Direct effects of oestradiol-17 β and prostaglandin E-2 in protecting pig corpora lutea from a luteolytic dose of prostaglandin F-2 α

S. P. Ford* and L. K. Christenson

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

Summary. Implants containing vehicle or oestradiol-17 β (10 mg) were placed into pairs of corpora lutea (CL) with and without prostaglandin F-2 α (PGF-2 α) (100 μ g) on Day 11 and CL were collected on Day 19, in cyclic gilts (Exp. 1). The results demonstrated that CL implanted with PGF-2 α with or without oestradiol-17 β had a markedly lower ($P < 0.01$) weight (mg) and progesterone concentration (ng/mg) than CL with vehicle- or oestradiol-17 β -implanted or unimplanted CL, which were similar (149 and 7.2 vs. 304 and 49.6, respectively). In Exp. 2, CL implanted with vehicle, oestradiol-17 β or PGE-2 remained fully functional until Day 19, whereas CL implanted with oestradiol-17 β $\pm$ PGF-2 α and PGE-2 + PGF-2 α exhibited lower ($P < 0.05$) weight and progesterone concentrations; CL implanted with PGE-2 + PGF-2 α were heavier ($P < 0.05$) and tended ($P < 0.10$) to have greater progesterone concentrations than CL implanted with oestradiol-17 β + PGF-2 α . In Exp. 3, a dose-dependent ($P < 0.05$) effect of PGE-2 on preventing regression induced by PGF-2 α was observed on Day 19. These data demonstrate a direct effect of PGE-2, but not of oestradiol-17 β in protecting the CL against luteolysis induced by PGF-2 α .

Keywords: oestradiol-17 β ; prostaglandin E-2; prostaglandin F-2 α ; intraluteal implants; corpus luteum; pig

Introduction

Evidence for a direct effect of an embryonic luteotrophin is provided by the observed high incidence of unilateral maintenance (Anderson, 1966; Niswender *et al.*, 1970; Christenson & Day, 1971) and high progesterone concentrations (Ford & Christenson, 1979) of corpora lutea (CL) adjacent to the gravid uterine horn in unilaterally pregnant pigs. Additional direct evidence of embryonic factors preventing the luteolytic effect of prostaglandin F-2 α (PGF-2 α) in pigs at the level of the CL is provided by Ball & Day (1982). In their study, luteal implants containing embryonic extracts and PGF-2 α were placed in individual CL on Days 30–35 of pregnancy. Implants with both embryonic extract and PGF-2 α were heavier and contained more progesterone than those containing PGF-2 α alone, which suggests that embryonic factors directly prevented the luteolytic response. Furthermore, Ball & Day (1982) observed that charcoal extraction of embryonic homogenates removed the luteotrophic factor(s), which indicated a compound of low M_r , such as a prostaglandin or steroid.

Pig embryos produce both oestradiol-17 β (Perry *et al.*, 1976) and prostaglandin E-2 (PGE-2; Geisert *et al.*, 1982a) at the time of maternal recognition of pregnancy (Days 11–13) and both compounds are known to be luteotrophic in this species (Geisert *et al.*, 1987; Akinlosotu *et al.*, 1986). The mechanism or mechanisms whereby these compounds extend luteal function are unclear. It is generally believed that the luteotrophic effect of oestrogen in pigs is indirect, resulting

*Reprint requests.

from a reduced uterine release of PGF-2 α into the peripheral circulation (Bazer *et al.*, 1986; Conley & Ford, 1989; Conley *et al.*, 1989). Prostaglandin E-2 has been shown to antagonize directly the luteolytic effects of exogenously administered PGF-2 α in ewes when both compounds are administered simultaneously into the ovarian artery (Henderson *et al.*, 1977) or into the mesovarium around the ovarian vascular pedicle (Reynolds *et al.*, 1981). Similar studies in pigs have not been reported.

The studies reported here evaluated directly the effects of the known porcine conceptus secretory products oestradiol-17 β and PGE-2 in preventing the luteolytic effect of PGF-2 α on individual pig CL.

Materials and Methods

Animals. Yorkshire gilts ($n = 19$) 6–8 months old and 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 Surital (Park-Davis, Morris Plains, NJ, USA) and maintained with Halothane (Halocarbon Laboratories Inc., Hackensack, NJ) and oxygen (Magness & Ford, 1982).

Ovaries were exposed by midventral laparotomy and gilts were assigned to one of 3 experiments.

