Oestradiol administration raises luteal LH receptor levels in intact and hysterectomized pigs*

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Summary. Occupied and unoccupied LH receptors in corpora lutea, and LH and progesterone concentrations in circulating plasma, were measured in non-pregnant gilts that had been treated with oestradiol-17 β benzoate to prolong luteal function. Oestradiol benzoate (5 mg, administered on Day 12 after oestrus) delayed luteal regression and the decline in LH receptor levels at luteolysis and raised unoccupied receptor levels from 11.8 ± 1.14 fmol/mg protein on Days 10–15 after oestrus to 31.8 ± 3.26 fmol/mg protein on Days 15–21. There was no simultaneous rise in occupied receptor levels and occupancy decreased from 29.8 ± 3.01 to $11.5 \pm 1.26\%$. Basal plasma LH concentrations were unchanged by oestradiol, but mean corpus luteum weight and plasma progesterone concentrations were slightly reduced. Oestradiol benzoate on Day 12 caused a similar increase in unoccupied receptor levels in gilts hysterectomized on Days 6–9 after oestrus, from 17.0 ± 5.83 to 34.5 ± 6.00 fmol/mg protein, determined on Days 15–18. Plasma concentrations of LH and progesterone were unchanged by oestradiol. Unoccupied receptor levels in corpora lutea and plasma LH and progesterone were unaltered by hysterectomy in untreated gilts. Occupied receptor levels were not influenced by hysterectomy or oestradiol. It is concluded that oestradiol-17 β raises luteal LH receptor levels by a mechanism independent of the uterus.

Introduction

Administration of oestrogens during the oestrous cycle prolongs luteal function in pigs (Kidder, Casida & Grummer, 1955; Gardner, First & Casida, 1963; Kraeling, Barb & Davis, 1975) but the mechanisms involved in this response are not clear. One possibility is that oestrogen reduces endometrial release of prostaglandin (PG) F-2 α into the circulation, thereby blocking the normal luteolytic action of the uterus (Frank, Bazer, Thatcher & Wilcox, 1977; Guthrie & Rexroad, 1981); such a mode of action has been suggested for the luteotrophic effect of endogenous oestrogen secreted by the conceptus early in gestation (Bazer & Thatcher, 1977). Alternatively, the luteolytic effect of the uterus may be reduced by a rise in uterine blood flow, in response to administered oestrogen, leading to a drop in the concentration of PGF-2 α in uterine venous blood by dilution (Ford, Christenson & Ford, 1982). However, there is evidence that a pituitary hormone may be involved in the luteotrophic action of oestrogens, since oestrogen alone fails to

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maintain corpora lutea after hypophysectomy or pituitary stalk section (du Mesnil du Buisson, 1966; Anderson, Dyck, Mori, Henricks & Melampy, 1967). The experiments described here were designed to investigate whether oestrogens have any effect on luteal concentrations of the luteinizing hormone (LH) receptor, and to determine whether any such response is mediated by the uterus.

Materials and Methods

Experiment 1. Forty-five mature crossbred gilts aged 11–17 months were distributed amongst 9 treatment groups (5 pigs/group). Each animal had been observed for 2 oestrous cycles of normal length (19–21 days) before being used for the study. Animals in Groups 1–4 (controls) were untreated, and were killed on Days 10, 12, 15 and 18 respectively after oestrus (first day of oestrus = Day 0). Groups 5–9 received 5 mg oestradiol benzoate (Intervet Laboratories Ltd, Cambridge, U.K.) subcutaneously in oil on Day 12 after oestrus, and were killed on Days 15, 18, 21, 24 and 27 respectively. At slaughter reproductive tracts were collected, chilled in ice, and transported to the laboratory. Receptors were prepared immediately (within 1 h of death). Blood for hormone measurement was collected after slaughter from a jugular vein into heparinized containers and transported to the laboratory on ice before preparing plasma. Plasma was stored at -20°C until assay.

Experiment 2. Twenty mature crossbred gilts, which had experienced 2 normal oestrous cycles, were assigned to 4 groups of 5 animals each. Animals in Groups 1 and 2 were intact and those in Groups 3 and 4 were hysterectomized on Days 6–9 after oestrus. Animals in Groups 2 and 4 received 5 mg oestradiol benzoate on Day 12 after oestrus. Group 1 animals were killed on Day 12 after oestrus and those in Groups 2–4 on Days 15–18. Reproductive tracts and blood were collected at slaughter as described for Exp. 1.

