvement of endometrial PGF-2 α in luteolysis in the pig.

Introduction

Evidence has accumulated that implicates prostaglandin (PG) F-2 α as the uterine luteolysin in the pig. Increased plasma PGF in the utero-ovarian vein on Days 14–16 of the oestrous cycle (Gleeson, Thorburn & Cox, 1974; Killian, Davis & Day, 1976; Moeljono *et al.*, 1977) corresponds to the stage of the cycle when the presence or absence of the uterus determines the regression or maintenance of the corpus luteum (Anderson, Butcher & Melampy, 1963). Injections of PGF-2 α (Gleeson, 1974; Hallford, Wettemann, Turman & Omtvedt, 1975) or its 16-aryloxy analogues (Guthrie & Polge, 1976, 1978) caused luteolysis when corpora lutea were about 10 days old (Day 12 of the oestrous cycle or pregnancy).

Patek & Watson (1976a) have proposed that luteal PGF-2 α synthesis *in vivo* might be involved in the process of luteolysis. They reported that luteal and endometrial PGF release *in vitro* was highest in tissues collected during the late luteal phase of the cycle. However, Guthrie, Rexroad & Bolt (1978) found that corpora lutea and endometrium collected on Days 15–16 of the cycle did not synthesize significantly more PGF than those collected on Days 7–8.

The purpose of this experiment was to determine (1) whether endometrial and luteal PGF release *in vitro* increased between Days 8 and 18 of the oestrous cycle and (2) how endometrial and luteal PGF release *in vitro* relates to declining plasma progesterone and luteal progesterone release *in vitro*.

Materials and Methods

Tissue recovery and incubation

Mature, crossbred pigs were assigned at random to be sampled (6/group) on Days 8, 12, 14, 16 or 18 of the oestrous cycle (Day 0 = first day of oestrus). On the assigned day, a blood sample was collected from a jugular vein, and then each pig was anaesthetized with an intravenous injection of 1.2–1.5 g sodium thiopental (33.3 mg/ml distilled water). A laparotomy was performed and the ovaries and uterus were excised. The ovaries and a 3-cm long section from the middle of each uterine horn were carried to the laboratory in ice-cold 0.9% (w/v) NaCl solution. Luteal and endometrial tissues were minced with scissors in ice-cold incubation medium (Krebs–Ringer–bicarbonate buffer, pH 7.4; 5 mM-glucose, 10 mM-sodium acetate and 1.3 μ M-calcium). Minced luteal and endometrial tissues (~100 mg) were weighed into separate 25-ml Erlenmeyer flasks containing 5 ml ice-cold incubation medium. Each tissue mince from each pig was incubated for 2 h with the following incubation treatments: (A) an atmosphere of air at 0°C; (b) an atmosphere of 95% O₂:5% CO₂ at 37°C; and (C) as for treatment B but indomethacin was added to the medium to make a concentration of 1.3×10^{-4} M. Tissues were incubated at 37°C with indomethacin or at 0°C to determine whether accumulation of PGF or progesterone in the medium at 37°C was due, at least in part, to synthesis. After incubation, the medium was pipetted from each incubation flask and frozen at –20°C.

Hormone assay

Aliquots of the medium from the luteal tissue incubations were diluted 10-fold with 0.01 M phosphate-buffered saline, pH 7.4, containing 0.1% gelatine. Progesterone was measured in petroleum ether extracts of 10–100 μ l blood plasma samples and the diluted medium by a previously validated radioimmunoassay (Guthrie, 1977; Guthrie *et al.*, 1978). The antiserum (465/5 provided by Dr B. J. A. Furr, I.C.I., Macclesfield, England) was produced in a goat to an 11 α -progesterone-succinyl-bovine serum albumin conjugate. The antiserum was used at a dilution of 1:5000 and 100 μ l bound 60% of 0.01 μ Ci [1,2,6,7-³H]progesterone in an incubation volume of 0.3 ml per assay tube. Only 17 α -hydroxyprogesterone, pregnenolone and 17 α -hydroxypregnenolone had cross-reactivity of >0.5% (1, 3.4 and 1.3%, respectively). The progesterone standards were dissolved in distilled water and extracted along with the samples. The sensitivity of the assay was 15–20 pg/tube at 90–95% binding, and the intra-assay and inter-assay coefficients of variability were 8.2 and 9.0%, respectively.