Experiment 1. Number of corpora lutea was determined on both ovaries of each gilt ($n = 4$) and an ovary with > 8 CL was selected to receive intraluteal silastic implants (treated ovary). Pairs of CL on the treated ovary were selected at random to receive implants containing (i) 10 mg oestradiol-17 β , (ii) 100 μ g PGF-2 α , (iii) 10 mg oestradiol-17 β + 100 μ g PGF-2 α or (iv) vehicle (silastic only). Pairs of implanted CL were then marked with different numbers of silk ligatures, depending upon treatment group. Ligatures were placed in the ovarian stroma between members of the pair. On the treated ovary, the CL in addition to the 8 implanted CL (1–3), as well as all CL on the other ovary, served as unimplanted controls.

Experiment 2. Two pairs of CL on each ovary of five gilts were used. Each pair was randomly assigned to receive intraluteal implants containing either (i) 10 mg oestradiol-17 β , (ii) 100 μ g PGE-2, (iii) 10 mg oestradiol-17 β + 100 μ g PGF-2 α or (iv) 100 μ g PGE-2 + 100 μ g PGF-2 α . An additional pair of CL, on the ovary with the greatest number of CL within each gilt, was selected to receive vehicle implants. All CL on each ovary not receiving implants served as unimplanted controls.

Experiment 3. The number of CL on each ovary of five gilts was determined and three pairs of CL on an ovary in each gilt with > 8 CL were selected to receive implants containing (i) 100 μ g PGE-2 + 100 μ g PGF-2 α , (ii) 200 μ g PGE-2 + 100 μ g PGF-2 α or (iii) 400 μ g PGE-2 + 100 μ g PGF-2 α . Four CL on the opposite ovary were selected to receive 10 mg oestradiol-17 β + 100 μ g PGF-2 α . All unimplanted CL on both ovaries served as controls.

After pairs of CL were marked for later identification, ovaries were returned to the body cavity, and gilts were allowed to recover. On Day 19, all gilts (Exps 1 to 3) were relaparotomized and their ovaries surgically removed. Corpora lutea on both ovaries of each gilt were immediately identified as to treatment group and excised from the ovaries. The weights of all CL were then recorded. Weights of implanted CL were recorded before and after the removal of the implant. Implant weights in these three studies were similar, averaging 50 ± 5 mg. All CL were individually homogenized and frozen at -20°C until determination of progesterone concentration by a specific radioimmunoassay.

Control animals. To confirm an effect of oestradiol-17 β on bilateral maintenance of CL at the dose used in these studies, two gilts subjected to sham surgery on Day 11, and three unoperated control gilts were slaughtered on Day 19. Corpora lutea were collected, weighed, and frozen as for treated gilts.

Implant preparation. Silastic compound was prepared by mixing equal quantities of Medical Grade Elastomer #382 and Medical Fluid #360 (Dow Corning Corporation, Midland, MI) with catalyst (stannous octoate: Dow Corning Corporation, Midland, MI) in a sterile tube containing hormone, which resulted in curing of the compound within 3 min. During this period, the compound remained fluid enough to pass through an 18-gauge needle, enabling the injection of 50 μ l of silastic compound into individual CL, and the in-situ formation of solid intraluteal implants. Implants containing oestradiol-17 β , PGE-2, PGF-2 α or combinations were prepared by combining 10 times the amount of crystalline hormone(s) required for each implant with silastic compound prior to addition of Medical Fluid and catalyst to a final volume of 0.5 ml.

Progesterone radioimmunoassay. Concentrations of progesterone in porcine luteal tissue were determined as described by Ford & Christenson (1979) and using the same fully characterized antisera (#GDN337). Sensitivity of the assay was defined as the amount of steroid that yielded 95% of the c.p.m. in the buffer control tubes, which averaged 50 pg. Intra- and interassay coefficients of variation for a luteal tissue pool ($27\,478 \pm 1\,126$ ng/mg; $n = 6$) were 9.1 and 10.0%, respectively.

Statistical analysis. Arithmetic means of CL weight and progesterone content and concentration were calculated for each set of implanted CL within a gilt, as well as all unimplanted CL on each ovary. These means were used to calculate treatment group means and for statistical comparisons. The effects of treatment on luteal weight and progesterone content and concentration were tested by analysis of variance for a randomized block in which animals served as blocks. Upon observation of a significant treatment effect ($P < 0.05$), treatments within a study were compared using Duncan's multiple range test.

