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Effects of LH, prostaglandin E_2, 8-bromo-cyclic AMP and forskolin on progesterone secretion by pig luteal cells

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The present study examined the effects of LH, prostaglandin E_2 (PGE_2), 8-bromo-cyclic AMP (cAMP) and forskolin on progesterone secretion by small and large pig luteal cells. Corpora lutea were isolated from gilts ($n \geq 3$ per day) on days 9, 12 and 14 of the oestrous cycle and days 9, 12, 14 and 30 of pregnancy. After enzymatic dissociation of the corpora lutea, small and large luteal cells were obtained by elutriation. Culture plates (24-well) were then seeded with 150 000 small luteal cells or 30 000 large luteal cells per well in 1 ml M199 medium in the absence or presence of LH, PGE_2, LH plus PGE_2, 8-bromo-cAMP or forskolin. After 12 h of incubation, culture plates were centrifuged, and the supernatant collected and frozen for subsequent assay of progesterone. Differences within day were not detected between cyclic and pregnant gilts, and thus, results were combined for days 9, 12 and 14. Basal progesterone secretion by small luteal cells was less ($P < 0.05$) on days 14 and 30 than days 9 and 12. Treatment with LH, PGE_2, 8-bromo-cAMP or forskolin increased ($P < 0.05$) progesterone secretion by small luteal cells on days 9 and 12; however, treatments had no effect on days 14 and 30. Basal progesterone production by large luteal cells was less ($P < 0.05$) on day 30 compared with other days. PGE_2 stimulated ($P < 0.001$) progesterone production by large luteal cells at all days. In contrast, 8-bromo-cAMP and forskolin inhibited progesterone production by large luteal cells on day 12 ($P < 0.05$), and day 14 ($P < 0.001$). These data show that pregnancy status does not alter luteal cell response to the aforementioned secretagogues. However, regulation of progesterone secretion differs between small and large luteal cells, and the age of the corpora lutea. Also, it is unlikely that the stimulatory actions of PGE_2 involve increased cAMP production in pig large luteal cells.

Introduction

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is recognized for its luteolytic actions in pig corpora lutea. In contrast, the role of prostaglandin E_2 (PGE_2) in corpus luteum function has received limited attention. Uterine prostaglandin secretion peaks earlier (days 11–12) in pregnant pigs than in cyclic pigs, with PGE_2 as the predominant prostaglandin (Christenson *et al.*, 1994). Intrauterine infusion of PGE_2 lengthens the lifespan of corpora lutea (Akinlosotu *et al.*, 1986), and intraluteal administration (Ford and Christenson, 1991) or intrauterine infusion (Akinlosotu *et al.*, 1988) of PGE_2 prevents luteolysis induced by $PGF_{2\alpha}$. In addition, PGE_2 stimulates progesterone production by luteal cells from gilts at day 12 of the oestrous cycle and during early pregnancy (Wiesak *et al.*, 1992). Therefore, PGE_2 is often considered luteotrophic and important in the function of the pig corpus luteum during the establishment of pregnancy.

The corpora lutea of pigs contain small (SLC) and large luteal cells (LLC) (Lemon and Loir, 1977). Pig SLC and LLC, collected on days 8–9 of the oestrous cycle, have been shown to be regulated in different ways by LH and PGE_2 (Richards *et al.*, 1994). LH was found to stimulate progesterone secretion by SLC but not by LLC. Conversely, PGE_2 stimulates progesterone secretion by LLC, but not by SLC (Richards *et al.*, 1994). However, there is conflicting data concerning the effects of LH on progesterone production by SLC and LLC. The confusion is exemplified by the observations that LH stimulated SLC, LLC or both (Hunter, 1981; Buhr, 1987; Tekpetey and Armstrong, 1991; Yuan *et al.*, 1993; Wiesak *et al.*, 1994). Despite the apparent stimulatory effects of LH on LLC, LH receptors have been found only on SLC (Meduri *et al.*, 1992, 1996). In contrast, PGE receptors are present primarily on LLC (Feng and Almond, 1996).

