Control of oestrus and fertility in gilts with accessory corpora lutea by prostaglandin analogues, ICI 79,939 and ICI 80,996*

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Treatment of cows or mares with prostaglandin (PG) F-2 α as early as Days 4 or 5 of the oestrous cycle causes regression of corpora lutea (CL) and results in oestrus 2–4 days after treatment (Lauderdale, 1972; Rowson, Tervit & Brand, 1972; Douglas & Ginther, 1972; Allen & Rowson, 1973). However, the CL of pigs do not regress in response to PGF-2 α until Days 11–12 of the cycle (Gleeson, 1974; Guthrie & Polge, 1976). Because the secretory activity of the CL in the pig declines rapidly by Days 14 or 15 of the cycle, the effective period for synchronization of oestrus by PGF-2 α treatment is too short to be of practical use. Exogenous gonadotrophins induce the formation of accessory CL at any stage of the oestrous cycle of the pig (Neill & Day 1964; Day, Neill, Oxenreider, Waite & Lasley, 1965; Caldwell, Moor, Wilmut, Polge & Rowson, 1969) and the CL formed on Days 8, 10 or 16 delayed oestrus for at least 15 days (Caldwell *et al.*, 1969). We therefore investigated the possibility of synchronizing oestrus in gilts by inducing accessory CL to delay oestrus and ovulation and then injecting PGF-2 α analogues to regress the accessory CL at a predetermined time.

Methods

Mature, crossbred Large White gilts were exposed once daily to a vasectomized boar to check for oestrus. The first day of oestrus was designated as Day 0 of the cycle and the gilts were assigned to one of three experiments after at least one oestrous cycle.

Accessory CL were induced by a single s.c. injection of PMSG (Folligon: Organon); 1000 or 1500 i.u. were given to 22 gilts in Exp. 1 and 1500 i.u. to 29 and 35 gilts in Exps 2 and 3, respectively. After 72 or 96 hr in Exp. 1 and 72 hr in Exps 2 and 3, an i.m. injection of 750 i.u. HCG (Chorulon: Organon) was given. The injections were administered in 3–4 ml 0.9% (w/v) NaCl solution. In Exps 1 and 3 the gonadotrophin treatments were administered at random to cyclic gilts so that accessory CL were formed in different stages of the cycle. In Exp. 2, PMSG was given on Days 1, 3, 7, 11, 13, 15 or 18 of the cycle to 4 gilts on each day except for 5 gilts on Day 15.

The PGF-2 α analogues (Imperial Chemical Industries Ltd) were injected i.m. in 1–2 ml 0.9% (w/v) NaCl solution 12 days after the HCG as follows; one injection of 0.5 mg ICI 79,939 (Exp. 1), two injections of 0.5 mg ICI 79,939 24 hr apart (Exp. 2), and injections of 1.0 mg and 0.5 mg ICI 80,996 24 hr apart (Exp. 3). In Exp. 2 an additional injection of 1000 i.u. PMSG was given to 2 gilts in each of the 7 experimental groups, at the time of the second ICI 79,939 injection, to control more precisely the onset of oestrus. These two PGF-2 α analogues have approximately equal luteolytic effects on Day 12 of the oestrous cycle (unpublished observations).

Luteal function was monitored by radioimmunoassay of plasma progesterone concentration by methods similar to those of Henricks, Hill, Dickey & Lamond (1973). Blood samples were withdrawn from the anterior vena cava into a heparinized syringe and the plasma was stored at –20°C until assayed. The antiserum (465/5), produced to an 11 α -succinyl progesterone-bovine serum albumin conjugate, was provided by Dr B. J. Furr of ICI Ltd. When 100 μ l antiserum were used at a dilution

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of 1:4000 about 65% of 0.01 μCi [1,2,6,7- ^3H]progesterone was bound. The antiserum was highly specific for progesterone: only 17 α -hydroxyprogesterone, Δ^5 -pregnenolone and 17 α -hydroxy- Δ^5 -pregnenolone had significant cross-reactivities (1.0, 3.4 and 1.3% respectively).

In Exp. 3, gilts that exhibited oestrus 4–7 days after the first ICI 80,996 injection were artificially inseminated once on the 2nd day of oestrus with 100–150 ml fresh, undiluted boar semen. Half of the gilts were subjected to laparotomy 5–7 days after the onset of oestrus to record the number of CL and to recover ova. The ova were flushed from the anterior part of each uterine horn with 40 ml sterile 0.9% (w/v) NaCl solution. The ova were prepared as whole mounts, fixed in 25% acetic alcohol, and stained with 1% lacmoid in 45% glacial acetic acid for cytological examination by phase-contrast microscopy. The remaining gilts were killed 27–30 days after the onset of oestrus to record the number of CL and embryos.

The results from Exps 1 and 3 were classified according to the stage of the oestrous cycle at which PMSG was given, Days 0–4, 5–9, 10–14 and 15 to the day before oestrus, for statistical comparison of the proportion of gilts exhibiting luteal regression and oestrus after treatment with the PGF-2 α analogue. Plasma progesterone concentrations were evaluated by analysis of variance. Treatment effects were tested at the 5% level of significance (Steel & Torrie, 1960).

Results

Gonadotrophin treatment started on or before Day 14 caused oestrus to be suppressed during the 12-day interval between the HCG and analogue injections. Treatment initiated between Day 15 and the day before the expected oestrus resulted in oestrous cycles of normal length (18–23 days) for 15 of 24 gilts which returned to oestrus 1–5 days after the PMSG injection.

