Direct luteotrophic effect of oestradiol-17 β on pig corpora lutea*

A. J. Conley† and S. P. Ford

Department of Animal Science, Iowa State University, Ames, IA 50011, USA

Summary. Five gilts each received intraluteal implants releasing about 4.4 ± 1.1 (Group 1), 15.0 ± 1.1 (Group 2) or 22.4 ± 1.0 (Group 3) μg oestradiol/day as determined by in-vitro incubation of implants of similar weight and oestradiol content. On Day 11 of the oestrous cycle (Day 0 = first day of oestrus), 3 CL in one ovary received oestradiol implants, 3 CL in the other ovary received vehicle implants, and all other CL in both ovaries served as uninjected control CL. An additional group of 6 animals served as controls and included 4 animals receiving bilateral vehicle implants (3 CL per ovary) on Day 11 and 2 unoperated gilts. All animals were slaughtered on Day 19 of their oestrous cycle, and the weight, progesterone content and concentration of each CL were determined. In Group 3 gilts, luteal weight, progesterone content and concentration were greater by 68.7 ± 24.0 mg, 6.54 ± 1.33 μg and 7.54 ± 2.00 ng/ml respectively ($P < 0.01$) in oestradiol-implanted CL than in vehicle-implanted CL, which appeared to be similar to uninjected control CL. No differences were seen between oestradiol-17 β and vehicle-treated CL in CL from gilts in Groups 1 or 2. All CL of Group 3 gilts were heavier and contained a greater content and concentration of progesterone ($P < 0.01$) than gilts in Groups 1 and 2, and in controls for which the values were similar. These results demonstrate that exogenous oestradiol can directly stimulate the growth of an individual CL; however, the oestradiol release rate must be high enough to maintain all CL bilaterally before this effect is seen.

Keywords: corpus luteum; oestradiol-17 β ; luteotrophin; progesterone; pigs

Introduction

Luteal maintenance in pigs is dependent on continued pituitary luteotrophic support (Anderson *et al.*, 1967) and the abrogation of the luteolytic influence of the uterus (Anderson *et al.*, 1965). Kidder *et al.* (1955) first reported that exogenous oestrogen extended luteal function in pigs, and since then, many studies have investigated the mechanism involved in this effect. Bazer *et al.* (1986) reviewed data that indicate that oestrogen decreases the release of prostaglandin (PG) F-2 α into the uterine vein, thereby reducing the luteolytic signal, but oestrogen may also have a direct effect on corpora lutea (CL) in the pig. It has been reported that oestrogen prevented PGF-2 α -induced luteolysis in hysterectomized gilts (Kraeling *et al.*, 1975) and increased luteal LH receptor numbers in pregnant and hysterectomized animals (Garverick *et al.*, 1982). Neither these nor other experiments performed so far, however, have been able to exclude the possibility of oestrogen-induced changes in pituitary luteotrophic support (Chakraborty *et al.*, 1972) or uterine-mediated effects in the same animal. In addition, most experiments to date have utilized high doses of oestrogen without regard to what might be a minimally effective dose by the route of administration chosen.

*Reprint requests to Dr S. P. Ford.

†Present address: Department of Obstetrics and Gynecology, University of Texas Health Science Center, 5323 Harry Hines Boulevard, Dallas, TX 75235, USA.

Therefore, the following experiment was performed to investigate the direct luteotrophic effect of oestradiol-17 β in gilts.

Materials and Methods

Experimental protocol. Fifteen gilts, 6–8 months of age weighing 120–150 kg, which had exhibited two oestrous cycles of normal duration (19–21 days), were assigned to surgery on Day 11 (Day 0 = first day of oestrus). Anaesthesia was induced with sodium thiamylal (Surital, Parke-Davis, Morris Plains, NJ, USA) and maintained with halothane (Halocarbon Laboratories, Inc., Hackensack, NJ, USA) and oxygen (Magness & Ford, 1982). Ovaries were exposed by midventral laparotomy, and numbers of CL were recorded. Three randomly selected CL in one ovary received oestradiol implants, and 3 CL in the opposite ovary received vehicle (Silastic only) implants. All other remaining CL in each ovary were left as uninjected control CL. Therefore, within each gilt, 4 sets of CL could be recognized, consisting of oestradiol-implanted and uninjected control CL in one ovary and vehicle-implanted and uninjected control CL in the other (Fig. 1). Each injected CL was marked with 3–0 silk for identification, and ovaries were replaced in the body cavity. At a subsequent laparotomy on Day 19, all gilts were ovariectomized. All CL were dissected from the ovaries and weighed. Implanted CL were weighed before and after implant removal. Each CL was then homogenized separately in saline (0.9% w/v NaCl) and stored at -70°C until analysed for progesterone by radioimmunoassay.

