Effect of prostaglandins on luteal function during early pregnancy in pigs

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Summary. Luteal cells were obtained by digestion of luteal tissue of cyclic (day 12) and early pregnant (days 12, 20 and 30) pigs. Suspensions of the dispersed luteal cells (5×10^4 cells ml^{-1}) were incubated for 2 h in minimum essential medium (MEM) alone (control) and MEM with different concentrations of prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) and PGE_2 (0.01, 0.1, 1, 10, 100 and 1000 ng ml^{-1}) and luteinizing hormone (LH) 100 and 1000 ng ml^{-1} , or with combinations of LH + $\text{PGF}_{2\alpha}$ and LH + PGE_2 . Net progesterone production was measured in the incubation media by direct radioimmunoassay.

The overall response pattern of the luteal cells to exogenous hormones on day 12 of the oestrous cycle and pregnancy differed ($P < 0.05$) from treatment on day 20 and 30 of pregnancy. In general progesterone production was higher ($P < 0.05$) and the response to $\text{PGF}_{2\alpha}$ and PGE_2 treatment was most obvious on day 12 of the oestrous cycle and pregnancy. Overall, $\text{PGF}_{2\alpha}$ stimulated progesterone production in a dose-dependent manner ($P < 0.05$). The response to PGE_2 was of a quadratic nature ($P < 0.05$) in which the lowest and the highest doses of PGE_2 were associated with a greater production of progesterone than were the intermediate doses. Treatment of luteal cells with $\text{PGF}_{2\alpha}$ + LH or PGE_2 + LH caused overall inhibition ($P < 0.05$) of progesterone production compared with treatment with each hormone alone. This interaction was not affected by the dose of LH used.

These findings indicate that $\text{PGF}_{2\alpha}$ and PGE_2 are involved in the autocrine control of corpus luteum function.

Keywords: pig; luteal cells; prostaglandins; pregnancy

Introduction

The mechanisms regulating the function of the corpus luteum during early pregnancy in pigs are not fully understood, but it is generally accepted that uterine prostaglandins influence luteal life-span in pigs (Anderson & Melampy, 1967; Gleeson *et al.*, 1974; Moeliono *et al.*, 1976, 1977) and other species (Bazer *et al.*, 1982). The pig corpus luteum seems to be refractory or insensitive to the luteolytic effect of exogenous prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) during the first 12 days of the oestrous cycle *in vivo* (Hallford *et al.*, 1974; Guthrie & Polge, 1976; Krzymowski *et al.*, 1976, 1978; Bazer *et al.*, 1982), presumably owing to the small number of luteal $\text{PGF}_{2\alpha}$ receptors (Gadsby *et al.*, 1990). An inhibitory effect of $\text{PGF}_{2\alpha}$, however, appears at about the time of luteolysis (day 11–13) in nonpregnant pigs (Guthrie & Polge, 1976; Moeliono *et al.*, 1976, 1977). During early pregnancy, days 13–17, endogenous uterine $\text{PGF}_{2\alpha}$ secretion into the uterine vein is reduced (Gleeson *et al.*, 1974; Moeliono *et al.*, 1977), and luteal $\text{PGF}_{2\alpha}$ receptor concentrations are considerably lower than in nonpregnant pigs (Gadsby *et al.*, 1990). However, the corpus luteum of cows

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(Shemesh & Hansel, 1975; Milvae & Hansel, 1983; Pate, 1988), ewes (Rexroad & Guthrie, 1979), women (Challis *et al.*, 1976), mares (Watson & Sertich, 1990), monkeys (Johnson *et al.*, 1988; Ottobre *et al.*, 1989), rabbits (Schlegel *et al.*, 1988; Schlegel & Daniels, 1989) and cyclic (Patek & Watson, 1976; Walker *et al.*, 1977; Guthrie *et al.*, 1978) or pregnant pigs (Watson & Patek, 1979; Guthrie & Rexroad, 1981) can synthesize prostaglandins.