It has been suggested that cAMP mediates LH-induced progesterone secretion by pig SLC (Tekpetey and Armstrong, 1991; Richards and Almond, 1994) and PGE_2-stimulated progesterone secretion by luteinized granulosa cells (Grimes *et al.*, 1993). The present study was designed to examine further the differential effects of LH and PGE_2 on pig SLC and LLC during the oestrous cycle and early pregnancy. In addition, 8-bromo-cAMP and forskolin were included to evaluate the potential role of cAMP in PGE_2-stimulated progesterone secretion by LLC.

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Materials and Methods

Materials

Hank's Balanced Salt Solution (HBSS; without calcium and magnesium), Hepes, sodium bicarbonate, PGE_2, forskolin, 8-bromo-adenosine 3′, 5′ cyclic monophosphate (8-bromo-cAMP), DNase (type I from bovine pancreas), hyaluronidase (type V from ovine testis), BSA (fraction V), Tris, penicillin–streptomycin and gentamicin were obtained from Sigma Chemical Co. (St Louis, MO). Pig LH (LER 786-3) was supplied by L E Reichert, Jr (Albany, NY). Collagenase (type IV) was purchased from Worthington Biochemical Corporation (Freehold, NJ). The Coat-a-Count progesterone radioimmunoassay kits were purchased from Diagnostic Products Corporation (Los Angeles, CA).

Animals and collection of ovaries

Twenty-nine gilts (6–7 months old) were obtained and housed at the North Carolina State University Swine Education and Research Facility. The onset of oestrus was detected by daily exposure to a mature boar. The first day of standing oestrus was considered day 0 of the oestrous cycle. Fifteen gilts were mated twice by artificial insemination beginning on the day after the onset of oestrus. The first day of insemination was considered day 1 of pregnancy. The experimental protocol was approved by the Institutional Animal Care and Use Committee of North Carolina State University.

Animals were killed by intravenous injections with Beuthanasia-D special (58.5 mg pentobarbital sodium kg^{-1} and 7.5 mg phenytoin sodium kg^{-1}; Schering-Plough Animal Health Corporation, Kenilworth, NJ) and the ovaries collected immediately, aseptically. Ovaries were collected from animals on day 9 (n = five gilts), day 12 (n = three gilts), and day 14 (n = six gilts) of the oestrous cycle and day 9 (n = four gilts), day 12 (n = four gilts), day 14 (n = four gilts) and day 30 (n = three gilts) of pregnancy. The corpora lutea from three of six nonmated gilts (day 14) were white and non-vascular. These corpora lutea were not used owing to the small numbers of LLC obtained after enzyme dissociation. The corpora lutea from the other three gilts, at day 14 of the oestrous cycle, were red and vascular and were used in this study. Uteri were removed and flushed with PBS (day 9, 12 and 14 pregnant gilts) or incised (gilts at day 30 of pregnancy) to confirm the presence of conceptuses. Immediately after removal from the animals, the ovaries were transported to the laboratory in chilled HBSS containing 20 mmol Hepes l^{-1}, 4.2 mmol sodium bicarbonate l^{-1}, 100 U penicillin ml^{-1}, 100 μg streptomycin ml^{-1} and 50 μg gentamicin ml^{-1}. The number of corpora lutea per gilt was 13.6 ± 0.7 (mean ± SEM, n = 26 gilts).

A blood sample was collected from each animal before death. This sample was used to determine serum progesterone concentrations. Blood was allowed to clot at room temperature before centrifugation (800 ***g***, 30 min) to collect serum. Serum samples were stored at − 20°C until assayed for progesterone concentrations.

Dissociation of corpora lutea

Corpora lutea were dissected from the ovaries and dissociated by collagenase (type IV, 60 units mg^{-1} dry weight) and hyaluronidase to obtain dispersed luteal cells as reported by Richards *et al.* (1994). Viability of dissociated luteal cells was greater than 80% as determined by Trypan blue exclusion.

