Journal of Reproduction and Fertility (1994) **102**, 27–34

Differential effects of LH and PGE_2 on progesterone secretion by small and large porcine luteal cells

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This study examined the effects of LH and PGE_2 on progesterone secretion by small and large porcine luteal cells with or without low-density lipoproteins. Corpora lutea were isolated from gilts 13–14 days after administering gonadotrophins; enzymatically dissociated and small and large cells were isolated by elutriation. Culture plates, 24-well, were then seeded with 150 000 small or 30 000 large luteal cells suspended in 1 ml M199 medium supplemented with 5 μg insulin ml^{-1}, 40 ng hydrocortisone ml^{-1} and with or without low-density lipoproteins (50 μg cholesterol ml^{-1}) or PGE_2. Cells were cultured for up to 24 h in a humidified incubator at 37°C under 5% CO_2 in air. The low-density lipoproteins stimulated ($P < 0.05$) progesterone secretion by large, but not small, luteal cells. Prostaglandin E_2 stimulated ($P < 0.05$) progesterone production by large luteal cells in a dose-dependent manner, and the stimulatory effects of PGE_2 were greater ($P < 0.05$) in the presence than in the absence of low-density lipoproteins. Progesterone secretion by small luteal cells was not significantly affected by PGE_2. Progesterone production by small luteal cells was enhanced ($P < 0.05$) by LH, and the stimulatory effects of LH were greater ($P < 0.05$) in the presence than in the absence of low-density lipoproteins. In the absence of these lipoproteins, LH had no effect on progesterone secretion by large luteal cells; however, in the presence of low-density lipoproteins, LH increased ($P < 0.05$) progesterone secretion by large cells, though to a lesser ($P < 0.05$) extent than the effect of LH on small cells. These data demonstrate that progesterone secretion by porcine luteal cells is stimulated differentially by LH and PGE_2 and that small luteal cells are more responsive to LH and PGE_2 acts primarily on large luteal cells.

Introduction

The corpus luteum contains at least two distinct steroidogenic cell types (Lemon and Loir, 1977; Ursely and Leymarie, 1979; Fitz *et al.*, 1982; Rodgers *et al.*, 1983; Hoyer *et al.*, 1986). The differential responses to LH of the two luteal cell types have been characterized in ruminants (Schwall *et al.*, 1986); however, the effect of LH on small and large luteal cells in the pig has not been clearly defined. For example, progesterone secretion by small and large porcine luteal cells cultured in a serum-free environment with and without low-density lipoproteins (LDL) for 24 h was either not affected (Buhr, 1987; Agu and Buhr, 1990) or was enhanced to the same extent (Yuan *et al.*, 1993) in the presence of LH. In comparison, small and large porcine luteal cells isolated from different stages of gestation (Lemon and Loir, 1977) or from animals at day 12–14 of the oestrous cycle (Hunter, 1981; Tekpetey and Armstrong, 1991) were differentially stimulated by LH: the gonadotrophin enhanced progesterone production by small luteal cells but had no or minimal stimulatory effect on large luteal cells.

Received 6 December 1993.

The secretion of progesterone by large, but not small, ovine luteal cells was increased by PGE_2 (Fitz *et al.*, 1984), supporting the observation that prostaglandin receptors are abundant on large luteal cells, but are essentially undetectable on small luteal cells (Fitz *et al.*, 1982). Addition of PGE_2 to mixed porcine luteal cell cultures enhances progesterone production (Wiesak *et al.*, 1992); however, the effects of PGE_2 on progesterone secretion by isolated small and large porcine luteal cells has not been characterized *in vitro*.

The objective of this study was to examine the effects of LH and PGE_2 on progesterone secretion by small and large porcine luteal cells cultured in a serum-free environment with and without LDL. The goal was to clarify the actions of LH further and to characterize the potential luteotrophic effects of PGE_2 on small and large luteal cells isolated from the pig corpus luteum following gonadotrophin-induced oestrus.

Materials and Methods

Materials

Medium 199 (M199; with Earle's Salts), Hank's Balanced Salt Solution (HBSS; without calcium and magnesium), Hepes,

sodium bicarbonate, insulin (from porcine pancreas), hydrocortisone, PGE_2, DNase (type I from bovine pancreas), hyaluronidase (type V from ovine testis), BSA (fraction V) and antibiotics were obtained from Sigma Chemical Co. (St Louis, MO). Collagenase (type IV) was purchased from Worthington Biochemical Corporation (Freehold, NJ). Porcine LH was generously provided by L. E. Reichert, Jr (Albany, NY). PG 600 (400 iu equine chorionic gonadotrophin, eCG and 200 iu hCG) was purchased from Intervet (Millsboro, DE), Beuthanasia-D Special from Schering-Plough Animal Health Corporation (Kenilworth, NJ) and the Coat-a-Count progesterone radioimmunoassay kits from Diagnostic Products Corporation (Los Angeles, CA).