Fig. 1. Diagram of placement of intraluteal oestradiol-17 β and vehicle implants in gilts.

Control animals. Four animals were used to investigate the effects of bilateral vehicle implants on luteal weight, progesterone content and concentration. Procedures were identical to those described above, except that vehicle implants were placed into 3 CL in each ovary of each animal. Two additional gilts were slaughtered at Day 19 of the oestrous cycle as nonsurgical controls.

Implant preparation. Silastic compound was prepared by mixing equal quantities of Medical Grade Elastomer #382 and Medical Fluid #360 (Dow Corning Corporation, Midland, MI, USA) with catalyst (stannous octoate, Dow Corning) in an ethanol-rinsed test tube, which resulted in curing of the compound within 3 min. During this period, the compound could be drawn into a 1-ml disposable syringe and remained fluid enough to pass through an 18-gauge needle. This allowed the injection of 25–50 μl of the Silastic compound into individual CL exposed at surgery and the subsequent formation *in situ* of solid intraluteal implants weighing ≈ 34 mg. Oestrogen implants were prepared by combining crystalline oestradiol-17 β (Sigma Chemical Company, St Louis, MO, USA) with Silastic compound at concentrations of 5, 15 and 50 mg oestradiol-17 β /ml of compound (Groups 1, 2 and 3, respectively). Implants of 34 mg were estimated to contain a total of about 150, 500 and 1500 μg oestradiol, respectively.

Implant release rate in vitro. Despite obvious differences between in-vivo and in-vitro conditions including temperature, surrounding fluid composition and hormonal milieu, release rate of oestradiol-17 β from implants of similar weight and hormone content to those used *in vivo* was estimated in the following manner. Implants, for each amount of oestradiol, were incubated at 37°C in 2 ml plasma from a pig castrated 7 days earlier. Plasma was changed daily, frozen and stored at -70°C until assayed for oestradiol by radioimmunoassay. All samples were included in a single assay. The slope of the line of release rate of oestradiol-17 β plotted against time was not different from zero by regression analysis ($P > 0.5$) at any amount of oestradiol, indicating that release was constant over the 7-day incubation period. Implants containing 150, 500 and 1500 μ g oestradiol released hormone at different rates 4.4 ± 1.1 , 15.0 ± 1.1 and 22.4 ± 1.0 μ g/day respectively (main effect of amount of oestradiol $P < 0.01$).

Radioimmunoassays. The progesterone and oestradiol radioimmunoassays were run as previously described and validated in this laboratory for pig luteal tissue (Ford & Christenson, 1979) and plasma (Magness & Ford, 1982). Sensitivity of these assays was defined as that amount of steroid that yielded 95% of the c.p.m. in the buffer control tubes; this amount was 2 pg and 50 pg for oestradiol and progesterone, respectively. Recovery rates averaged over 90% for both steroids. A single assay for oestradiol-17 β included all samples and plasma pools which measured 13.7 ± 5.1 pg/ml with an intra-assay coefficient of variation of 9.2%. All samples from individual gilts were assayed for progesterone together. Progesterone pools measuring 27.22 ± 4.60 ng/ml yielded intra- and inter-assay coefficients of variation of 11.3 and 10.0%.