Preparation of small and large cells

Small and large cells were isolated by elutriation (Gadsby *et al.*, 1990; Richards *et al.*, 1994), immediately after cell dispersal. Dissociated luteal cells were elutriated using HBSS containing 20 mmol Hepes l^{-1}, 4.2 mmol sodium bicarbonate l^{-1}, 1 mg BSA ml^{-1}, 0.02 mg DNase ml^{-1}, 100 U penicillin ml^{-1}, 100 μg streptomycin ml^{-1} and 50 μg gentamicin ml^{-1}. Five fractions (200 ml per fraction) were collected in succession at buffer flows of 12 ml min^{-1} at 1360 ***g***, 24 ml min^{-1} at 1360 ***g***, 30 ml min^{-1} at 1360 ***g***, 30 ml min^{-1} at 1020 ***g*** and 30 ml min^{-1} at 680 ***g***. A sample from each fraction was examined with light microscopy. The first fraction contained mainly erythrocytes. The second fraction contained only small cells (< 23 μm in diameter), while the third and fourth fractions contained small cells, small cell clumps and large cells. The fifth fraction contained large cells (> 23 μm in diameter) and 8.6 ± 1% small cells (n = 26 pigs). Viabilities of small (fraction 2) and large cells (fraction 5) were greater than 85%.

Cell culture and treatments

Cell cultures were performed using established procedures (Richards *et al.*, 1994). Incubation media was medium 199 containing 20 mmol Hepes l^{-1}, 4.2 mmol NaHCO3 l^{-1}, 5 μg insulin ml^{-1}, 40 ng hydrocortisol ml^{-1}, 50 μg low-density lipoprotein (LDL)-carried cholesterol ml^{-1}, 0.1% BSA, 50 μg gentamicin ml^{-1}, 100 U penicillin ml^{-1} and 100 μg streptomycin ml^{-1}, pH 7.38. The LDL was prepared as described by Richards *et al.* (1994). The dissociation and elutriation procedures typically produce a mixed cell fraction with 15% LLC and 85% SLC (Feng and Almond, 1996). Therefore, fivefold more SLC than LLC were used in each culture well. The SLC (150 000 SLC) or LLC (30 000 LLC) were seeded in 24-well plates with 1 ml incubation media per well in the absence or presence of 50 ng LH ml^{-1}, 100 ng PGE_2 ml^{-1}, 50 ng LH ml^{-1} plus 100 ng PGE_2 ml^{-1}, 0.01, 0.1, 1 mmol 8-bromo-cAMP l^{-1} or 0.5, 5, 50 μmol forskolin l^{-1} in triplicate wells. Incubations were at 37°C with 5% CO_2 in a humid environment for 12 h. Cell viability did not change after 12 h of culture.

Progesterone radioimmunoassay

Commercial radioimmunoassay kits, validated for pig serum (Almond and Dial, 1990; Richards and Almond, 1994), were used to determine serum progesterone concentrations. The assay requires no extraction. The progesterone concentration in each serum sample was determined in triplicate and 100 μl serum was used in each replicate. The intra-assay coefficients of variation (CV) for both high (24 ng ml^{-1}) and low (4 ng ml^{-1}) progesterone reference sera were < 5%. Sensitivity of the progesterone radioimmunoassay was 10 pg per tube.

For each treatment and day, progesterone production by SLC and LLC was determined independently for cyclic animals

and pregnant animals. Within day, the cells from pregnant and cyclic animals were not combined. Culture medium was collected from luteal cell incubations and centrifuged at 250 ***g*** for 7 min. After centrifugation, the supernatant was decanted and stored at − 20°C until assayed. Progesterone concentrations in the decanted media were determined using established radioimmunoassay procedures (Richards *et al.*, 1994). The intra-assay CV for high (10.5 ng ml^{-1}) and low (0.3 ng ml^{-1}) progesterone reference media were 6.4% and 8.9%, respectively. The interassay CV value was 5.8% for the high progesterone reference media and 8.2% for the low media. Sensitivity of the radioimmunoassay was 10 pg per tube.

Statistical analyses

Differences in serum progesterone concentrations during the oestrous cycle and early pregnancy were analysed by least-squares analysis of variance (ANOVA) using the General Linear Model (GLM) procedure (SAS, 1988). Effects included in the model were pregnancy status (cyclic versus pregnant), day and their interaction. The difference of serum progesterone concentrations between the cyclic and pregnant gilts within day was detected by contrasts (SAS, 1988).