Animals and collection of ovaries

Oestrus was induced by a single injection of PG 600 into prepubertal (5–6 months of age), crossbred gilts ($n = 4$) averaging 100 kg in body mass. Ovaries were collected aseptically 13–14 days after the administration of PG 600 (on day 8–9 of the oestrous cycle) from gilts killed by i.v. injection of Beuthanasia-D Special (58.5 mg pentobarbital sodium kg^{-1} and 7.5 mg phenytoin sodium kg^{-1}). Ovaries were obtained within 15 min of death and 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 corpora lutea isolated from ovaries of an individual gilt were qualitatively (i.e. colour, size) similar, and there was no indication of ovaries bearing two populations of corpora lutea as a result of the administration of PG 600.

Dissociation of corpora lutea

The enzymatic dissociation was performed as described by Gadsby *et al.* (1990, 1993). Briefly, corpora lutea (6–15 corpora lutea per animal) were dissected from the ovaries, diced with scalpel blades, placed in 40 ml HBSS in an Erlenmeyer flask, and incubated for 30 min at 37°C in a water bath with gentle stirring. The medium was discarded and replaced with 40 ml of an enzyme solution containing 1 mg collagenase ml^{-1}, 1 mg hyaluronidase ml^{-1}, 0.06 mg DNase ml^{-1} and 10 mg BSA ml^{-1} in HBSS, and the luteal tissue was incubated for 1 h at 37°C with gentle stirring. After incubation, the tissue clumps were allowed to settle, and the supernatant was removed. Dispersed cells were collected by centrifuging the supernatant at 300 ***g*** for 5 min, washed twice with HBSS and resuspended in 10 ml HBSS. The enzyme dissociation was repeated twice to obtain the maximal yield of luteal cells. The dissociated luteal cells were resuspended in 20 ml HBSS containing 1 mg BSA ml^{-1}, filtered through a 150 μm nylon mesh to remove undigested tissue debris and maintained on ice. The number and viability of luteal cells were determined by a haemocytometer and Trypan blue exclusion. The viability of the dissociated luteal cells was greater than 80%.

Elutriation of luteal cells

Dissociated small and large luteal cells were separated by elutriation (Gadsby *et al.*, 1990). Briefly, luteal cells were subjected to elutriation using HBSS containing 1 mg BSA ml^{-1} and 0.02 mg DNase ml^{-1}, and five fractions of 200 ml each were collected in succession. The first fraction contained predominantly erythrocytes and was collected using a buffer flow of 12 ml min^{-1} at 1360 ***g***. The second fraction contained more than 95% small luteal cells (< 25 μm in diameter) and was collected at a buffer flow of 24 ml min^{-1} at 1360 ***g***. A third fraction containing single small luteal cells and small cell clumps mixed with large luteal cells was collected at a buffer flow of 30 ml min^{-1} at 1360 ***g***. The fourth fraction contained small cell clumps mixed with large luteal cells and was harvested using a buffer flow of 30 ml min^{-1} at 1020 ***g***. The fifth and final fraction, a highly enriched large luteal cell (> 25 μm in diameter) fraction containing no more than 20% small nucleated cells, remained after fractions 1–4 had been eluted and was harvested by maintaining a buffer flow of 30 ml min^{-1} at 680 ***g***. The viability of small (fraction 2; 93.8 ± 0.8%) and large (fraction 5; 85 ± 2.1%) luteal cells was determined within 1 h after elutriation.

Incubation of luteal cells

Individual wells of 24-well plates were seeded with 150 000 small or 30 000 large luteal cells suspended in 1 ml incubation medium (M199 containing 20 mmol Hepes l^{-1}, 1 mg BSA ml^{-1}, 5 μg insulin ml^{-1}, 40 ng hydrocortisone ml^{-1}, 10 000 U penicillin ml^{-1}, 10 mg streptomycin ml^{-1} and 50 mg gentamicin ml^{-1}; pH 7.4). The plates were centrifuged at 250 ***g*** for 5 min at 4°C to sediment the cells on the bottom of the well, and 100–200 μl aliquots of medium were removed from each well receiving treatments. Various quantities of LDL in the presence or absence of LH or PGE_2 were applied (100 μl aliquots) in wells containing small or large luteal cells. Plates were incubated in a humidified incubator at 37°C under 5% CO_2 in air. Trypan blue exclusion was used to assess the viability of small and large luteal cells cultured in medium containing LDL (50 μg cholesterol ml^{-1}) after 0, 12, 18, 24 and 36 h of incubation. Each treatment was applied in triplicate wells containing luteal cells prepared from ovaries of an individual gilt, and all treatments were replicated four times with luteal cells from different gilts (i.e. $n = 4$).