Locally produced prostaglandins could play a role in regulating luteal function and may have an impact on luteal steroidogenesis (Fitz *et al.*, 1984a, b; Hahlin *et al.*, 1988; Pate & Nephew, 1988; Alila *et al.*, 1988; Wiltbank *et al.*, 1989, 1990). PGF_{2α} has a direct antisteroidogenic effect only on large ovine luteal cells, which is mediated through the phospholipase C – protein kinase C, second messenger pathway (Wiltbank *et al.*, 1989, 1990). Other prostaglandins, such as E₂, I₂ and D₂ are also produced by luteal tissue and prolong the lifespan of the corpus luteum in ewes (Huecksteadt & Weems, 1978), cows (Milvae & Hansel, 1983; Alila *et al.*, 1988) and humans (Bennegard *et al.*, 1990). Prostaglandin E₂ (PGE₂) also counteracts the effects of PGF_{2α} in indomethacin-treated cyclic gilts (Akinlosotu *et al.*, 1986, 1988), but the mechanism of action of prostaglandins on luteal function in pigs is still unknown.

It is well established that the corpus luteum of the oestrous cycle of pigs does not require gonadotrophic support after the preovulatory surge of luteinizing hormone (LH) (Sammelwitz *et al.*, 1961; Anderson & Melampy, 1967) and treatment of gilts with LH antiserum during the luteal phase does not disrupt luteal function (Spies *et al.*, 1967). The pig corpus luteum is not highly responsive to LH (Cook *et al.*, 1967) in contrast to the corpus luteum of ruminants. However, it has been reported that LH may stimulate hydrolysis of phosphatidylinositol in isolated luteal membranes of pigs (Allen *et al.*, 1988). Interaction between the two signal transducing systems cAMP and inositol may be important in the integrated control of luteal function.

The aim of this study was to elucidate the effects of PGF_{2α} and PGE₂ on progesterone production by the corpus luteum at different stages of early pregnancy in pigs and to determine whether the interaction of LH with prostaglandins has an impact on steroidogenesis.

Materials and Methods

Animals

Sexually mature pigs (hybrid Landrace × Large White) were assigned randomly to four groups as follows: group 1, day 12 of the oestrous cycle; group 2, day 12, group 3, day 20 and group 4, day 30 of pregnancy. Oestrus was checked daily using a vasectomized boar and the day of mating to fertile boars and the first day of behavioural oestrus was designated day 0 in pregnant and cyclic gilts, respectively. Pregnancy was confirmed by recovery of spherical, filamentous or expanded embryos after dissection of the uterus.

Cell preparation and incubation

Ovaries were removed immediately after slaughter, placed in ice-cold Eagle's minimum essential medium MEM (Flow Laboratories, UK) and transported to the laboratory. Corpora lutea were dissected from the ovaries and the surrounding tissue was removed. Luteal cells were dispersed with 0.1% collagenase (Sigma, Poole, UK) and gentle mechanical agitation of the luteal tissue. Luteal cell viability, as determined by trypan blue (0.2%) exclusion, was 75–80%. Suspensions of dispersed luteal cells were incubated in a shaking water bath at 5×10^4 cells in 1 ml Eagle's minimum essential medium (MEM) at 39°C for 2 h without and with different doses of hormones (experimental treatments). At the end of incubation, cells were sedimented by centrifugation at 1000 g for 10 min and medium was stored at –20°C until estimation of net progesterone accumulation by radioimmunoassay (Foxcroft *et al.*, 1987). The intra- and interassay coefficients of variation were 5.3 and 15.8%, respectively; assay sensitivity was 20 pg per tube.