The mean progesterone concentration in the culture medium from triplicate wells was used for the statistical analysis. The statistical analysis was performed separately for small and large cells to test the effect of each secretagogue on progesterone secretion during the oestrous cycle and early pregnancy. In both cases, the same split-plot design was used. Pregnancy status (cyclic versus pregnant), day and their interaction were included as whole plot effects. Treatment was included as a split-plot effect. Natural log transformation of data was used based on a normal distribution test (Shapiro–Wilks W-test, SAS, 1988) and homogeneous variance test (Barlett's test) (JMP, 1989). The variance of the data from different days of the oestrous cycle and pregnancy, including day 30 of pregnancy, were homogeneous after transformation. Transformed data were subjected to least-squares ANOVA using the GLM procedure (SAS, 1988). The variation between pigs within each whole plot was used as the error term to test the significance of whole plot effect. The mean square of error from the model was used to test the significance of treatment and interaction of treatment with whole plot effects. Differences in progesterone production by SLC or LLC between treatment and control within day were compared by contrasts (SAS, 1988). The means of basal progesterone secretion by SLC or LLC collected at different stages of the oestrous cycle and early pregnancy were compared by least significance difference (SAS, 1988).

Results

Day, and interaction of day with pregnancy status ($P < 0.05$), but not pregnancy status ($P > 0.05$) affected serum progesterone concentrations (Fig. 1). On day 14, serum progesterone concentrations were lower ($P < 0.05$) in cyclic gilts than in pregnant gilts. Serum progesterone concentrations in the three gilts with white, non-vascular corpora lutea on day 14 were not included in the analysis.

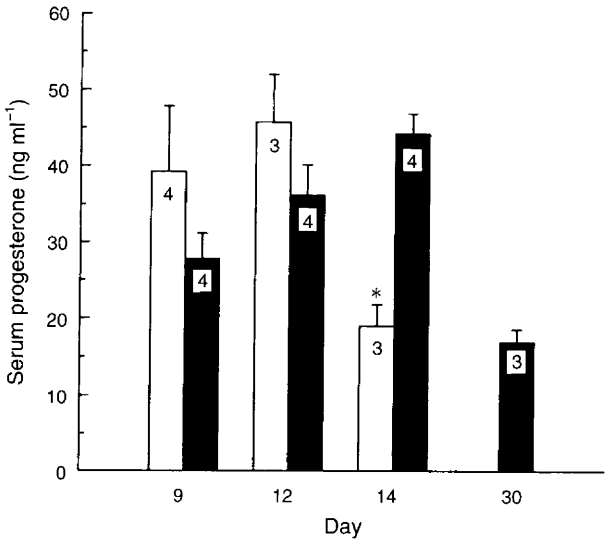

Fig. 1. Serum progesterone concentrations (means + SEM) in (□) cyclic (days 9, 12 and 14) and (■) pregnant gilts (days 9, 12, 14 and 30). The first day of standing oestrus was designated day 0 of the oestrous cycle or pregnancy. The number in each bar represents the number of gilts. Day and interaction of day with pregnancy status but not pregnancy status affected ($P < 0.05$) serum progesterone concentrations. *Indicates that cyclic and pregnant groups differed significantly ($P < 0.05$) within day.

Day, treatment, and the interaction of day with treatment affected ($P < 0.001$) the secretion of progesterone by SLC and LLC. Pregnancy status and other interactions had no effect ($P > 0.05$) on progesterone secretion by SLC or LLC. Thus, the results for progesterone production by SLC or LLC from both cyclic and pregnant gilts were combined.

Basal progesterone secretion by SLC collected at days 14 and 30 was lower ($P < 0.05$) compared with days 9 and 12 after oestrus. Progesterone secretion by SLC collected on days 9 and 12 was stimulated ($P < 0.05$) by LH, PGE_2, LH plus PGE_2, 8-bromo-cAMP and forskolin (Fig. 2). Stimulation of progesterone secretion by 8-bromo-cAMP or forskolin on day 9 and by 8-bromo-cAMP on day 12 was dose-dependent (linearly, $P < 0.05$). LH, PGE_2, 8-bromo-cAMP and forskolin had no effects on progesterone secretion by SLC collected on days 14 and 30 after oestrus (Fig. 2).