Lipoprotein preparation

The LDL were harvested by differential ultracentrifugation of plasma obtained from barrows (Havel *et al.*, 1955). Blood was collected in bottles containing 1 mg EDTA ml^{-1} and the plasma decanted after centrifugation. The specific gravity of plasma was adjusted by adding potassium bromide, and the LDL fraction was harvested after centrifugation for 24 h at 200 000 ***g***. The LDL (specific gravity 1.006–1.063) fraction was dialysed, sterilized by passing it through a 0.22 μm filter, stored at 4°C and used within 3 months of preparation. The cholesterol content (1.09–1.93 mg ml^{-1}) of the LDL fraction was determined enzymatically (Allain *et al.*, 1974).

Progesterone radioimmunoassay

Medium was collected from luteal cell incubations, centrifuged at 250 ***g*** for 5 min to remove cellular debris, and

immediately stored at −20°C until the progesterone content was determined by radioimmunoassay using a commercially available kit. The standard curve obtained with standards diluted in human serum was parallel to a standard curve obtained using progesterone-supplemented PBS containing 1 mg gelatin ml^{-1}. In incubation medium supplemented with various quantities (0.125–2.1 ng ml^{-1} per tube) of progesterone, the average percentage of observed divided by expected progesterone values was 108.7 ± 4.7. The intra-assay coefficients of variation for high (2.5 ng per tube) and low (0.2 ng per tube) progesterone reference media were 3.4% and 7.2%, respectively. The interassay coefficients of variation of eight assays were 6.7% and 8.6% for high and low media, respectively. The sensitivity of the assay was 10 pg per tube.

Statistical analyses

Data were subjected to least-squares analysis of variance using the General Linear Model procedure (SAS, 1988). The statistical model for the time-course experiments was a split-plot design and included pig as a block, treatment as a whole-plot effect, time as a split-plot effect and a time by treatment interaction. Effects were tested against the appropriate mean square, and time interval means were compared by contrast (SAS, 1988). The statistical model for the remaining experiments was a randomized complete block design and included pig and treatment, or pig and cell as sources of variation. Treatment means were compared by the Student–Newman–Keuls test (SAS, 1988). Natural log transformation of data used in each analysis resulted in a normal distribution (Shapiro–Wilks W test; SAS, 1988) and homogeneous variance (Barlett's test; JMP, 1989).

Results

The viability of small luteal cells after 24 h of incubation was similar to that at the start of the experiment (0 h) (87.6 ± 0.9% versus 77 ± 1.7% for 0 and 24 h, respectively). However, by 36 h, the viability of small cells was significantly less than that at 0 h (87.6 ± 0.9% versus 64.4 ± 7.3% for 0 and 36 h, respectively; $P < 0.05$). The viability of large luteal cells (84.2 ± 1.3%) did not change over the 36 h incubation. Because the viability of small luteal cells had decreased after 36 h of incubation, but not after 24 h, luteal cells in subsequent experiments were incubated for 24 h.

LDL stimulated ($P < 0.05$) progesterone secretion by large, but not by small, luteal cells (Fig. 1). Because a maximal response was observed at approximately 50 μg ml^{-1}, subsequent experiments were conducted in the presence of an LDL supplementation of 50 μg cholesterol ml^{-1}.

To determine the time-course of progesterone secretion by small and large luteal cells under maximal stimulatory conditions during the 24 h incubation, luteal cells were incubated in medium supplemented with LDL in the presence or absence of LH or PGE_2 for 3, 6, 12, 18 and 24 h. In the presence of LDL, progesterone secretion by small and large luteal cells increased ($P < 0.05$) temporally during the 24 h incubation. Throughout the 24 h incubation, progesterone secretion by small and large luteal cells was greater ($P < 0.05$) in medium supplemented with LDL and LH or PGE_2 than in medium supplemented with LDL alone (Fig. 2).

