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 × 10⁴ cells ml⁻¹) were incubated for 2 h in minimum essential medium (MEM) alone (control) and MEM with different concentrations of prostaglandin F₂α (PGF₂α) and PGE₂ (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 + PGF₂α and LH + PGE₂. 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 PGF₂α and PGE₂ treatment was most obvious on day 12 of the oestrous cycle and pregnancy. Overall, PGF₂α stimulated progesterone production in a dose-dependent manner (P < 0.05). The response to PGE₂ was of a quadratic nature (P < 0.05) in which the lowest and the highest doses of PGE₂ were associated with a greater production of progesterone than were the intermediate doses. Treatment of luteal cells with PGF₂α + LH or PGE₂ + 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 PGF₂α and PGE₂ 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 lifespan in pigs (Anderson & Melampy, 1967; Gleeson et al., 1974; Moeljono 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 F₂α (PGF₂α) 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 PGF₂α receptors (Gadsby et al., 1990). An inhibitory effect of PGF₂α, however, appears at about the time of luteolysis (day 11–13) in nonpregnant pigs (Guthrie & Polge, 1976; Moeljono et al., 1976, 1977). During early pregnancy, days 13–17, endogenous uterine PGF₂α secretion into the uterine vein is reduced (Gleeson et al., 1974; Moeljono et al., 1977), and luteal PGF₂α 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$_{2\alpha}$ 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 E$_2$, I$_2$ and D$_2$ are also produced by luteal tissue and prolong the lifespan of the corpus luteum in ewes (Huecksteadt & Wecms, 1978), cows (Milvae & Hansel, 1983; Alila et al., 1988) and humans (Bennoncard et al., 1990). Prostaglandin E$_2$ (PGE$_2$) also counteracts the effects of PGF$_{2\alpha}$ 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$_{2\alpha}$ and PGE$_2$ 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$_{2\alpha}$ or E$_2$ (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$_{2\alpha}$ and LH.
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or PGE₂ and LH. Additionally, PGF₂α 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 F₂α 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 PGF₂α plus PGE₂ were combined with the control (0) and the appropriate prostaglandins E₂ or F₂α. 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

<table>
<thead>
<tr>
<th>ANOVA source</th>
<th>Contrast</th>
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<tbody>
<tr>
<td>LH</td>
<td>LH 0 versus (LH 100 ng ml⁻¹ + LH 1000 ng ml⁻¹)</td>
</tr>
<tr>
<td>Group*LH</td>
<td>[(group 1 + group 2) versus (group 3 + group 4)]*(LH 0 versus (LH 100 ng ml⁻¹ + LH 1000 ng ml⁻¹))</td>
</tr>
<tr>
<td>PGF₂α</td>
<td>PGE₂ (linear)</td>
</tr>
<tr>
<td>Group*PGF₂α</td>
<td>(group 1 versus group 2)*PGE₂ (group 1 + group 2) versus (group 3 + group 4)*PGE₂</td>
</tr>
<tr>
<td>LH*PGF₂α</td>
<td>[LH 0 versus (LH 100 ng ml⁻¹ + LH 1000 ng ml⁻¹)]*PGE₂</td>
</tr>
<tr>
<td>PGE₂</td>
<td>PGE₂ (quadratic)</td>
</tr>
<tr>
<td>Group*PGE₂</td>
<td>[(group 1 + group 2) versus (group 3 + group 4)]*PGE₂</td>
</tr>
<tr>
<td>LH*PGE₂</td>
<td>[LH 0 versus (LH 100 ng ml⁻¹ + LH 1000 ng ml⁻¹)]*PGE₂</td>
</tr>
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LH: luteinizing hormone; PGF₂α: prostaglandin F₂α; PGE₂: prostaglandin E₂; LH 0: without LH treatment (control).

Results

The initial analysis of variance revealed group (P < 0.05) and LH, PGE₂ and PGF₂α 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 PGF₂α (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 × PGE₂ - 0.000024 × (PGE₂)² (r² = 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, PGF₂α and PGE₂ treatment (P < 0.05) than on days 20 and 30 of pregnancy. Treatment of luteal cells with PGF₂α 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$_2$a (PGF$_2$a), luteinizing hormone (LH) and PGF$_2$a 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$_2$a 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$_2$a 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$_2$a stimulates progesterone production \textit{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 \textit{et al.}, 1990), as the small luteal cells are known to be the predominant cell type in our suspension (data not shown). Mattioli \textit{et al.} (1985) have shown that in short-term incubations, PGF$_2$a stimulated progesterone production by dispersed luteal cells (mid-luteal phase) of pigs in a dose-dependent manner. Similarly, PGF$_2$a stimulated progesterone secretion in bovine small luteal cells \textit{in vitro}, presumably acting through the phospholipase-C–phosphatidylinositol hydrolysis and mobilization of intracellular Ca$^{2+}$.
Fig. 2. Effect of prostaglandin E\textsubscript{2}, luteinizing hormone (LH) and PGE\textsubscript{2} 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\textsubscript{2a} 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 PGE\textsubscript{2}; this may explain why we could not inhibit progesterone production in vitro by PGF\textsubscript{2a} treatment of luteal cells. Furthermore, the lack of a luteolytic effect of PGF\textsubscript{2a} could be due to the presence of only a small number of large luteal cells (the target for the luteolytic effects of PGF\textsubscript{2a}) in our incubation samples or because the incubation time was too short to permit luteolytic effects of PGF\textsubscript{2a} to occur. It has been suggested that the cytotoxic effect of PGF\textsubscript{2a} 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\textsubscript{2a} 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\textsubscript{2a} 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
between the early stimulatory and the later inhibitory (luteolytic) actions of PGF$_{2\alpha}$ 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$_{2\alpha}$ 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$_{2\alpha}$ 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$_{2\alpha}$ 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$_{2\alpha}$ 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...
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arachidonic 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 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 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 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 PGF$_{2\alpha}$ in the corpus luteum in different physiological states.

Our studies also demonstrated that LH and prostaglandins 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 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 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 E₂ and F₂α are important in the control of progesterone synthesis during early pregnancy in pigs.

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