Statistical analysis. Arithmetic means were calculated for each set of CL within each gilt (Fig. 1). The variable number of remaining uninjected control CL among gilts resulted in greatly unbalanced data, so only means and standard errors have been included in tables for comparison. Differences between oestradiol- and vehicle-implanted CL were evaluated by analysis of variance of a completely randomized split plot in which dose of oestradiol was the main plot, and luteal treatment (oestradiol or vehicle implants) was the subplot. Within-group differences were examined further by paired *t* test. The in-vitro release of oestradiol from implants was analysed by one-way analysis of variance to determine the differences among doses, and linear regression was conducted to test linear and quadratic trends of release over time. Vehicle implant effects on luteal weight, progesterone content and concentration of control sows and gilts with bilateral vehicle implants were tested by analysis of variance for a randomized block for which animals were considered to be blocks.

Results

Weight, progesterone content and concentration of all CL were significantly elevated ($P < 0.01$) in Group 3 gilts when compared with those in either of the other two groups, which were similar (Table 1). Significant ($P < 0.01$) group by treatment interactions were obtained for luteal weight, progesterone content and concentration. By paired *t* test oestradiol-implanted CL were heavier (68.7 ± 24.0 mg, $P < 0.05$) and had higher progesterone content (6.54 ± 1.33 μ g, $P < 0.01$) and concentration (7.54 ± 2.00 ng/mg, $P < 0.01$) in Group 3 gilts. No differences between oestradiol- and vehicle-implanted CL were seen for luteal weight, progesterone content or concentration in gilts in either Group 2 (-7.6 ± 13.3 mg, -0.75 ± 0.34 μ g and -4.54 ± 1.69 ng/mg, oestradiol versus vehicle, $P > 0.05$) or Group 1 (-10.3 ± 9.5 mg, -0.13 ± 0.05 μ g and -0.40 ± 0.52 ng/mg, oestradiol versus vehicle, $P > 0.05$). The slightly higher means and the increased variation seen in luteal characteristics in Group 2 gilts were due to a single animal, gilt 2404, in which CL were maintained. In this gilt, luteal weights, progesterone contents and concentrations for oestradiol- and vehicle-implanted CL were 353.6 vs 333.8 mg, 13.93 vs 15.92 μ g and 39.39 vs 47.69 ng/mg, respectively.

When a frequency distribution of luteal weight was compiled (Fig. 2) among Groups 1, 2 and 3, a bimodal distribution was obtained, suggesting that CL were either maintained (Group 3) or not (Groups 1 and 2). No linear effect of level of oestradiol on CL was obtained. Except for Gilt 2404 in Group 2, all CL weighing over 200 mg were obtained from Group 3 gilts (Fig. 2). In addition, ovaries of gilts in Groups 1 and 2 consistently contained populations of 8–12-mm follicles, suggestive of the approach of oestrus. Follicular development in ovaries from Group 3 gilts, however, consisted exclusively of 3–6-mm follicles, consistent with the presence of functional CL. There were no consistent differences in weight or progesterone content or concentration between vehicle-implanted CL and uninjected CL in either ovary within groups of gilts (Table 1).

In control pigs, there were no differences between vehicle-implanted and unimplanted CL in luteal weight, progesterone content or concentration, which averaged 152.8 mg, 1.35 μ g and

Table 1. Means $\pm$ s.e.m. of luteal weight (minus implant weight), luteal tissue progesterone (P_4) content and concentration of groups of CL within gilts given intraluteal oestradiol-17 β (E_2) implants‡