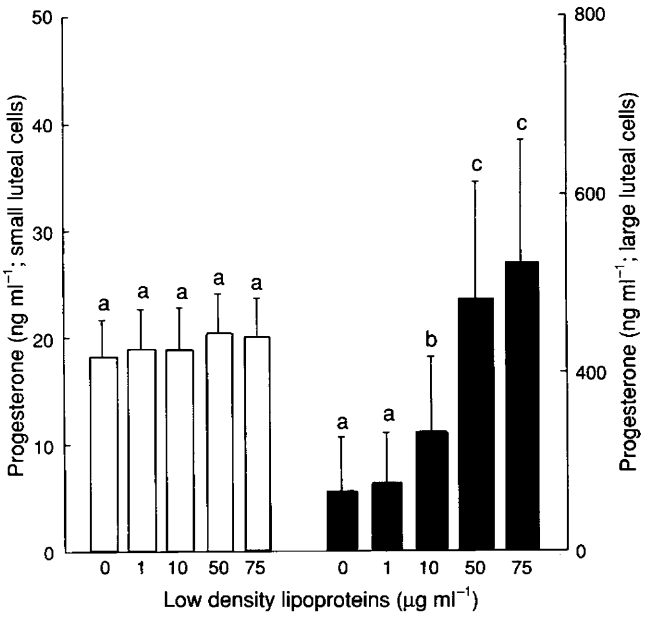

Fig. 1. Effect of various amounts of low density lipoproteins (0–75 μg cholesterol ml^{-1}) on progesterone secretion by small (□) and large (■) porcine luteal cells after 24 h of culture. Results are means + SEM of four replicates. Within each replicate, treatments were conducted in triplicate. Within each cell group, bars with different superscripts are significantly different ($P < 0.05$) values.

Small and large cells were incubated with medium containing different quantities of LH and PGE_2 with and without LDL for 24 h to examine the effects of LH and PGE_2 on progesterone secretion by luteal cells (Figs 3 and 4). Progesterone secretion by small luteal cells was enhanced ($P < 0.05$) by LH, and the stimulatory effect of LH was greater ($P < 0.05$) in the presence than in the absence of LDL (Fig. 3). Progesterone secretion by small luteal cells was not affected by PGE_2 either in the presence or absence of LDL (Fig. 3).

In the absence of LDL, LH had no effect on progesterone secretion by large luteal cells, but each dose of LH enhanced ($P < 0.05$) progesterone secretion in the presence of LDL to the same extent (Fig. 4). In the presence of LDL, the percentage change in progesterone secretion by large luteal cells (131.6 ± 2.0%) in response to LH relative to the progesterone content of medium with LDL only after 24 h of culture was less than the relative LH-induced response in small luteal cells (252.5 ± 20.6%; $P < 0.05$). The addition of PGE_2 stimulated ($P < 0.05$) progesterone secretion by large luteal cells in the absence and presence of LDL, with a maximal stimulatory dose of 100 ng ml^{-1} and an apparent half-maximal stimulatory dose of 6 ± 0.5 ng ml^{-1} (Fig. 4). PGE_2 had the greatest ($P < 0.05$) effect on progesterone secretion in the presence of LDL.

Discussion

Similar to the actions of PGE_2 on subpopulations of ovine luteal cells (Fitz *et al.*, 1984), the present data demonstrated that