Basal progesterone secretion by LLC was similar between days 9, 12 and 14. However, progesterone secretion was lower ($P < 0.05$) by LLC collected at day 30 than by LLC at previous days. PGE_2 and PGE_2 plus LH stimulated ($P < 0.05$) progesterone secretion by LLC at all stages examined (Fig. 3). LH inhibited ($P < 0.05$) progesterone secretion by LLC at day 14 after oestrus, but had no effect on LLC at other days. Neither 8-bromo-cAMP nor forskolin altered progesterone secretion by LLC collected on day 9; however, 8-bromo-cAMP and forskolin inhibited ($P < 0.05$) progesterone secretion by LLC from day 12 gilts. Both 8-bromo-cAMP and forskolin further inhibited ($P < 0.001$) progesterone production by LLC collected from day 14 gilts. 8-Bromo-cAMP had a linear and quadratic dose–effect, and forskolin had a linear dose–effect ($P < 0.05$). On day 30 of pregnancy, 8-bromo-cAMP (0.1 and 1 mmol l^{-1}) and forskolin (5 μmol l^{-1}) stimulated ($P < 0.05$) progesterone secretion by LLC (Fig. 3).

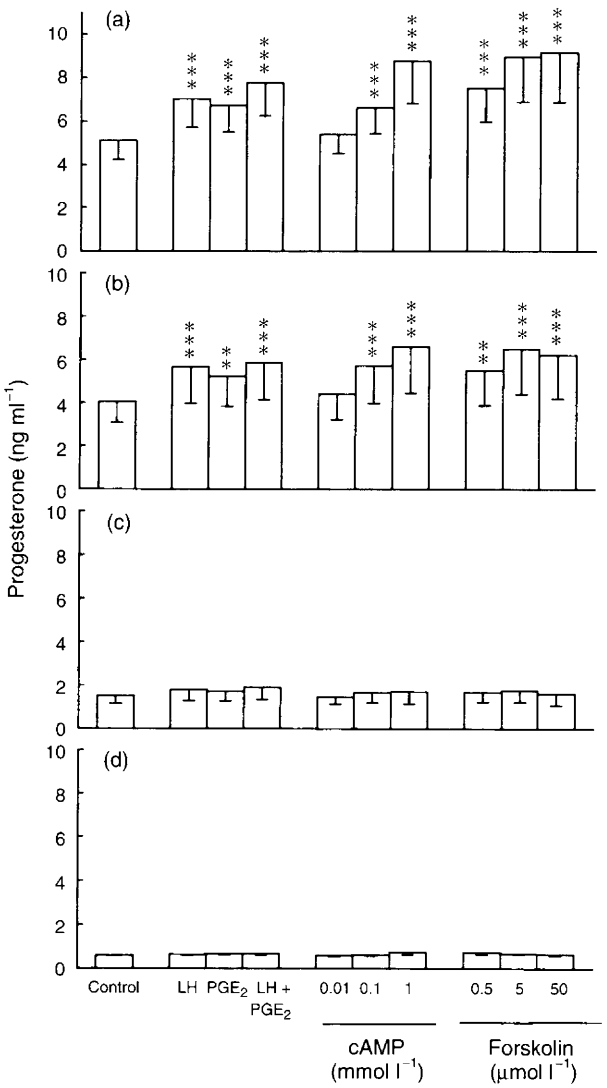

Fig. 2. Progesterone secretion (means – SEM) by small luteal cells (SLC) collected on (a) day 9, (b) day 12, (c) day 14 and (d) day 30 after oestrus. SLC from cyclic and pregnant gilts were cultured for 12 h in the absence (control) or presence of 50 ng LH ml^{-1}, 100 ng prostaglandin E_2 (PGE_2) ml^{-1}, 50 ng LH ml^{-1} plus 100 ng PGE_2 ml^{-1}, 0.01, 0.1, 1 mmol 8-bromo-cAMP (cAMP) l^{-1} or 0.5, 5, 50 μmol forskolin l^{-1}. Within day, ** and *** indicate differences of $P < 0.01$, and $P < 0.001$, respectively, compared with basal progesterone production by control SLC.