In addition to the induced accessory CL, the spontaneous CL in many gilts may have remained functional after Day 14 of the treatment oestrous cycle. Ovulation probably occurred between 40 and 42 hr after the HCG injection (Dziuk, Polge & Rowson, 1964; Hunter, 1967) or about 5 days after the PMSG injection, and the high progesterone concentrations on Days 16 and 18 in 16/21 gilts in Exp. 2 (Table 1) were presumably due to maintenance of the spontaneous CL.

Table 1. Effect of gonadotrophin treatment of gilts on the induction of accessory CL and on luteal function (Exp. 2)

Day of treatment		Condition of CL	No. of gilts	Plasma progesterone (ng/ml, mean $\pm$ S.E.M.)		
				Day of the oestrous cycle		
PMSG	HCG			14	16	18
3	6	Maintained	4	52.4 $\pm$ 15.9	56.2 $\pm$ 32.3	47.1 $\pm$ 28.2
7	10	Maintained	4	67.5 $\pm$ 8.7	81.6 $\pm$ 16.0	77.6 $\pm$ 12.0
11	14	Maintained	3	61.7 $\pm$ 7.7	46.7 $\pm$ 0.9	72.5 $\pm$ 4.4
		Regressed	1	32.3	10.2	3.3
13	16	Maintained	3	48.8 $\pm$ 11.6	44.9 $\pm$ 1.9	55.1 $\pm$ 8.7
		Regressed	1	23.9	2.8	3.1
15	18	Maintained	2	53.9 $\pm$ 9.7	52.2 $\pm$ 3.6	37.4 $\pm$ 1.6
		Regressed	3	26.5 $\pm$ 11.7	8.7 $\pm$ 6.9	2.1 $\pm$ 0.4

The effects of the analogue treatment on the number of gilts exhibiting luteal regression and oestrus are shown in Table 2. Luteal regression, based on plasma progesterone concentrations and the occurrence of oestrus, appeared to be complete in 54, 83 and 89% of the gilts in Exps 1, 2 and 3, respectively and the sensitivity of the accessory CL to ICI 79,939 and ICI 80,996 was similar to that of spontaneous CL in gilts treated with ICI 79,939 on Days 11 and 12 of the cycle (Guthrie & Polge, 1976).

Table 2. Effect of treatment with PGF-2 α analogues, ICI 79,939 or ICI 80,996, on luteal function and oestrus in gilts with accessory CL

Exp.	Analogue (dose)	No. of gilts treated	No. of gilts showing:		
			Induced luteal regression	Oestrus within 4-7 days	Delayed luteal regression
1	79,939 (0.5 mg)	22	13*	11	7
2	79,939 (0.5 mg + 0.5 mg)	29	24	19	5
3	80,996 (1.0 mg + 0.5 mg)	35	31	30	4
Totals		86	68	60	16

* Two gilts had exhibited luteal regression before treatment and were not included.

The failure of 16 gilts to exhibit a positive oestrous response was due to incomplete luteal regression. The progesterone concentrations before the first analogue injection were higher in these gilts than in those in which the CL had regressed completely (Table 3). Eight gilts did not exhibit oestrus until after the 4-7 day interval following the analogue-induced luteal regression. Plasma progesterone concentrations in these gilts were < 1 ng/ml, indicating that ovulation had also been delayed.

Table 3. The luteal response of gilts to treatments with PGF-2 α analogues

Exp.	Analogue (dose)	Condition of CL	No. of gilts	Plasma progesterone (ng/ml, mean $\pm$ S.E.M.)		
				Before treatment	After treatment	
					48 hr	96 hr
1	79,939 (0.5 mg)	Regressed	13*	44.0 $\pm$ 7.2	4.6 $\pm$ 1.5	—
		Maintained	7	49.8 $\pm$ 6.9	33.9 $\pm$ 3.3	—
2	79,939 (0.5 mg + 0.5 mg)	Regressed	24	58.7 $\pm$ 8.6	3.8 $\pm$ 0.5	2.1 $\pm$ 0.2
		Maintained	5	113.1 $\pm$ 33.5	57.4 $\pm$ 37.7	41.1 $\pm$ 26.3
3	80,996 (1.0 mg + 0.5 mg)	Regressed	31	71.2 $\pm$ 7.9	—	3.3 $\pm$ 0.2
		Maintained	4	165.2 $\pm$ 66.6	—	65.5 $\pm$ 22.9

* Two gilts had exhibited luteal regression before treatment and were not included.

Administration of 1000 i.u. PMSG at the time of the second ICI 79,939 injection in Exp. 2 significantly increased the proportion of gilts that returned to oestrus: 11 exhibited luteal regression and 10 of these returned to oestrus in 4-5 days compared to only 1 of 8 gilts exhibiting luteal regression and not given additional PMSG.

The conception rate for the 30 inseminated gilts in Exp. 3 was 80%. Thirteen of 15 gilts killed on Days 5-7 of pregnancy were pregnant with a mean $\pm$ S.E.M. number of 12.0 $\pm$ 0.9 embryos and 13.7 $\pm$ 0.6 CL per gilt. By Days 27-30, 11 of 15 gilts were pregnant with 11.5 $\pm$ 1.0 embryos and 15.2 $\pm$ 1.6 CL per gilt. Although these results are preliminary, they are similar to those of experiments using methallibure to synchronize oestrus (Gerrits & Johnson, 1964; Polge, 1964) and could offer the basis for a new method of oestrous synchronization in gilts.

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