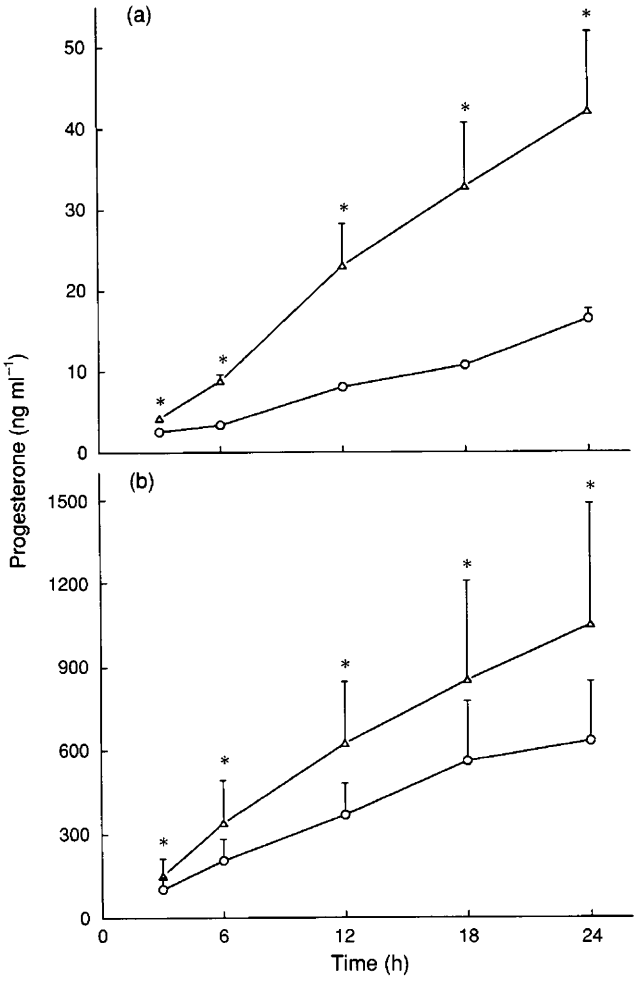

Fig. 2. Effect of (a) porcine LH [0 ng ml^{-1} (○); 50 ng ml^{-1} (△)] or (b) PGE_2 [0 ng ml^{-1} (○); 100 ng ml^{-1} (△)] on progesterone secretion by (a) small and (b) large porcine luteal cells in the presence of low density lipoproteins (50 μg cholesterol ml^{-1}) throughout a 24 h incubation period. Results are means + SEM of four replicates. Within each replicate, treatments were conducted in triplicate. Within each time point, bars with an asterisk (*) indicate a significant difference ($P < 0.05$) in values between treatments.

progesterone production by large, but not by small, luteal cells was enhanced by PGE_2 in a dose-dependent manner. Progesterone secretion by mixed porcine luteal cells is stimulated by PGE_2 (Wiesak *et al.*, 1992), and the present results extend this observation by demonstrating the differential effect of PGE_2 on the two types of luteal cell. Fitz *et al.* (1982) reported that in sheep the majority of specific PGE_2-binding sites are located on the large luteal cell rather than on the small luteal cell. On the basis of these data, it is postulated that the luteotrophic actions of PGE_2 on the corpus luteum are directed primarily towards the large luteal cell.

During the oestrous cycle of the pig, serum concentrations of PGE_2 in the utero–ovarian vein and posterior vena cava range from concentrations no greater than approximately 0.3 ng ml^{-1} on days 2–13 to peak values of approximately 10 ng ml^{-1} on days 14–16 (Bell *et al.*, 1990; Christenson *et al.*, 1994). In addition, porcine luteal cells have been shown to secrete PGE_2 throughout the luteal phase of the oestrous cycle (Jones and Gadsby, 1993). The uterus or corpus luteum are therefore potential sources of PGE_2, especially during the late luteal phase of the oestrous cycle.

It has been postulated that PGE_2 plays a role in maintaining the corpus luteum during early pregnancy in pigs. The chronic i.u. infusion of PGE_2 to gilts beginning on day 7 of the oestrous cycle (Akinlosotu *et al.*, 1986) or the intraluteal administration of PGE_2 to gilts on day 11 of the oestrous cycle (Ford and Christenson *et al.*, 1991) gives protection against the luteolytic actions of $PGF_{2\alpha}$. In addition, Akinlosotu *et al.* (1986) noted that PGE_2 increases the plasma concentation of progesterone, indicating a direct luteotrophic effect of PGE_2 on luteal cells. In unilaterally pregnant gilts, PGE_2 concentrations are greater in utero–ovarian venous blood draining the gravid uterine horn compared with the non-gravid horn. This increase in the concentration of PGE_2 is associated with a high progesterone content and mass of corpora lutea in the ipsilateral ovary, suggesting a role for conceptus-associated increases in uterine PGE_2 production in the local stimulation of luteal function during early pregnancy in the pig (Christenson *et al.*, 1994). In contrast, the chronic i.u. infusion of PGE_2 to gilts on day 12 of the oestrous cycle (Schneider *et al.*, 1983) or the insertion of beads containing oestradiol and PGE_2 into the uterine lumen of gilts on day 10 after oestrus (Laforest and King, 1992) has no effect on plasma progesterone concentrations or on the duration of the oestrous cycle.

Grimes *et al.* (1993) addressed the potential intracellular mechanisms of action of PGE_2 and reported that the prostaglandin stimulates the production of cAMP by luteinized, porcine granulosa cells and acts in a manner similar to cAMP in enhancing progesterone production; this suggests that the luteotrophic actions of PGE_2 may be mediated by cAMP. In addition, PGE_2 induces progesterone synthesis and transcription of the genes encoding the steroidogenic enzymes 3β-hydroxysteroid dehydrogenase and P450 side-chain cleavage in luteinized porcine granulosa cells (Li *et al.*, 1993). The authors also noted that PGE_2 reverses the inhibition of steroidogenesis and transcription of the genes encoding steroid enzymes caused by $PGF_{2\alpha}$.