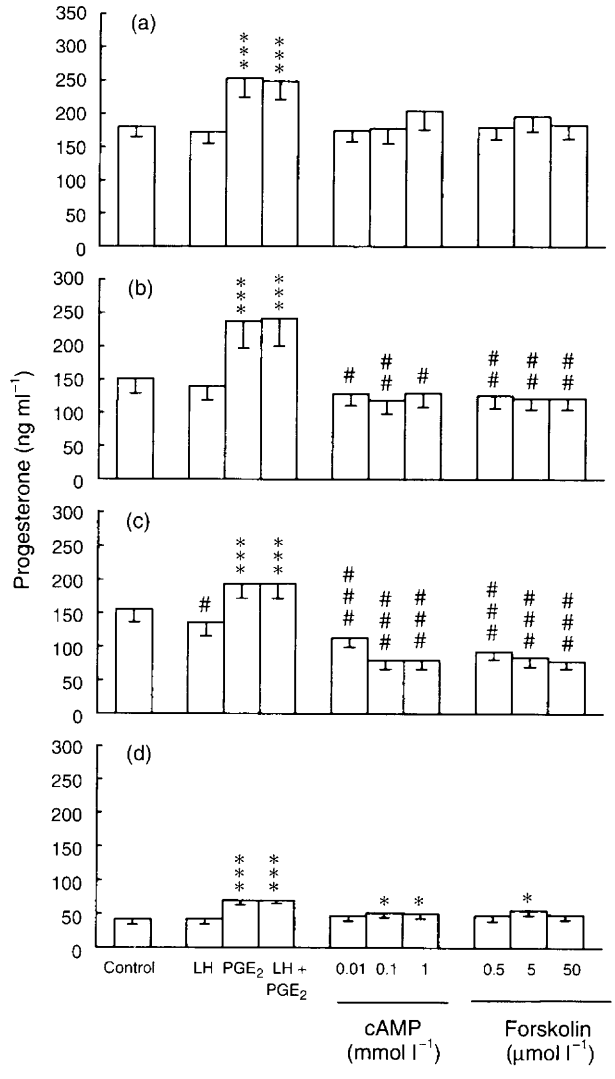

Fig. 3. Progesterone secretion (means – SEM) by large luteal cells (LLC) collected on (a) day 9, (b) day 12, (c) day 14 and (d) day 30 after oestrus. LLC from cyclic and pregnant gilts were cultured for 12 h in the absence (control) or presence of 50 ng LH ml^{-1}, 100 ng prostaglandin E_2 (PGE_2) ml^{-1}, 50 ng LH ml^{-1} plus 100 ng PGE_2 ml^{-1}, 0.01, 0.1, 1 mmol 8-bromo-cAMP (cAMP) l^{-1} or 0.5, 5, 50 μmol forskolin l^{-1}. Within day, *, ** and *** indicate stimulation at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; while #, ## and ### indicate inhibition at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, compared with basal progesterone production by control LLC.

Discussion

By day 14, SLC had lost their responsiveness to the hormones and basal progesterone secretion had decreased by this time. Forskolin and 8-bromo-cAMP inhibited progesterone secretion by LLC on days 12 and 14, although basal progesterone production did not change. These data suggest that pig luteal cells change markedly around days 12 and 14. Correspondingly, pig corpora lutea become sensitive to the luteolytic effect of $PGF_{2\alpha}$ after day 12 of the oestrous cycle (Gleeson, 1974; Diehl and Day, 1974) and maternal recognition of pregnancy occurs during this interval (Bazer *et al.*, 1992). However, little information is available about the intracellular changes during this stage of pregnancy or the oestrous cycle. Unexpectedly, pregnancy status and the interaction of pregnancy status with treatment had no effect on progesterone secretion by cultured SLC or LLC collected on day 14, even though the concentrations of serum progesterone declined in cyclic, but not in pregnant, animals. It is possible that the collection of corpora lutea for *in vitro* studies removes, or reduces, the effects of endogenous hormones on progesterone production by luteal cells. At days 12–14 in cyclic pigs, concentrations of $PGF_{2\alpha}$ are higher in the utero–ovarian vein compared with those of PGE_2. Conversely, PGE_2 concentrations are higher than $PGF_{2\alpha}$ concentrations in utero–ovarian veins of pregnant pigs (Christenson *et al.*, 1994). Therefore,

differences observed in serum progesterone concentrations reflect the influence of endogenous hormones; however, the functional status of luteal cells from cyclic and pregnant gilts appears to be similar under *in vitro* conditions.