Prostaglandin F was measured in aliquots of undiluted medium from the luteal and endometrial tissue incubations without extraction (Rexroad, 1978). The procedure was a double antibody radioimmunoassay with goat anti-PGF-2 α as the first antibody (10142-JCC-50, a gift of Dr K. T. Kirton, Upjohn Co., Kalamazoo) and rabbit anti-goat globulin (DJB 3 $\times$ 1, provided by Dr D. J. Bolt, this laboratory) as the second antibody. The antibody cross-reacted about 16% with PGF-1 α and <0.016% with PG A-1, A-2, B-2, E-1 and E-2. Recovery of 0.2–1.6 ng PGF-2 α added to laboratory standards (see below) and assayed directly was $110 \pm 5\%$ ($n = 14$, $r = 0.99$), and recovery from a pool of medium samples from pig luteal tissue incubations was $119 \pm 14\%$ ($n = 15$, $r = 0.92$). The laboratory standards were supernatants of ovine uterine myometrium homogenates that had low (indomethacin-treated) or high (cycloheximide-treated) concentrations of PGF in relation to the standard curve. The laboratory standards were included in all assays and had intra- and inter-assay coefficients of variability of 8.6 and 14.1%, respectively.

The hormone concentration of the incubation medium was analysed as a split-plot design; least squares analysis of variance was used. The whole-unit analysis consisted of the 5 days of the oestrous cycle when tissues were collected, and the sub-unit analysis was based on the 3 incubation treatments. Plasma progesterone concentrations were analysed as a completely

randomized design; the 5 days of the cycle sampled for blood and tissue were used as the classification variable. Means from statistically significant treatment combinations were compared by Duncan's New Multiple Range Test. Linear correlation coefficients were calculated to determine the interrelationships among plasma progesterone, luteal medium progesterone and PGF and endometrial medium PGF concentrations after incubation at 37°C. Partial correlation coefficients (Snedecor & Cochran, 1967) were also calculated to determine whether these variables were correlated merely because of their common association with day of the cycle or whether there was a real relationship when the effect of day of the cycle was eliminated.

Results

Measurements of plasma progesterone and luteal progesterone release *in vitro* are shown in Table 1. At 37°C, luteal progesterone release decreased from the highest value on Day 8 to a value of approximately 50% by Day 14 ($P < 0.05$). In contrast, plasma progesterone increased from Day 8 to the highest value on Day 12 ($P < 0.05$); progesterone decreased markedly from Day 14 to 16 ($P < 0.05$). The decrease in luteal progesterone release *in vitro* was also greater between Days 14 and 16 than at any other time. Incubation of luteal tissue at 0°C significantly reduced progesterone release *in vitro* when compared with incubation at 37°C on Days 8, 12 and 14 ($P < 0.05$), but not on Days 16 and 18. The presence of indomethacin during incubation at 37°C had no significant effect on progesterone release at 37°C for any day of the cycle.

Table 1. Plasma progesterone and progesterone released *in vitro* during a 2-h incubation period from luteal tissue of pigs on various days of the oestrous cycle

Day of cycle	Plasma progesterone (ng/ml)	Progesterone (ng/mg tissue)		
		0°C	37°C	37°C + indomethacin
8	25.1 ± 1.9 ^a	2.5 ± 0.4 ^a	18.0 ± 4.6 ^a	14.1 ± 2.7 ^a
12	42.8 ± 4.2 ^b	3.6 ± 0.5 ^b	12.6 ± 2.2 ^{ab}	12.1 ± 2.6 ^{ab}
14	36.3 ± 2.9 ^{ab}	3.0 ± 0.4 ^{ab}	9.4 ± 1.8 ^{bc}	7.3 ± 1.1 ^{bc}
16	5.9 ± 3.1 ^c	0.8 ± 0.2 ^c	2.7 ± 0.8 ^c	2.9 ± 0.9 ^c
18	4.5 ± 3.4 ^c	0.6 ± 0.4 ^c	2.5 ± 1.6 ^c	2.7 ± 1.5 ^c

Values are mean ± s.e.m. for 6 observations/group.

Means within a column with no superscript letter in common differ significantly by Duncan's Multiple Range Test ($P < 0.05$).

Means for endometrial and luteal PGF release *in vitro* are shown in Table 2. Endometrial PGF release *in vitro* at 37°C increased from Day 8 to the highest value on Day 16, the mean for Day 16 being significantly greater than those for the preceding days ($P < 0.05$). Luteal PGF release *in vitro* at 37°C increased after Day 14, but only the means for Days 8 and 18 differed significantly ($P < 0.05$). Incubation of endometrial or luteal tissue at 0°C or at 37°C plus indomethacin reduced PGF release on all days of the cycle sampled ($P < 0.01$).