The present study demonstrated that LH, in the presence of LDL, induces a greater increase in progesterone secretion by small luteal cells (2.5 times) compared with large luteal cells (1.3 times); this is comparable to the effects of LH on small and large porcine luteal cells isolated from different stages of gestation (Lemon and Loir, 1977) and days 12–14 (but not day 6.5) of the oestrous cycle (Hunter, 1981; Tekpetey and Armstrong, 1991). In contrast, Buhr (1987) reported that porcine LH (10–100 μg ml^{-1}) after 24 h of culture either in the presence or absence of LDL has no effect on progesterone secretion by small and large porcine luteal cells isolated on day 10 of the oestrous cycle. However, Yuan *et al.* (1993) noted that LH (1 μg ml^{-1}) in the presence of LDL induces a similar increase (approximately 1.4 times) in progesterone secretion by small and large luteal cells from day 10 of the oestrous cycle. The studies of Buhr (1987) and Yuan *et al.* (1993) *in vitro* used a 12–16 h preincubation before adding treatments, which may have accounted for the lack of, or minimal, steroidogenic response to LH by the small luteal cell. Bovine theca cells that had undergone luteinization *in vitro* were unable to maintain

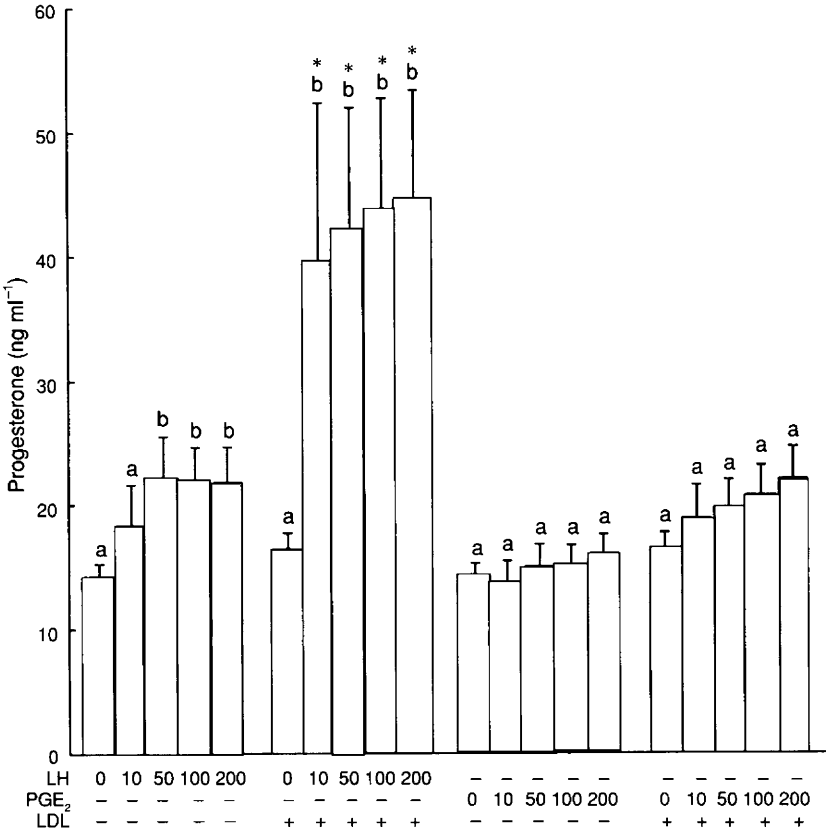

Fig. 3. Effect of various amounts of porcine LH (0–200 ng ml^{-1}) and PGE_2 (0–200 ng ml^{-1}) on progesterone secretion by small porcine luteal cells in the absence and presence of low density lipoproteins (LDL; 50 μg cholesterol ml^{-1}) after 24 h of culture. Results are means + SEM of four replicates. Within each replicate, treatments were conducted in triplicate. Within each treatment group, bars with different superscripts represent significantly different ($P < 0.05$) values. Bars with an asterisk (*) indicate a significant difference ($P < 0.05$) in values between cells treated with LH only and LH plus LDL.

progesterone production and synthesis of cholesterol side-chain cleavage enzymes in the absence of chronic cAMP stimulation (Aflalo and Meidan, 1993). The absence of a cAMP stimulant for an extended period may cause a disruption in the steroidogenic machinery of the small luteal cell that renders these cells unresponsive to a subsequent challenge with LH.