Experimental treatments

Treatments across all groups were prostaglandins F_{2α} or E₂ (both from Sigma) at 0, 0.01, 0.1, 1, 10, 100 and 1000 ng ml^{–1}, porcine LH (NIH-pLH, Bethesda, USA) at 100 or 1000 ng ml^{–1} and combinations of PGF_{2α} and LH

or PGE₂ and LH. Additionally, PGF_{2α} and PGE₂ each at equal concentrations of 10, 100 and 1000 ng ml⁻¹ were added to incubation tubes. Experimental treatments were performed in duplicate for each pig.

Statistical analysis

The experiment was analysed using split-plot analysis of variance (Steel & Torrie, 1980). Whole plots were the groups ($G = 4$) with five animals per group. The split plots were the combinations of prostaglandins (either F_{2α} or E₂; $P = 7$) and LH ($L = 3$). Specific contrasts among the means were computed as shown in Table 1. Within each of the three concentrations of prostaglandins (10, 100 and 1000 ng ml⁻¹), the data for PGF_{2α} plus PGE₂ were combined with the control (0) and the appropriate prostaglandins E₂ or F_{2α}. The data were analysed as a split plot with groups ($G = 4$) as the whole plots and five animals per group. The split plot contained one concentration of each prostaglandin and co-treatment.

Table 1. Summary of specific contrasts made in the analysis of variance for the split-plot design

ANOVA source	Contrast
LH	LH 0 versus (LH 100 ng ml ⁻¹ + LH 1000 ng ml ⁻¹)
Group*LH	[(group 1 + group 2) versus (group 3 + group 4)]*[LH 0 versus (LH 100 ng ml ⁻¹ + LH 1000 ng ml ⁻¹)]
PGF _{2α}	PGF _{2α} (linear)
Group*PGF _{2α}	(group 1 versus group 2)*PGF _{2α} [(group 1 + group 2) versus (group 3 + group 4)]*PGF _{2α}
LH*PGF _{2α}	[LH 0 versus (LH 100 ng ml ⁻¹ + LH 1000 ng ml ⁻¹)]*PGF _{2α}
PGE ₂	PGE ₂ (quadratic)
Group*PGE ₂	[(group 1 + group 2) versus (group 3 + group 4)]*PGE ₂
LH*PGE ₂	[LH 0 versus (LH 100 ng ml ⁻¹ + LH 1000 ng ml ⁻¹)]*PGE ₂

LH: luteinizing hormone; PGF_{2α}: prostaglandin F_{2α}; PGE₂: prostaglandin E₂; LH 0: without LH treatment (control).

Results

The initial analysis of variance revealed group ($P < 0.05$) and LH, PGE₂ and PGF_{2α} effects ($P < 0.0001$) and significant interactions (at least $P < 0.02$). Specific contrasts in the split-plot analysis (see Table 1) demonstrated that treatment of luteal cells with increasing doses of PGF_{2α} (Fig. 1) produced an overall linear increase ($P < 0.05$) in progesterone production, whereas PGE₂ treatment (Fig. 2) produced an overall quadratic response ($P < 0.05$) described by the equation $y = 12.20 + 0.026 \times \text{PGE}_2 - 0.000024 \times (\text{PGE}_2)^2$ ($r^2 = 0.27$). The overall LH effect ($P < 0.05$) was independent of the dose used except on day 12 of pregnancy when there was a significant ($P < 0.05$) dose-dependent increase in progesterone production in response to LH treatment. There were no differences in the pattern of response of luteal cells between the cyclic and pregnant pigs on day 12 or days 20 and 30 of pregnancy; in subsequent analyses data from appropriate groups were therefore combined. Progesterone secretion on day 12 of the oestrous cycle and pregnancy was significantly higher ($P < 0.05$) and more responsive to exogenous LH, PGF_{2α} and PGE₂ treatment ($P < 0.05$) than on days 20 and 30 of pregnancy. Treatment of luteal cells with PGF_{2α} plus LH or PGE₂ plus LH produced overall ($P < 0.05$) inhibition of progesterone production compared with incubations with LH or with prostaglandin alone. The doses of LH used did not influence the overall response to these combined treatments.