The results of the present study indicate that LH and PGE_2 act via different second message pathways in SLC and LLC. The results also confirm previous reports (Tekpetey and Armstrong, 1991; Richards *et al.*, 1994) that showed that 8-bromo-cAMP and forskolin stimulate progesterone secretion by pig SLC in a similar fashion to LH. This observation suggests that cAMP mediates LH-induced progesterone secretion as previously demonstrated in ovine corpora lutea (Hoyer and Niswender, 1986). Four PGE receptor subtypes, EP_1, EP_2, EP_3 and EP_4, have been identified and cloned from various tissues and species (Coleman *et al.*, 1994; Regan *et al.*, 1994; Katsuyama *et al.*, 1995; Negishi *et al.*, 1995). EP_1 couples to an unidentified G protein to increase intracellular Ca^{2+}. EP_2 and EP_4 couple to Gs to increase cAMP, whereas EP_3 couples to Gi, Gs or Gq to increase cAMP, decrease cAMP or activate the phospholipase C pathway, respectively, depending on the isoform of the receptor (Coleman *et al.*, 1994). The present results show that 8-bromo-cAMP and forskolin inhibit progesterone production by LLC collected on days 12 and 14. These data suggest that PGE_2 does not act by increasing cAMP and that the receptor subtype may not be EP_2 and EP_4 in pig LLC. EP_3 receptors have been demonstrated in ovine (Pierce *et al.*, 1995) and bovine (Anderson and Wiltbank, 1995) corpora lutea. It is of interest that pig LLC differ from the luteinized granulosa cells that produce cAMP in response to PGE_2 and that PGE_2 acts like cAMP to enhance progesterone production (Grimes *et al.*, 1993).

The protein kinase C pathway is another signal transduction pathway in luteal cells. Protein kinase C activity increases nearly threefold in mature corpora lutea relative to postovulatory follicles (DeManno *et al.*, 1992). In a short (4 h) culture without LDL, phorbol-12 myristate-13 acetate, a potent stimulant of the protein kinase C pathway, induced progesterone secretion by pig LLC (Gadsby and Earnest, 1994). However, after 24 h culture, activation of protein kinase C inhibited progesterone production by pig mixed luteal cells in the presence of LDL (Brannian *et al.*, 1995). Further studies are needed to elucidate the intracellular signal pathway for PGE_2-stimulated progesterone production by LLC.

The present study also showed that 8-bromo-cAMP and forskolin inhibited progesterone production by LLC collected on days 12 and 14. The mechanism of this inhibition is not clear. One possibility is that 8-bromo-cAMP and forskolin induced apoptosis. Although the viability of LLC collected on days 12–14 did not change after 12 h culture, apoptosis may already be in progress. Aharoni *et al.* (1995) reported that cAMP and forskolin stimulated rat granulosa cell differentiation to luteal cells, with an initial increase in progesterone secretion, followed by apoptosis and suppression of progesterone secretion. Forskolin and 8-bromo-cAMP may induce or hasten apoptosis in the LLC collected from gilts at days 12 and 14, with a subsequent decrease in progesterone production.

A previous study by Tekpetey and Armstrong (1991) reported that dbcAMP and forskolin had no effect on progesterone secretion by LLC. The age of the corpora lutea was considered as mid-cycle (days 12–14) based on the morphological appearance of the samples collected at an abattoir. In the present study, days 9–12 appeared to be mid-cycle and day 14 as late-cycle, since the corpora lutea collected from half of the day 14 pigs were non-vascular and white. It is possible that hormones that stimulate cAMP production stimulate the growth of follicles and at the same time enhance regression of aged corpora lutea, and specifically the LLC. This suggestion is supported by our observation that LH inhibited progesterone secretion by LLC collected on day 14 and FSH inhibited progesterone secretion by LLC collected on day 15 but not by those collected on day 10 (Yuan *et al.*, 1993). It is well known that LH and FSH act by increasing cAMP production.