Hypophysectomy (see Anderson and Melampy, 1967) or administration of LH antiserum (Spies *et al.*, 1967) after the preovulatory surge of LH does not disrupt the subsequent function of the porcine corpus luteum, indicating that LH is not essential for maintaining the porcine corpus luteum during the oestrous cycle. However, high-affinity LH and hCG receptors have been identified in the porcine corpus luteum throughout the luteal phase (Ziecik *et al.*, 1980; Rao and Edgerton, 1984), and Ekstrom and Hunzicker-Dunn (1990) demonstrated that pig luteal membranes extracted with urea display LH- or hCG-responsive, GTP-dependent adenylate cyclase activity. These studies support the postulate that LH has a physiological role, if only supplemental, in regulating the steroidogenic function of the porcine corpus luteum during the oestrous cycle.

The physiological differences, if any, between induced and spontaneous corpora lutea have not been thoroughly elucidated. Segal and Baker (1973) reported that corpora lutea are normal in structure and progesterone content on days 5 and 10 after gonadotrophin-induced ovulation in prepubertal gilts compared with spontaneous corpora lutea, but had partially regressed by day 20 in pregnant gilts. In hysterectomized gilts, induced corpora lutea were maintained for 30 days after the administration of gonadotrophins, indicating that the failure of the prepubertal gilt to maintain pregnancy may be due to an imbalance between the luteolytic and luteotrophic mechanisms (Rampacek *et al.*, 1976). It has been shown that corpora lutea induced by administering superovulatory doses of eCG (1000–1500 iu) and hCG (500 iu) to gilts (120–130 days of age) have an increased susceptibility to uterine luteolysin (Puglisi *et al.*, 1978) and a decreased sensitivity to gonadotrophins (Kineman *et al.*, 1987). In addition, daily injections of 500 iu hCG cause regression of induced, accessory corpora lutea but not of spontaneous corpora lutea located on the same ovary of mature cyclic gilts (Rampacek *et al.*, 1992), as well as regression of gonadotrophin-induced corpora lutea of prepubertal gilts (Rampacek *et al.*, 1985). These data suggest that

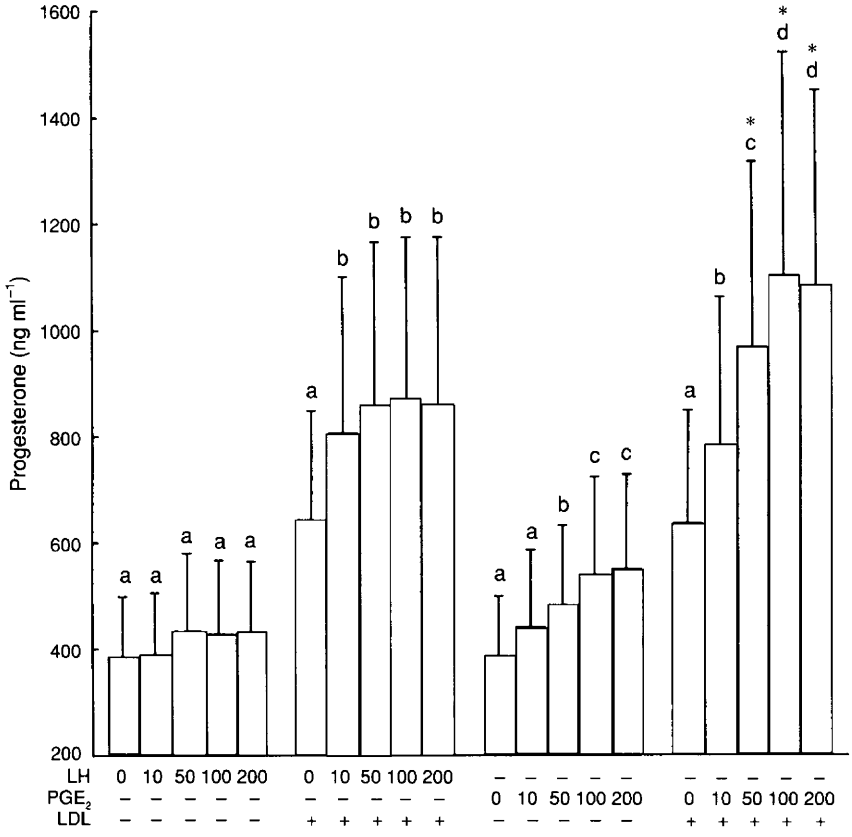

Fig. 4. Effect of various quantities of porcine LH (0–200 ng ml^{-1}) and PGE_2 (0–200 ng ml^{-1}) on progesterone secretion by large porcine luteal cells in the absence and presence of low density lipoproteins (LDL; 50 μg cholesterol ml^{-1}) after 24 h of culture. Results are means + SEM of four replicates. Within each replicate, treatments were conducted in triplicate. Within each treatment group, bars with different superscripts represent significantly different ($P < 0.05$) values. Bars with an asterisk (*) indicate a significant difference ($P < 0.05$) in values between cells treated with PGE_2 only and PGE_2 plus LDL.

induced corpora lutea, regardless of the physiological age of the gilt, are functionally different from spontaneous corpora lutea.