Measurement of luteal LH receptor. Unoccupied and occupied LH receptor levels and dissociation constants were determined as previously described and validated by Zieck, Shaw & Flint (1980). For preparation of receptor, corpora lutea were dissected from the ovaries, trimmed of excess connective tissue, weighed, minced with scissors and homogenized with a Polytron homogenizer in 4 volumes (ml/g tissue) of 25 mM-Tris-HCl, pH 7.3, containing 0.25 M-sucrose. Corpora lutea, all of the same age, from each animal were homogenized together. Homogenates were centrifuged at 2000 g for 30 min, and the resulting supernatants were centrifuged again at 27 000 g for 1 h. The final supernatants were discarded and the sediments were resuspended in 1 volume 25 mM-Tris-HCl, pH 7.3, containing 5 mM-MgCl₂. Resuspended receptor fractions were divided into 1 ml samples and stored in liquid N₂ until assayed for receptor concentration and occupancy; a further sample (0.2 ml) of each preparation was stored at -20°C for protein estimation (Lowry, Rosebrough, Farr & Randall, 1951). Receptor fractions kept in liquid N₂ showed no loss of activity after storage for 1 year.

Unoccupied LH receptor levels and dissociation constants were measured by equilibrium saturation assay and binding parameters determined from Scatchard plots. Each assay tube contained receptor protein (3 mg/ml), one of 9 different concentrations of LH in the range 0–250 ng/ml and ¹²⁵I-labelled LH, 20 000–25 000 c.p.m., in a final volume of 0.5 ml; LH (porcine LH, potency 0.63 × NIH-LH-S1) was iodinated using chloramine T to a specific activity of 25–40 µCi/µg. All additions were made in 25 mM-Tris-HCl containing 5 mM-MgCl₂ and 0.1% bovine serum albumin, pH 7.3. Incubations were performed overnight at 25°C. Non-specific binding was determined in the presence of excess unlabelled LH (1000 ng for Exp. 1; 500 ng in Exp. 2), and was usually <3% of the total ¹²⁵I-labelled LH added (equivalent to ~1 fmol/mg protein). Correlation coefficients (*r*) obtained by linear regression analysis of Scatchard plots averaged 0.89 ± 0.02 for the 64 determinations made.

Occupied receptor concentrations were estimated by eluting endogenous LH from the luteal receptor preparations and measuring LH by radioimmunoassay. Preparations (2 ml) containing

30–50 mg protein were incubated overnight at 4°C in 6 ml of freshly prepared 1 N-formic acid, pH 2.4. Insoluble material was removed by centrifugation at 10 000 g for 30 min and the resulting supernatant was decanted and freeze-dried. After freeze-drying residues were dissolved in 2 ml radioimmunoassay buffer and assayed for LH using 0.2 ml of each preparation as described below for determination of LH in plasma, material from each experiment being determined in one assay. Intra-assay coefficients of variation were 9.3 and 6.7% for Exps 1 and 2 respectively. Parallelism of eluted LH in the radioimmunoassay and recovery efficiency for the elution procedure were assessed by extracting 1, 2 and 3 ml of receptor preparation; further 2 ml samples of the same preparation were extracted after adding 0.25 ng and 1.00 ng LH. Measurement of LH after extraction of 1, 2 and 3 ml of receptor preparation gave 0.24, 0.54 and 0.75 ng LH respectively (correlation coefficient, $r = 0.99$; 6 determinations). Recovery of LH added to receptor preparations or buffer was 88% ($n = 4$). Occupied receptor levels were not determined in Day-18 untreated and Day-24 or -27 oestradiol benzoate-treated gilts, since limited amounts of luteal extract were prepared from the regressed corpora lutea of these animals.

Hormone assays. Plasma progesterone concentrations were determined by radioimmunoassay as described by Newcomb, Booth & Rowson (1977). Intra- and inter-assay coefficients of variation were 7.1 and 11.1% respectively, and the limit of sensitivity was 1 ng/ml. Plasma LH was measured by radioimmunoassay as described by Niswender, Reichert & Zimmerman (1970), using porcine LH (LH-GPZ-1, potency $0.63 \times \text{NIH-LH-S1}$; Ziecik, Goralska, Krzymowski & Pogorzelski, 1978) as standard and tracer. LH was measured in plasma from each experiment in one assay; intra-assay coefficients of variation for Exps 1 and 2 were 9.1 and 8.4% respectively. Parallelism was determined by assaying 50 μl , which gave 0.15 ng, 100 μl (0.33 ng) and 200 μl (0.54 ng) of pooled plasma; recovery of added LH was 86%. Sensitivity of the assay was 0.2 ng/ml.