Fig. 1. Effect of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), luteinizing hormone (LH) and $PGF_{2\alpha}$ plus LH on progesterone production for 2 h incubation by luteal cells dissociated from the corpora lutea of (a) day 12 cyclic and days 12 (b), 20 (c) and 30 (d) pregnant pigs. Each bar represents the mean derived from duplicate observations from five gilts; SEM for whole plot = 1.48; vertical bars indicate SEM.

The results provide preliminary evidence for an interaction between equal concentrations of $PGF_{2\alpha}$ and PGE_2 affecting progesterone production by luteal cells (Fig. 3). When data for all groups were combined, there were significant interactions ($P < 0.0001$) between $PGF_{2\alpha}$ and PGE_2 at doses of 10, 100 and 1000 $ng\ ml^{-1}$ incubation medium except on day 30 of pregnancy.

Discussion

This study showed that $PGF_{2\alpha}$ stimulates progesterone production *in vitro* by luteal cells of mid-luteal phase and early pregnant pigs. We suggest that this stimulatory effect could be mediated through the low-affinity receptors present on small luteal cells (Gadsby *et al.*, 1990), as the small luteal cells are known to be the predominant cell type in our suspension (data not shown). Mattioli *et al.* (1985) have shown that in short-term incubations, $PGF_{2\alpha}$ stimulated progesterone production by dispersed luteal cells (mid-luteal phase) of pigs in a dose-dependent manner. Similarly, $PGF_{2\alpha}$ stimulated progesterone secretion in bovine small luteal cells *in vitro*, presumably acting through the phospholipase-C-phosphatidylinositol hydrolysis and mobilization of intracellular Ca^{2+} .

Fig. 2. Effect of prostaglandin E₂, luteinizing hormone (LH) and PGE₂ plus LH on progesterone production for 2 h incubation by luteal cells dissociated from the corpora lutea of (a) day 12 cyclic and days 12 (b), 20 (c) and 30 (d) pregnant pigs. Each bar represents the mean derived from duplicate observations from gilts; SEM for whole plot = 1.47; vertical bars indicate SEM.

(Davis *et al.*, 1987, 1989). Prostaglandin F_{2α} inhibits luteal steroidogenesis *in vitro* when lipoproteins are present in the culture medium (Pate & Nephew, 1988; Wiltbank *et al.*, 1990) and incubation times were longer than 4–6 h (Fritz *et al.*, 1991). The cells in our study were incubated in MEM without any supplementation (bovine serum albumin or serum) to avoid interactions between blood proteins and PGE₂; this may explain why we could not inhibit progesterone production *in vitro* by PGF_{2α} treatment of luteal cells. Furthermore, the lack of a luteolytic effect of PGF_{2α} could be due to the presence of only a small number of large luteal cells (the target for the luteolytic effects of PGF_{2α}) in our incubation samples or because the incubation time was too short to permit luteolytic effects of PGF_{2α} to occur. It has been suggested that the cytotoxic effect of PGF_{2α} on small luteal cells is mediated through indirect mechanisms, such as intercellular communication from large cells (Braden *et al.*, 1988); the disruption of these intercellular communications may therefore have had an effect. Data reported here are consistent with the recent demonstration that injection of a luteolytic dose of PGF_{2α} to both pregnant and pseudopregnant gilts produced a rapid and transient increase in serum progesterone concentrations lasting approximately 1 h (Gadsby *et al.*, 1991). PGF_{2α} may stimulate progesterone synthesis or secretion in porcine luteal cells *in vivo* before the subsequent decline in serum concentrations of progesterone associated with luteolysis in most pregnant animals. Gadsby *et al.* (1991) suggest that there may be a causal relationship