In contrast, the ratio of the number of large luteal cells to the total number of luteal cells and the basal lipoprotein-stimulated progesterone production by luteal cells are similar in induced and spontaneous corpora lutea (Kineman *et al.*, 1987; Agu and Buhr, 1990). Ovarian cyclicity, reproductive performance and the ability to initiate and maintain pregnancy are similar in eCG-treated (400 iu), hCG-treated (200 iu) gilts and gilts mated during spontaneous oestrus (Britt *et al.*, 1989). Karalus *et al.* (1990) reported that more of the heavier and older gilts (> 160 days of age, 75–85 kg in body mass) that had been induced to ovulate with gonadotrophins continued to be cyclic or were pregnant compared with lighter and younger gilts, indicating that reproductive performance is dependent on age and mass of the prepubertal gilt at the time of gonadotrophin treatment. Moreover, our preliminary experiments (data not shown) showed no differences in the stimulatory effects of LDL, LH or PGE_2 on progesterone production by small and large luteal cells from eCG-induced (400 iu) or hCG-induced (200 iu) or spontaneous corpora lutea of gilts (160–180 days of age, 100–120 kg body mass). These data indicate that gonadotrophin-induced corpora lutea of prepubertal gilts, especially of older and heavier gilts like those used in the present study, are functionally comparable to spontaneous corpora lutea.

Studies *in vitro* and *in vivo* have indicated that maximal rates of progesterone secretion by luteal cells depend on the availability of relevant lipoprotein-carried cholesterol (Grummer and Carroll, 1988). In pigs, LDL appears to be the preferred lipoprotein for progesterone synthesis by the corpus luteum (Buhr, 1987). In addition, porcine granulosa cells are highly responsive to LDL despite the absence of this lipoprotein in follicular fluid, suggesting that granulosa cells develop an ability to use LDL to facilitate progesterone synthesis upon luteinization (Veldhuis *et al.*, 1984). In the present study, LDL enhanced basal progesterone secretion by large, but not by small, luteal cells, which is similar to results of earlier studies in pigs (Buhr, 1987) and sheep (Wiltbank *et al.*, 1990). In addition, progesterone secretion by small ovine luteal cells was stimulated in a dose-dependent manner by lipoproteins in the presence of LH, indicating that LH may be required to activate some essential rate-limiting step for steroidogenesis within small luteal cells (Wiltbank *et al.*, 1990).

In the presence of LDL, LH stimulated progesterone secretion by large luteal cells, whereas LH had no effect in the

absence of LDL. In addition, the small and large luteal cells were most responsive to LH and PGE_2, respectively, in the presence of LDL. Pretreatment of porcine luteal cells with LH significantly enhances the amount of surface-bound ^{125}I-labelled LDL; this suggests that LH enhances the binding of LDL to membranes, thus increasing the uptake and metabolism of cholesterol by luteal cells (Rajkumar *et al.*, 1987). The uptake and metabolism of radiolabelled LDL and synthesis of the LDL receptor by human granulosa-lutein cells is increased by hCG (Golos *et al.*, 1985, 1986). The response of small and large luteal cells to secretagogues could therefore be enhanced in the presence of LDL because of an increased availability of cholesterol that stems from a stimulant-induced increase in the expression of the LDL receptor.

In summary, progesterone production by large, but not small, luteal cells is stimulated by PGE_2, suggesting that the luteotrophic effects of PGE_2 in pigs *in vivo* (Akinlosotu *et al.*, 1986; Christenson *et al.*, 1994) and *in vitro* (Wiesak *et al.*, 1992) are directed primarily at the large luteal cell. Small and large luteal cells were more responsive to the luteotrophic agents in the presence of LDL. These data suggest that maximal, stimulated progesterone synthesis by porcine luteal cells cultured in a serum-free environment requires supplementation of the medium with LDL, which supplies the luteal cell with an effective and usable form of cholesterol.

The authors gratefully acknowledge the technical assistance of P. Routh. They thank J. Jayawickrama for her assistance with statistical analysis of data, and K. Esbenshade and J. Britt for their helpful criticism during the preparation of this manuscript. This work was supported by the North Carolina Veterinary Competitive Grants Program.

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