Statistical analyses. In Exp. 1, data were first analysed by a one-way analysis of variance, before a 2×3 factorial analysis of variance was carried out to compare untreated animals on Days 10, 12 and 15 with treated animals on Days 15, 18 and 21 after oestrus. Data in Exp. 2 were analysed by 2×2 factorial analysis of variance.

Results

Experiment 1: effects of oestradiol in intact pigs

Corpora lutea were maintained approximately 6 days longer in oestradiol benzoate-treated gilts than in untreated animals, as indicated by gross morphological appearance, weight and peripheral plasma progesterone concentrations at slaughter (Table 1). Luteal regression was evident on Day 18 in control animals and on Days 24 and 27 in treated gilts.

One-way analysis of variance was used to compare variables measured in untreated gilts on Day 18 with those on Days 10, 12 and 15; in treated gilts on Days 24 and 27 with those on Days 15, 18 and 21; and in untreated gilts on Day 18 with those of treated gilts on Days 24 and 27. Mean corpus luteum weight and level of unoccupied LH receptors, and mean concentration of progesterone in plasma all decreased at luteal regression ($P < 0.001$; Table 1), and no significant differences were found when Day-18 untreated gilts were compared with Day-24 and -27 treated gilts (except that mean corpus luteum weight was greater, $P < 0.05$, for Day-18 controls). The only other significant difference was that plasma LH concentrations were raised ($P < 0.01$) in treated animals on Day 27 compared with earlier days, probably because the animals were approaching oestrus.

Data were subsequently analysed to compare untreated animals on Days 10, 12 and 15 with treated gilts on Days 15, 18 and 21. There were no significant differences in the number of corpora lutea or the mean plasma LH concentrations between treatment groups. However, mean

Table 1. Number and weight of corpora lutea, luteal levels of occupied and unoccupied LH receptors, and concentration of LH and progesterone in jugular venous plasma of non-pregnant pigs during the oestrous cycle before and after treatment with oestradiol benzoate on Day 12 of the cycle

Treatment	Group	Day of cycle at slaughter	No. of animals	No. of CL/pig	Mean wt of CL (mg)	Receptor conc. (fmol/mg protein)		Hormone conc. at slaughter (ng/ml)	
						Unoccupied receptors	Occupied receptors	LH	Progesterone
Control	1	10	5	16.2 ± 2.0	433 ± 27	11.8 ± 1.30	4.34 ± 0.34	3.1 ± 0.5	24.7 ± 2.0
	2	12	5	14.0 ± 0.9	486 ± 19	12.2 ± 3.13	5.77 ± 0.80	1.7 ± 0.1	22.4 ± 1.8
	3	15	5	13.8 ± 1.7	410 ± 42	11.5 ± 1.44	3.93 ± 0.33	2.1 ± 0.1	15.6 ± 3.8
	4	18	5	14.4 ± 2.0	241 ± 62	2.56 ± 0.87	—	2.1 ± 0.1	2.1 ± 1.5
Oestradiol	5	15	5	14.2 ± 0.7	399 ± 26	32.8 ± 7.90	5.10 ± 0.68	1.9 ± 0.1	18.5 ± 1.7
	6	18	5	13.6 ± 1.2	401 ± 45	34.0 ± 3.43	3.91 ± 0.76	1.6 ± 0.3	18.1 ± 2.2
	7	21	5	12.4 ± 1.3	347 ± 46	28.4 ± 5.73	2.69 ± 0.48	1.9 ± 0.3	12.9 ± 3.8
	8	24	5	15.6 ± 0.8	186 ± 53	6.2 ± 4.11	—	1.9 ± 0.1	1.4 ± 0.9
	9	27	4	13.0 ± 0.9	114 ± 27	1.4 ± 0.24	—	5.6 ± 1.5	<1

Values are means ± s.e.m.

corpus luteum weight and plasma progesterone concentrations were reduced ($P < 0.05$) in oestrogen-treated animals, and progesterone levels decreased significantly ($P < 0.05$) with time in both groups. There were no significant interactions between treatment and time for any of the variables tested.