Two pigs, one each on Days 16 and 18, had active corpora lutea as indicated by high plasma progesterone values of 21.2 and 21.3 ng/ml on Days 16 and 18, respectively. The hormone values from these 2 pigs were included in the statistical analyses and in data represented in Tables 1–4 and Text-fig. 1. Endometrial PGF release *in vitro* for these 2 pigs with active corpora lutea was low on Days 16 and 18 (13.0 and 7.5 ng/100 mg tissue, respectively).

The means for plasma progesterone and release of progesterone and PGF *in vitro* at 37°C were plotted in Text-fig. 1 to show their temporal relationships. Calculation of the simple linear

Table 2. Release of PGF *in vitro* during a 2-h incubation period from endometrial and luteal tissues of pigs on various days of the oestrous cycle

Day of cycle	Endometrial PGF (ng/100 mg tissue)			Luteal PGF (ng/100 mg tissue)		
	0°C	37°C	37°C + indomethacin	0°C	37°C	37°C + indomethacin
8	4.8 ± 0.8 ^a	22.4 ± 4.0 ^a	7.9 ± 1.0 ^a	6.3 ± 1.8 ^a	18.5 ± 2.6 ^a	10.0 ± 1.5 ^a
12	6.1 ± 1.6 ^a	28.2 ± 6.5 ^{ab}	7.6 ± 1.0 ^a	3.9 ± 0.9 ^a	20.9 ± 2.6 ^{ab}	9.2 ± 0.7 ^a
14	7.6 ± 1.8 ^a	43.7 ± 11.2 ^{ab}	14.5 ± 1.9 ^a	5.9 ± 1.3 ^a	20.8 ± 1.9 ^{ab}	9.6 ± 1.6 ^a
16	18.9 ± 7.2 ^b	103.5 ± 28.6 ^c	28.3 ± 7.2 ^b	5.3 ± 1.2 ^a	33.3 ± 6.5 ^{ab}	13.5 ± 1.8 ^a
18	9.0 ± 1.7 ^{ab}	80.2 ± 21.5 ^{bc}	16.6 ± 3.1 ^a	7.2 ± 1.8 ^a	37.8 ± 10.3 ^b	14.0 ± 3.3 ^a

Values are mean ± s.e.m. for 6 observations/group.

Means within a column with no superscript letter in common differ significantly by Duncan's Multiple Range Test ($P < 0.05$).

Table 3. Simple correlations for 30 observations for plasma progesterone and release *in vitro* at 37°C for progesterone and PGF

Variables		Variables			
		Luteal progesterone	Luteal PGF	Endometrial PGF	Day of the cycle
Plasma progesterone	<i>r</i>	0.512	-0.489	-0.487	-0.547
	<i>P</i>	0.004	0.007	0.007	0.0018
Luteal progesterone	<i>r</i>		-0.472	-0.533	-0.700
	<i>P</i>		0.0085	0.0024	0.0001
Luteal PGF	<i>r</i>			0.278	0.455
	<i>P</i>			0.135	0.0115
Endometrial PGF	<i>r</i>				0.527
	<i>P</i>				0.0028

Text-fig. 1. Temporal relationships of plasma progesterone and release *in vitro* at 37°C of luteal progesterone and luteal and endometrial PGF during the oestrous cycle of the pig.

correlation coefficients (Table 3) showed that plasma progesterone and luteal progesterone release *in vitro* were negatively correlated with luteal and endometrial PGF release *in vitro*. However, when the effect of day of the cycle was eliminated, the partial correlation coefficients for luteal progesterone release *in vitro* with PGF release *in vitro* were not significant for either luteal or endometrial tissue (Table 4). Plasma progesterone retained a negative correlation with endometrial PGF release *in vitro* but not with luteal PGF release *in vitro*. Luteal and endometrial PGF release *in vitro* were not significantly correlated.

Table 4. Partial correlations for 24 observations for plasma progesterone and release *in vitro* at 37°C for progesterone and PGF when day of the cycle is held constant

Variables		Variables		
		Luteal progesterone	Luteal PGF	Endometrial PGF
Plasma progesterone	<i>r</i>	0.438	-0.214	-0.396
	<i>P</i>	0.025	0.290	0.045
Luteal progesterone	<i>r</i>		-0.220	-0.201
	<i>P</i>		0.280	0.330
Luteal PGF	<i>r</i>			-0.027
	<i>P</i>			0.910

Discussion

Accumulation of endometrial PGF in the incubation medium may be an index of the intrinsic potential of the endometrium to synthesize PGF-2 α *in vivo*. Endometrial PGF release *in vitro* increased from Day 12 to Day 16 of the oestrous cycle in conjunction with a decrease in plasma progesterone during the same time interval. Release of endometrial PGF *in vitro* was also reported to be higher in the late luteal phase, Days 14–15, than in any other stage of the cycle in guinea-pigs (Poyser, 1972) and ewes (Patek & Watson, 1976b; Roberts, McCracken, Gavagan & Soloff, 1976); the increased release of PGF coincided with the time of luteolysis.