Results

Experiment 1

Uninjected control CL were similar in weight and progesterone content and concentration on Day 19, regardless of location (treated or control ovary); thus, values were pooled within a gilt (Fig. 1). On the treated ovary, CL containing silastic implants releasing oestradiol-17 β were similar in weight and progesterone content and concentration to vehicle-implanted and unimplanted, control CL. Pairs of CL receiving implants containing PGF-2 α with or without oestradiol-17 β appeared to be regressing, as shown by their blanched appearance and their significantly ($P < 0.01$) lower weights and progesterone content and concentrations than those of the other CL groups. These CL implanted with PGF-2 α were similar in weight and in progesterone content and concentration to the CL of Day-19 surgical and nonsurgical control gilts, whose values were similar and averaged 112 ± 15 mg, 695 ± 172 ng, and 3.1 ± 0.2 ng/mg, respectively.

Fig. 1. Average weights ($\square$) and progesterone content (hatched) and concentrations (dotted) of pairs of corpora lutea (CL) collected on Day 19 of the oestrous cycle from gilts ($n = 4$) in Exp. 1. Values are means $\pm$ s.e.m. Within a measurement, means with different superscripts differ ($P < 0.01$).

Experiment 2

Changes in progesterone content of CL followed those of progesterone concentration. As observed in Exp. 1, uninjected, vehicle-injected and oestradiol-17 β -injected CL were similar in weight and in progesterone content and concentration (Fig. 2). Corpora lutea injected

Fig. 2. Average weights ($\square$) and progesterone concentrations ($\blacksquare$) of pairs of corpora lutea (CL) collected on Day 19 of the oestrous cycle from gilts ($n = 5$) in Exp. 2. Values are means $\pm$ s.e.m. Within a measurement, means with different superscripts differ ($P < 0.05$).

with PGE-2 alone were similar in weight and in progesterone content and concentration to CL injected with vehicle or oestradiol-17 β . Corpora lutea injected with PGF-2 α , in combination with oestradiol-17 β or PGE-2, had lower ($P < 0.05$) weights and progesterone content and concentrations than CL injected with oestradiol-17 β or PGE-2 alone. Corpora lutea injected with PGE-2 + PGF-2 α , however, had significantly greater ($P < 0.05$) weights and tended ($P < 0.10$) to have greater progesterone content and concentrations than CL injected with oestradiol-17 β + PGF-2 α .

Experiment 3

As observed in the previous studies, CL injected with oestradiol-17 β + PGF-2 α were lighter ($P < 0.01$) and had lower ($P < 0.01$) progesterone content and concentrations than uninjected control CL (Fig. 3); but PGE-2 protected the CL in a dose-dependent manner against reductions induced by PGF-2 α in weight and progesterone content and concentration. At a ratio of 4:1 (PGE-2:PGF-2 α), the weight of injected CL was equal to that of uninjected control CL; and these CL had greater ($P < 0.05$) progesterone content and concentrations than those injected with oestradiol-17 β + PGF-2 α .

Discussion

These studies are the first to investigate the specific effects of oestradiol-17 β and PGE-2 in protecting individual pig CL from the luteolytic effects of PGF-2 α . Because treatments were administered to individual CL, we eliminated any indirect effect(s) of oestradiol-17 β or PGE-2 at either the uterus or the hypothalamic-pituitary axis. These studies used intraluteal administration of oestradiol-17 β on Day 11, to maintain all CL bilaterally until Day 19 of the oestrous cycle, as

Fig. 3. Average weights (□) and progesterone content (▨) and concentration (▤) of pairs of corpora lutea (CL) collected on Day 19 of the oestrous cycle from gilts ($n = 5$) in Exp. 3. Values are means $\pm$ s.e.m. Within a measurement, means with different superscripts differ ($P < 0.05$).

reported by Conley & Ford (1989). The studies reported here used a dose of 40 mg (10 mg/CL in 4 CL) per gilt which is approximately four times the dose of oestradiol-17 β (1.5 mg/CL in 6.5 CL) previously shown (Conley *et al.*, 1989) to suppress PGF-2 α release from the uterus until Day 19 in gilts.