Unoccupied LH receptor levels were raised 2- to 3-fold by oestrogen administration, and remained elevated for 3–9 days after treatment. Dissociation constants for binding of LH to unoccupied receptors increased slightly ($P < 0.01$) after oestrogen treatment (means: 2.4 and 2.8×10^{-10} M in Groups 1 and 2 compared with 5.0 and 5.8×10^{-10} M in Groups 5 and 6 respectively). Occupied receptor levels were not affected by treatment and the percentage of sites occupied decreased ($P < 0.01$) after oestrogen treatment, reflecting the increased number of unoccupied sites; there were 29.8 ± 3.0 and $11.6 \pm 1.3\%$ of sites occupied in untreated and oestradiol benzoate-treated gilts, respectively. Receptor occupancy declined ($P < 0.05$) with time after treatment.

Experiment 2: effects of oestradiol in hysterectomized pigs

Preliminary experiments with 2 hysterectomized gilts (data not shown) indicated, from gross appearance, weight and peripheral plasma progesterone concentrations, that corpora lutea were maintained until Day 30 after oestrus following hysterectomy on Days 6–9. Of the 10 gilts in the hysterectomized–untreated and hysterectomized–oestrogen-treated groups, corpora lutea were maintained in all except one animal, in which plasma progesterone had declined to below 1 ng/ml. Corpora lutea were maintained in all intact, oestrogen-treated gilts.

Hysterectomy had no effect on any characteristic examined; levels of unoccupied and occupied receptors and the dissociation constants of unoccupied receptors were similar for untreated intact and hysterectomized gilts (Table 2). There were no significant interactions between hysterectomy and oestrogen treatment. Similarly, the number of corpora lutea, total luteal weight and concentrations of LH and progesterone were not affected by oestrogen treatment. However, the concentration of unoccupied LH receptors was increased ($P < 0.01$) more than 2-fold after oestrogen treatment in intact and hysterectomized gilts. Levels of occupied receptors on the other hand were not affected by treatment, and 37.5 ± 5.1 and $20.1 \pm 3.6\%$ of receptors were occupied by LH in untreated and oestrogen-treated gilts, respectively ($P < 0.01$). The dissociation constant for unoccupied receptors increased ($P < 0.05$) after oestrogen treatment.

Table 2. Number and weight of corpora lutea, level of occupied and unoccupied luteal LH receptors and concentrations of LH and progesterone in jugular venous plasma of non-pregnant pigs after hysterectomy and/or treatment with oestradiol benzoate on Day 12 of the cycle

	Group 1 (control)	Group 2 (oestradiol treated)	Group 3* (hysterectomized)	Group 4* (hysterectomized + oestradiol)
Day of cycle at slaughter	10–12	15–18	15–18	15–18
No. of CL/pig	13.8 ± 1.3	14.0 ± 0.7	14.8 ± 0.9	14.2 ± 0.6
Mean wt of CL (mg)	462 ± 28	352 ± 8	452 ± 16	398 ± 54
Level of receptors (fmol/mg protein)				
Unoccupied	17.7 ± 0.64	37.8 ± 8.99	17.0 ± 5.83	34.5 ± 6.00
Occupied	6.54 ± 0.77	5.85 ± 0.67	5.75 ± 1.01	5.80 ± 0.24
Plasma hormone conc. at slaughter (ng/ml)				
LH	2.11 ± 0.23	2.68 ± 0.27	2.74 ± 0.64	2.14 ± 0.29
Progesterone	33.8 ± 2.4	27.4 ± 2.9	28.0 ± 7.2	30.3 ± 1.5
Dissociation constants of unoccupied receptors (M × 10 ⁻¹⁰)	2.1 ± 0.21	3.3 ± 0.27	1.9 ± 0.10	2.9 ± 0.12

Values are mean ± s.e.m. for 5 animals/group.

* Hysterectomy performed on Days 6–9 of the cycle.

Discussion

Oestradiol benzoate given as a single injection on Day 12 after oestrus extended the life-span of the corpora lutea in non-pregnant gilts in the present study, as reported previously. Prolongation of luteal function was associated with an increased level of unoccupied LH receptor in the corpora lutea which occurred within 3 days of treatment and was maintained for up to 9 days. The proportion of receptors occupied by LH was similar to that reported in the sheep corpus luteum (Suter, Fletcher, Sluss, Reichert & Niswender, 1980).