Ovary	CL group†	Luteal characteristic	Treatments*		
			Group 1 (4 μ g E_2 /day) (N = 5)	Group 2 (15 μ g E_2 /day) (N = 5)	Group 3 (23 μ g E_2 /day) (N = 5)
1	Oestradiol implanted	Weight (mg)	119.9 $\pm$ 10.9	164.7 $\pm$ 47.7	345.0 $\pm$ 20.7 ^a
		P_4 content (μ g)	0.4 $\pm$ 0.2	3.0 $\pm$ 2.7	23.0 $\pm$ 2.7 ^a
		P_4 conc. (ng/mg)	3.3 $\pm$ 1.3	10.1 $\pm$ 7.4	66.2 $\pm$ 5.5 ^a
2	Vehicle-implanted	Weight (mg)	130.2 $\pm$ 5.5	172.3 $\pm$ 43.4	276.4 $\pm$ 18.7 ^b
		P_4 content (μ g)	4.8 $\pm$ 0.1	3.8 $\pm$ 3.0	16.5 $\pm$ 2.2 ^c
		P_4 conc. (ng/mg)	3.7 $\pm$ 0.9	14.7 $\pm$ 8.6	58.7 $\pm$ 5.6 ^c
1	Unimplanted control	Weight (mg)	103.3 $\pm$ 9.4	151.0 $\pm$ 41.0	278.6 $\pm$ 19.1
		P_4 content (μ g)	0.4 $\pm$ 0.2	2.5 $\pm$ 2.2	18.4 $\pm$ 1.9
		P_4 conc. (ng/mg)	3.7 $\pm$ 1.6	9.4 $\pm$ 6.9	65.6 $\pm$ 4.7
2	Unimplanted control	Weight (mg)	92.3 $\pm$ 6.9	139.8 $\pm$ 51.9	266.4 $\pm$ 14.3
		P_4 content (μ g)	0.4 $\pm$ 0.1	3.5 $\pm$ 2.9	18.4 $\pm$ 1.6
		P_4 conc. (ng/mg)	4.1 $\pm$ 1.1	15.5 $\pm$ 8.6	68.9 $\pm$ 3.7

*Luteal characteristics of Group 3 gilts were significantly different ($P < 0.05$) from those of Group 1 and Group 2, which were similar.
†Unimplanted control CL from Ovary 1 and Ovary 2 not included in statistical analysis.
‡For each luteal tissue characteristic, means within a column differ: ^{a,b} $P < 0.05$; ^{a,c} $P < 0.01$.

Fig. 2. Frequency distribution of weights of individual CL collected from oestradiol-treated gilts in Groups 1, 2 and 3 on Day 19 of the oestrous cycle after placement of intraluteal implants on Day 11. The proportion of the height of each bar contributed to by each group is shown as stippled or cross-hatched. Data include implanted (minus implant weight) and unimplanted CL.

9.64 ng/mg, respectively ($P > 0.05$). These values were similar to those observed in the 2 un-operated control gilts for which average luteal weight, progesterone content and concentration were 115.5 mg, 0.55 μ g and 4.57 ng/mg, respectively. As for gilts in Groups 1 and 2, ovarian follicular development indicated approaching oestrus. Thus, in comparing the luteal characteristics of gilts in Groups 1, 2 and 3 with those receiving bilateral vehicle implants and non-surgical control gilts, with the exception of Gilt 2404, only CL in Group 3 gilts appeared to have been maintained.

Discussion

This study is the first to demonstrate *in vivo* a direct luteotrophic effect of oestrogen on pig CL. The results show that intraluteal implantation of oestradiol selectively increased weight and progesterone content of those CL when compared with other CL in the same ovary or in the opposite ovary in gilts exhibiting luteal maintenance. Luteal hypertrophy is detectable by Day 24–26 after oestrus in pregnant and hysterectomized gilts that have been unilaterally ovariectomized early in the luteal phase (Rathmacher *et al.*, 1967; Staigmiller *et al.*, 1972, 1974). Because compensatory ovarian hypertrophy in the pig is accompanied by increases in follicular size and ovarian venous oestrogen concentrations (Redmer *et al.*, 1984), it is tempting to speculate that a similar local exposure of CL to these increases in oestrogen may result in an increase in luteal weight. Although no changes in systemic LH concentrations occur after unilateral ovariectomy (Redmer *et al.*, 1984) or during oestradiol benzoate-induced pseudopregnancy (Flowers *et al.*, 1987), Garverick *et al.* (1982) reported that oestradiol increased LH receptor levels in CL of pregnant and hysterectomized gilts. Therefore, it is possible that the increase in luteal weight observed in the present study may be due in part to increased LH sensitivity and response, perhaps through increased luteal LH receptor populations. Whether this reflects hyperplasia or simply luteal hypertrophy is not known; however, both LH and hCG have been shown to induce increases in luteal cell size in mid-luteal-phase ewes (Farin *et al.*, 1988).