These studies demonstrate that, even at a ratio of 100:1, oestradiol-17 β was unable to protect a CL against the simultaneous intraluteal administration of PGF-2 α . These data support the conclusions of Bazer *et al.* (1986), who suggested that the predominant effect of oestradiol-17 β in extending luteal function in gilts is through its effect on inhibiting uterine release of PGF-2 α . In support of this concept, Conley & Ford (1989) reported that intraluteal oestradiol-17 β could directly stimulate the growth of an individual CL, but only if the release rate of oestradiol-17 β was high enough to maintain all CL bilaterally. In contrast, Kraeling *et al.* (1975) reported that oestrogen prevented CL regression induced by PGF-2 α in hysterectomized gilts. Unfortunately, no details were provided by these researchers on how luteal maintenance was assessed.

Significant experimental evidence suggests that pig embryos may exert a direct luteotrophic effect. This evidence includes the high incidence of unilateral maintenance (Christenson & Day, 1971) and high progesterone concentrations (Ford & Christenson, 1979) of CL adjacent to the gravid uterine horn in unilaterally pregnant pigs. In addition, Ford *et al.* (1982) observed transient increases in luteal blood flow in gilts at the time of maternal recognition of pregnancy. Ball & Day (1982) used intraluteal implants containing porcine embryonic extracts to maintain the function of individual CL on an ovary. In contrast to the results obtained by Conley & Ford (1989) with oestradiol-17 β , no systemic luteotrophic effect seemed necessary to elicit this response, suggesting that embryonic extract prevented the luteolytic effects of the uterus at the level of the CL. The active principle or principles were heat stable and charcoal extractable, indicating that they were of a low M_r and thus possibly a steroid or prostaglandin. The data obtained in this study suggest that, if involved, oestradiol-17 β is not the only antiluteolytic factor produced by pig embryos.

Unlike the intraluteal administration of oestradiol-17 β , intraluteal administration of PGE-2 protected individual CL against the concomitant administration of PGF-2 α and this effect was dose dependent. At PGE-2:PGF-2 α ratios of 4:1, CL weight and P4 content and concentration were significantly greater than in CL implanted with PGF-2 α alone and weights of implanted CL were similar to that of unimplanted control CL. Few reports have measured PGE-2 concentrations in the reproductive tissues of pregnant and nonpregnant pigs. Patek & Watson (1983) demonstrated that arachidonic acid stimulated more PGE-2 than PGF-2 α secretion from Day-20 pregnant pig endometrium. Geisert *et al.* (1982a) reported that PGE-2 and PGF increased in uterine luminal flushings from pregnant gilts on Day 11 to 14 after mating in association with blastocyst elongation and occurred simultaneously with blastocyst oestrogen production; this increase may reflect secretion of PGE-2 and PGF from the uterine epithelium and/or blastocyst. Evidence suggesting the uterus as a major source of these prostaglandins was presented by Geisert *et al.* (1982b), who observed that treatment of gilts with oestrogen on Day 11 of an oestrous cycle increased PGF-2 α and PGE-2 concentrations in uterine luminal flushings in a manner similar to that observed in the presence of embryos. The embryo itself may also contribute to the observed increase in uterine luminal PGE-2; Paria *et al.* (1987) observed that Day-5 porcine blastocysts are capable of releasing significant amounts of PGE-2 in the medium during 48 h of culture.

In contrast to the negative results obtained by others (Sander *et al.*, 1982; Schneider *et al.*, 1983), Akinlosotu *et al.* (1986) maintained luteal function in gilts by a constant or a pulse (every 4 h)-infusion of PGE-2 from Day 7 to Day 23. The failure of previous attempts to maintain luteal function with PGE-2 may be due to the delayed start of the infusion period or to the less rigorous infusion regime over those days. Further, because intra-uterine infusion of PGE-2 on Days 12, 15 or 18 of the oestrous cycle is known to stimulate PGF-2 α release by the pig uterus (Okrasa *et al.*, 1985), PGF-2 α may have overcome the luteotrophic effect of PGE-2.

These data suggest that the major antiluteolytic effect of oestradiol-17 β in oestrogen-induced pseudopregnant gilts is to decrease uterine release of PGF-2 α into the peripheral circulation, rather than a direct protective effect at the level of the CL. When uterine release of PGF-2 α is reduced by oestradiol-17 β , PGE-2 protects individual CL from the luteolytic effects of exogenously administered PGF-2 α . Since both oestradiol-17 β and PGE-2 are produced synchronously with PGF-2 α during early pregnancy, an independent role for each compound in protecting the CL from luteolysis induced by PGF-2 α can be envisaged.

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