In an earlier experiment (Guthrie *et al.*, 1978), we found that endometrial synthesis of PGF *in vitro* was greater on Days 15–16 of the cycle than on Days 7–8 although the difference was not statistically significant. Plasma progesterone averaged 11.2 ng/ml on Days 15–16, indicating that luteolysis had not proceeded to the degree found in the present experiment. In our present experiment, the highest level of endometrial PGF release coincided with very low plasma progesterone and progesterone release *in vitro*. In the pig (Gleeson *et al.*, 1974; Killian *et al.*, 1976; Moeljono *et al.*, 1977) and ewe (Cox, Thorburn, Currie, Restall & Schneider, 1972), PGF secretion, i.e. concentrations in the utero-ovarian vein, generally increased gradually after Day 12, but the fact that quite large amounts of PGF were secreted periodically after plasma progesterone values had fallen to very low levels indicates maximum synthesis of endometrial PGF-2 α *in vivo* after luteolysis was almost completed. Thus, the decrease in plasma progesterone, resulting from early PGF-2 α secretion and perhaps increased oestradiol secretion, may facilitate the increased secretion of endometrial PGF-2 α in the pig as has been reported for the ewe (Scaramuzzi, Baird, Boyle, Land & Wheeler, 1977).

The pattern of progesterone release *in vitro* appears to differ from that of the secretion of progesterone *in vivo* into peripheral plasma (Text-fig. 1). However, this apparent difference in secretion patterns may have limited significance. The means for progesterone secretion *in vitro* on Days 8 and 12 did not differ significantly, and the variation between animals within days can be high. In addition, progesterone concentrations in plasma from the peripheral circulation or

ovarian vein differ among pigs, and reports differ with respect to which day of the cycle the progesterone secretion rate is highest (Masuda, Anderson, Henricks & Melampy, 1967; Brinkley & Young, 1968; Henricks, Guthrie & Handlin, 1972).

Whether luteal PGF-2 α synthesis has a role in the uterine luteolytic process is still not clear. Patek & Watson (1976a) indicated that both endometrial and luteal PGF release *in vitro* were higher during the late luteal phase of the cycle than at any other time. However, their conclusion was apparently derived without appropriate statistical analysis of the data, and they estimated the day of the cycle by the morphological appearance of the corpora lutea in ovaries recovered from pigs slaughtered at an abattoir. When we consider the difficulty in dating pig corpora lutea by their morphological appearance (Masuda *et al.*, 1967), an error of 48 h in dating would not be unexpected. The results of our study do not support the proposal (Patek & Watson, 1976a) of a role for luteal PGF-2 α secretion in the process of luteolysis initiated by the uterus. Endometrial PGF release *in vitro* was not significantly correlated with luteal PGF release *in vitro*. We detected an increase in the mean luteal PGF release *in vitro* from Days 14 to 16, but the increase was not consistent enough to be significant. In addition, when corrected for the effect of day of the cycle, luteal PGF release *in vitro* was not correlated with plasma progesterone or luteal progesterone release *in vitro*. In contrast, we have shown that increased luteal PGF release *in vitro* (Guthrie, Rexroad & Bolt, 1979) was associated with luteolysis induced by the PGF-2 α analogue, Cloprostenol (ICI-80,996). We have not determined whether increased PGF release from pig luteal tissue *in vitro* was an artefact of induced luteolysis or a step in the process of induced luteolysis.

The evidence that PGF-2 α is the natural luteolysin in the pig is not conclusive. Watson & Maule Walker (1977) reported that endometrial superfusates from the late luteal phase of the cycle decreased luteal progesterone secretion *in vitro* by 90%. The luteolytic effects of the endometrial superfusate did not appear to be due to PGF-2 α release by endometrial or luteal tissue. More work is required to determine which factors originating in the uterine endometrium, besides PGF-2 α , may be luteolytic in the pig and to what degree, if any, luteal PGF-2 α synthesis is involved in luteolysis.

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