Conflicting data exist on the role of LH in the pig corpora lutea. At mid-cycle, LH stimulates progesterone secretion by SLC, but has no, or minimal, effect on progesterone secretion by LLC (Hunter, 1981; Tekpetey and Armstrong, 1991; Richards *et al.*, 1994). Wiesak *et al.* (1994) showed that LH stimulated progesterone production by LLC, but not by SLC. LH has no effect (Buhr, 1987) or induces similar, albeit minimal, progesterone production by both SLC and LLC (Yuan *et al.*, 1993). The reason for these discrepancies was discussed by Richards *et al.* (1994). In spite of these differences, the aforementioned studies reveal that LH stimulation of progesterone secretion by pig luteal cells is less than twofold, which is lower than the stimulatory effects of LH on ovine luteal cells (two- to fortyfold) (Niswender *et al.*, 1994). Hypophysectomy (Anderson and Melampy, 1967) or administration of LH antiserum (Spies *et al.*, 1967) after the preovulatory surge of LH did not interrupt subsequent function of the corpora lutea. The results of the present study showed that LH stimulated progesterone production by SLC collected on days 9 and 12, but had no effect on production by SLC collected at days 14 and 30. In addition, LH had an inhibitory effect on production by LLC collected on day 14. Similarly, it has been reported that LH has no effect on progesterone production by pig SLC and LLC at day 15 of the oestrous cycle after 24 h culture (Buhr, 1987; Yuan *et al.*, 1993). However, progesterone secretion was acutely stimulated when pig SLC, collected from sows on day 30 and day 60 of pregnancy, were superfused with a high dose of LH (10 μg in 3 ml) (Lemon and Loir, 1977). Anderson (1987) also found that LH is required from about day 14 to day 50 of pregnancy. The concentration of LH receptors on pig corpora lutea increases from early to mid-cycle (day 10), and decreases after day 12 of the oestrous cycle. Concentrations of LH receptors also increase between days 12 and 30 of pregnancy; however, the affinity of the receptor decreases at day 30 of pregnancy (Ziecik *et al.*, 1980). LH receptors exist only on SLC (Meduri *et al.*, 1992, 1996). Furthermore, the activity of adenylate cyclase, which is presumably involved in LH signal transduction in corpora lutea, increases from day 3 to day 8, and then decreases on day 13 of the pig oestrous cycle (Ritzhaupt *et al.*, 1986). Overall, the results of the present study and previous investigations suggest that LH has a moderate stimulatory role on SLC at mid-cycle, but that progesterone production by LLC is independent of LH.

Richards *et al.* (1994) reported that PGE_2 increases progesterone secretion by pig LLC but not SLC. In the present study, PGE_2 had a moderate stimulatory effect on progesterone secretion by pig SLC on days 9 and 12, while PGE_2 stimulated progesterone secretion by LLC at all stages examined. The

same *in vitro* culture system was used in the two studies. However, the previous study used prepubertal gilts and oestrus was induced by PG-600® (400 iu eCG and 200 iu hCG; Intervet, Millsboro, DE), while the present study used postpubertal, naturally cyclic gilts, which could contribute to some of the differences observed in the two studies. PGE receptors were sixtyfold more abundant on LLC than on SLC in pigs (Feng and Almond, 1996). Taken together, these data suggest the primary targets of PGE_2 are LLC.

The present study showed that progesterone secretion by SLC or LLC was less at day 30 of pregnancy than at days 9 and 12 after oestrus. Wiesak *et al.* (1994) found that mixed luteal cells from pigs at day 16 of pregnancy released less progesterone than did cells from pigs at day 12 of pregnancy or of the oestrous cycle. Thus, peripheral progesterone concentrations have consistently been found to decline on day 30 of pregnancy compared with day 10 (Bazer *et al.*, 1982) or with days 9–14 (present study).

In summary, day, treatment and interaction of day with treatment, but not pregnancy status and other interactions affected progesterone production by SLC and LLC in response to secretagogues before day 14 of the oestrous cycle or of pregnancy. These data demonstrate that SLC and LLC are regulated in different ways during the oestrous cycle and early pregnancy and suggest that PGE_2 does not act by increasing cAMP in pig LLC.

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