Increased LH receptor levels coincided with luteal maintenance in oestrogen-treated animals, although it is not certain that this was the cause of the prolongation of luteal activity. Maintenance of the corpora lutea is dependent on LH in the pig from about Day 14 after oestrus (du Mesnil du Buisson, 1966, 1973; Anderson *et al.*, 1967; Spies, Slyter, & Quadri, 1967) and therefore it might be assumed that luteal function required LH during the period of oestrogen-stimulated progesterone secretion after Day 15. This would be consistent with the lack of effectiveness of oestrogens in maintaining luteal function after hypophysectomy (du Mesnil du Buisson, 1966) or pituitary stalk section (Anderson *et al.*, 1967). However, the proportion of receptors occupied by LH, which presumably reflects the action of LH on the tissue (Suter *et al.*, 1980), was reduced during the period of prolonged luteal function suggesting that the relationship between LH receptor level and luteal activity is complex.

PGF-2 α is luteolytic in pigs when given late in the cycle, and in other species leads to loss of luteal LH receptors (Grinwich, Ham, Hichens & Behrman, 1976a; Grinwich, Hichens & Behrman, 1976b). Since oestrogens reduce uterine PGF-2 α release (see 'Introduction'), the rise in LH receptor concentrations in response to oestrogen could reflect, at least in part, the reverse of this effect. But the present experiments have raised the possibility that oestrogen may also have a direct effect on the corpus luteum, since unoccupied LH receptor concentrations were elevated in hysterectomized animals. A direct effect would be consistent with the stimulatory actions of oestrogen on progesterone production by porcine granulosa cells in culture (Goldenberg, Bridson & Kohler, 1972; Veldhuis, Klase & Hammond, 1981) and of stilboestrol on binding of LH by isolated porcine granulosa cells (Nakano, Akahori, Katayama & Tojo, 1977). These two mechanisms (via the uterus and direct) are not necessarily mutually exclusive and both may be involved in maintaining luteal function in early pregnancy. An effect mediated through reduced

uterine PGF-2 α production might be expected to precede any action exerted directly in view of the small amounts of oestrogen secreted by the conceptus into the systemic circulation before Day 12 of gestation, the time of the maternal recognition of pregnancy (Dhindsa & Dziuk, 1968; Ford *et al.*, 1982) and the intimate spacial relationship between the conceptus and the endometrium. This sequence would be consistent with the later rise in luteal LH receptor observed between Days 20 and 30 of gestation (Ziecik *et al.*, 1980), which coincides with the peak in circulating oestrone sulphate levels in early pregnancy (Robertson & King, 1974; Heap *et al.*, 1981). Investigations of the luteotrophic effects of embryo extracts, which maintain luteal function in unilaterally pregnant pigs and retard PGF-2 α induced luteal regression when administered into the uterine lumen (Longenecker & Day, 1972; Ball & Day, 1982a, b), also suggest a local action. The heat stability and removal by charcoal of the active principle in such extracts (Ball & Day, 1982a) are consistent with it being an oestrogen.

An alternative mechanism by which oestrogens may raise luteal LH receptor levels without involving the uterus is through effects on circulating concentrations of pituitary hormones. Levels of the LH receptor are raised by prolactin in rat corpora lutea (Grinwich *et al.*, 1976b; Gibori & Richards, 1978), but prolactin is not luteotrophic in the pig during the cycle (du Mesnil du Buisson, 1973). Suter *et al.* (1980) have demonstrated in sheep that administration of LH reduces luteal LH receptor levels (by 'down regulation') for 2–24 h; a fall in LH might be expected to result in a rise in LH receptor concentration in the corpus luteum by the inverse of this effect. A drop in the circulating concentration of LH would be in agreement with the decline in receptor occupancy found in the present study after oestrogen treatment. However, there are several pieces of evidence which are inconsistent with such an indirect action of oestrogen. Plasma LH levels at slaughter were unchanged by oestrogen in the present study. In a separate experiment, administration of oestradiol to mature cyclic pigs on Day 12 after oestrus had no effect on LH concentrations in samples obtained daily thereafter, although a decline in LH was noted after treatment on Days 3 or 7, and a rise was observed after treatment on Day 16 (C. Polge & H. D. Guthrie, unpublished observations). Furthermore, luteal LH receptor levels in the present study were raised for at least 9 days after oestrogen administration, which is considerably longer than the time course of the effect of LH observed in sheep (Suter *et al.*, 1980). Lastly, administration of antiserum to LH, which presumably lowers available plasma LH, causes luteal regression (Spies *et al.*, 1967), whereas treatment with oestrogen prolongs luteal function. It is therefore concluded that oestrogens may have a direct effect on the corpus luteum in the pig.

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