Two other important points emerged from the present study. Firstly, a minimally effective luteotrophic dose of intraluteal oestradiol was determined which, even when administered unilaterally, still resulted in bilateral luteal maintenance. Saunders *et al.* (1983) did not observe any increased potency of oestradiol in inducing luteal maintenance when 100 μ g oestradiol/day were infused into the uterine lumen as compared with the same daily treatment administered subcutaneously. However, Ford *et al.* (1982a) demonstrated bilateral luteal maintenance and reduced utero-ovarian venous PGF-2 α concentrations in gilts after intrauterine infusion of just 1.5 μ g oestradiol/day. Therefore, it is unlikely that Saunders *et al.* (1983) could have detected any such sensitivity difference at the levels of oestradiol used in that study even though they were about 2% of those commonly used to induce pseudopregnancy in pigs (Geisert *et al.*, 1987).

The minimally effective luteotrophic dose of oestradiol used in the present study (67 μ g/ovary/day) was also much higher than that found to be necessary to stimulate CL function when administered by an intrauterine route (Ford *et al.*, 1982a). Therefore, it seems probable that the systemic effect of oestradiol, inducing bilateral luteal maintenance in this study, is due to an effect on the uterus, possibly through a bilateral reduction of PGF-2 α release into the utero-ovarian vein such as that noted by Ford *et al.* (1982a) and as first described by Frank *et al.* (1977). This supports the conclusions of Bazer *et al.* (1986) that the primary effect of oestrogen that results in the maintenance of luteal function in pigs is a decrease of the luteolytic signal.

At present, there seem to be few published reports refuting the notion that oestrogen maintains luteal function by an effect on the uterus. However, some evidence does suggest that the embryo may exert a direct luteotrophic effect. This evidence includes the high incidence of unilateral maintenance (Christenson & Day, 1971) and elevated progesterone concentrations (Ford & Christenson, 1979) of CL adjacent to the gravid horn in unilaterally pregnant pigs. In addition, Ford *et al.* (1982b) observed transient increases in luteal blood flow in gilts at the time of maternal recognition of pregnancy. More importantly, Ball & Day (1982) used intraluteal implants containing pig embryonic extract to maintain the function of individual CL in an ovary. In contrast to the results of the present study, no systemic luteotrophic effect seemed to be necessary to elicit this response, suggesting that embryonic extract directly prevented the luteolytic effects of the uterus at the level of the CL. Furthermore, the active principle(s) was heat-stable and charcoal-extractable, indicating that it was of low molecular weight, possibly a steroid. The results obtained in the present study would suggest that, if involved, oestradiol is not the only luteotrophic factor produced by the pig embryo. The identity of additional factors such as prostaglandin E-2 (Akinlosotu *et al.*, 1986) as embryonic luteotrophins remains to be determined.

The second important point to emerge from these studies is that the results clearly demonstrate the all-or-none effect of oestradiol on luteal maintenance (Fig. 2) previously described by Gardner *et al.* (1963). Similarly, an identical study to that reported here was performed in sows and included the same oestradiol treatments plus an additional higher level, but still no linear effect of level of oestradiol exposure of CL was seen for any luteal characteristic measured (Conley & Ford, 1988). Whether or not Gilt 2404 maintained CL in response to the implanted oestrogen is conjectural. If so, this gilt may not have received sufficient oestradiol to elicit any of the direct effects seen in Group 3 gilts. None the less, in this gilt, the luteal characteristics measured indicated bilateral luteal maintenance similar to that seen overall in CL from Group 3 gilts, again supporting an all-or-none systemic, rather than a graded local effect of oestradiol-17 β in the prevention of luteal regression in pigs. At present, no explanation can be offered for why no graded response is seen.

Finally, the results of this study suggest that implantation of CL as described had no measurable effect on luteal weight, progesterone content or concentration. It was suggested by Ball & Day (1982) that the implantation procedures utilized in their study may have caused some luteal damage. Luteal implants used in the present study weigh 10% of average CL weight, but still release adequate levels of oestradiol to maintain luteal function, and release rate is relatively constant. Therefore, this procedure seems to provide an effective means by which to investigate the effects of local exposure of CL to steroids and possibly other compounds on pig luteal function.

In conclusion, this study demonstrates both direct and indirect effects of oestradiol on luteal maintenance in pigs. Further studies will be necessary to determine the physiological relevance of the local exposure of CL to oestradiol and other blood-borne factors released by the porcine conceptus.

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