Fig. 3. Effect of prostaglandins F_{2a} (■) and E₂ (▨) alone or combined in equal doses (□) on progesterone production *in vitro* by luteal cells recovered from the groups of (a) day 12 cyclic and days 12 (b), 20 (c) and 30 (d) pregnant gilts. The horizontal line represents the progesterone production by luteal cells without any treatment. Overall SEM for prostaglandins doses: 1.58 for 10 ng ml⁻¹, 1.46 for 100 ng ml⁻¹, 1.69 for 1000 ng ml⁻¹; vertical bars indicate SEM.

between the early stimulatory and the later inhibitory (luteolytic) actions of PGF_{2a} on porcine luteal cells. Similarly, Watson & Maule-Walker (1977) used superfused porcine luteal tissue slices to show that a transient stimulation in progesterone output preceded the inhibitory effect of PGF_{2a} on progesterone production.

The pattern of response of luteal cells to exogenous hormones on day 12 of the oestrous cycle and pregnancy was different to that on days 20 and 30 of pregnancy. The progesterone deficiency (low basal progesterone production and reduced responsiveness to prostaglandins, LH or LH plus prostaglandins treatment) in the early pregnancy of pigs (day 20 and 30) may have been caused by the initiation of luteolysis on day 11 (Bazer *et al.*, 1982) by prostaglandins of luteal origin, as suggested by Rothchild (1981). Guthrie & Rexroad (1981) demonstrated that the peak of PGF_{2a} secretion during the late luteal phase in nonpregnant pigs usually occurs after the initial decrease in plasma progesterone. The results from studies *in vivo* (Bazer *et al.*, 1982) showed that peripheral progesterone in the blood decreased to 10–25 ng ml⁻¹ on days 20–30 of pregnancy, whereas prostaglandin concentrations in the utero-ovarian vein were very low and similar to those in hysterectomized pigs (King, 1990). The extent of the initiation of a transient period of luteolysis in early pregnancy would not be expected to mimic fully the changes seen during the oestrous cycle due to: (i) the considerably smaller number of PGF_{2a} receptors on the corpus luteum of pregnant compared with nonpregnant pigs (Gadsby *et al.*, 1990), (ii) the lack of luteolytic factors from the uterus, as it is well documented that during pregnancy uterine PGF_{2a} is sequestered in the uterus (Bazer *et al.*, 1982) and (iii) it is likely that the corpus luteum would already have received luteotrophic support of embryonic (Dhindsa & Dziuk, 1968; Ball & Day, 1979; Van der Meulen *et al.*, 1988) or pituitary (Brinkley *et al.*, 1964, authors' unpublished observations) origin.

Unfortunately, we do not know the origin of prostaglandins within the pig corpus luteum *in vivo* or within luteal cell suspensions *in vitro*, or what regulates the synthesis and balance of these

arachidonate metabolites. These issues are currently under investigation. If we assume that the luteal production of prostaglandins could be the physiological signal triggering a transient luteolysis in early pregnancy, then the corpus luteum has to be rescued for pregnancy to continue in pigs. It has been suggested (Hahlin *et al.*, 1988; Bennegard *et al.*, 1990) that the hormone that rescues or protects the corpus luteum from the luteolytic effects of $\text{PGF}_{2\alpha}$ during early pregnancy is PGE_2 in humans (Balmaceda *et al.*, 1981). The role of PGE_2 in corpus luteum function during early pregnancy of pigs is unknown. However, PGE_2 can counteract the luteolytic effect of $\text{PGF}_{2\alpha}$, since chronic intrauterine infusions of PGE_2 from day 7 in cyclic gilts (Akinlosotu *et al.*, 1986, 1988), and day 9 in cows (Gimenez & Henricks, 1981) and ewes (Pratt *et al.*, 1979), inhibits luteolysis, maintains luteal progesterone production and prolongs the oestrous cycle. Shelton *et al.* (1990) have indicated that PGE_2 can stimulate progesterone synthesis and may also have inhibitory effects on bovine luteal cells during early pregnancy. Our results are generally in agreement with these observations, with the exception that the porcine luteal cells did not respond to PGE_2 treatment in a simple dose dependent manner. Large, as well as pools of small and large, ovine luteal cells (Fitz *et al.*, 1984b; Balapure *et al.*, 1989) also exhibited a quadratic pattern of response to PGE_2 but in a reverse manner to pig luteal cells, with maximum stimulation resulting from media concentrations of PGE_2 between 10 and 50 ng ml⁻¹. The physiological basis for these different dose-dependent quadratic effects may be related to a number of factors: (i) the different developmental status of the target tissues due to differences between the species or culture conditions; (ii) the functional status of PGE_2 and $\text{PGF}_{2\alpha}$ receptors on luteal cells which can regulate crossreactivities of different prostaglandins with their receptors and (iii) the relative proportions of PGE_2 to $\text{PGF}_{2\alpha}$ in the corpus luteum in different physiological states.

Our studies also demonstrated that LH and prostaglandins $\text{F}_{2\alpha}$ and E_2 interact to inhibit progesterone production by pig luteal cells. Shelton *et al.* (1990) showed that low concentrations of PGE_2 (0.01–10 ng ml⁻¹) inhibit progesterone production stimulated by lower doses of LH (0.1 ng ml⁻¹), but this was overcome by higher concentrations of PGE_2 (>100 ng ml⁻¹). In addition higher doses of LH did not inhibit the effect of low doses of PGE_2 upon progesterone synthesis. Nevertheless, PGE_2 was as efficient as $\text{PGF}_{2\alpha}$ in inhibiting LH stimulated progesterone synthesis in dispersed rat luteal cells (Thomas *et al.*, 1978). A similar antagonistic effect of PGE_2 on human chorionic gonadotrophin stimulation of luteal cells from rhesus monkeys has been reported (Stouffer *et al.*, 1979). Finally, LH and $\text{PGF}_{2\alpha}$ interact in large luteal cells of cows to produce Ca^{2+} concentrations that are higher than the sum of Ca^{2+} produced by each hormone separately (Alila *et al.*, 1989). Results reported here and in other studies indicate that the interaction of the two intracellular second messenger pathways (LH and prostaglandins) has a negative effect on progesterone production by luteal cells. This conclusion is further supported by the work of Davis *et al.* (1987, 1989) who showed that LH can activate the inositol phospholipid–phospholipase C signalling system in bovine luteal cells.

It is generally recognized that LH is a luteotrophic hormone in many species and its effect is mediated through an intracellular cAMP mechanism, but the luteotrophic influence of LH on the pig corpus luteum is still uncertain. Ekstrom & Hunzicker-Dunn (1990) demonstrated that pig luteal membranes were resistant to *in vitro*, hormone-induced, desensitization of LH/hCG responsive adenyl cyclase when compared with follicular membranes; hormonal stimulation was readily observed by extracting the membranes with urea, and GTP was required during the assay. They also suggested that some auxiliary factor in the corpus luteum could be responsible for the resistance of pig luteal adenyl cyclase to desensitization.

In this study, the effect of LH alone on progesterone production was not consistent across the groups and was dose dependent only on day 12 of pregnancy. This supports the suggestion of Ekstrom & Hunzicker-Dunn (1990) that the activity of LH receptors, or changes in the classes of LH/hCG receptors, may be of critical importance in regulating the response to gonadotrophic stimulation. It is also in agreement with our unpublished observation that incubation conditions and the physiological state of the ovary (i.e. day of pregnancy) play important roles in responsiveness to LH.

We conclude that prostaglandins exert both stimulatory and inhibitory effects on the activity of porcine luteal cells. They can also interact with LH and with each other, indicating multiple sites of action. We therefore suggest that the ratios of LH, prostaglandins E_2 and F_{2a} are important in the control of progesterone synthesis during early pregnancy in pigs.

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