EFFECT OF LIPOPROTEINS AND LUTEINIZING HORMONE ON PROGESTERONE PRODUCTION BY LARGE AND SMALL LUTEAL CELLS THROUGHOUT THE PORCINE ESTROUS CYCLE

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ABSTRACT

Large and small cells were isolated from porcine corpora lutea (CL) on d 10, 15 or 18 of the estrous cycle. They were incubated 13 to 16 h in cholesterol- and serum-free media and then supplied with 0, 10, 50 or 100 µg of either porcine low density lipoprotein (LDL) or porcine high density lipoprotein (HDL). Each dose was supplemented with 0, 10, 50 or 100 ng of porcine LH. Media progesterone (P₄) content was assessed immediately before and 2 and 24 h after addition of lipoproteins and LH. Production of P₄ by large cells always exceeded that of small cells. Day 10 large cells were stimulated by LDL, unaffected by LH, and either inhibited or unaffected by HDL. No treatment affected d 10 small cells. Day 15 large cells and small cells were stimulated by both lipoproteins (LDL>HDL). The large cells were stimulated to a small extent by LH at 2 h (P<.05). Large cells could be isolated from only two of five preparations of d 18 CL. Day 18 small cells produced a small quantity of P₄ in a response which qualitatively resembled that of d 15 small cells. Cell type, cycle stage and lipoprotein (particularly LDL) were the major effectors of P₄ production. The minimal response to LH supports the theory of autonomy of the porcine CL with respect to P₄ production. Days 10 and 15 bracket the period of commitment to luteolysis, and the nature of P₄ production by each cell type changed over that period. However, responsiveness to lipoproteins and LH, and cell presence over the cycle, hint that large cells may be more closely correlated than small cells with porcine luteal function.

(Key Words: Pigs, Corpus Luteum, Estrous Cycle, Lipoproteins, LH, Progesterone.)

Introduction

Corpora lutea (CL) of the pregnant pig contain two cell types (generally referred to as large or small) of differing steroidogenic capacity (Lemon and Loir, 1977; Lemon and Mauleon, 1982). Stage of the estrous cycle affects progesterone (P₄) production by mixed porcine luteal cells in short-term culture (Hunter, 1981; Mattioli et al., 1985; Kineman et al., 1987) and by each type of ovine luteal cell (Schwall et al., 1986). However, steroidogenesis in each type of porcine cell over the cycle has not been characterized. Large and small ovine luteal cells have different binding affinities for LH and PGF₂α (Fitz et al., 1982), but the porcine CL is regarded as autonomous with respect to LH (Hunter, 1981) and resistant to PGF₂α over much of the cycle. In an autonomous system, substrate utilization could have a large impact on P₄ production. Serum lipoproteins are precursors for luteal steroidogenesis in the cow (Pate and Condon, 1982), rat and hamster (Kim and Greenwald, 1985) and are also used by porcine granulosa cells (Veldhuis et al., 1984). The effect of homologous lipoproteins on P₄ production characteristics of large and small porcine luteal cells at various stages of the estrous cycle could elucidate factors influencing luteal function and regression in the pig.

Materials and Methods

All CL were taken from sows of proven fertility which had displayed an estrous cycle of 19 to 22 d prior to ovariotomy. The first day of standing estrus was designated d 0 and ovaries were removed on 1) d 9 or 10, 2) d 15 and 3) d 17 or 18. For convenience, these are
referred to as d 10, d 15 and d 18. Four or five cell preparations were examined for each stage. Each preparation was made from the paired ovaries of a single sow, except for d 18, which contained paired ovaries from two sows.

**Lipoprotein Preparation.** Blood was taken by jugular venipuncture from sows between d 3 and 5 of the estrous cycle. Lipoproteins were isolated from serum according to Terpstra et al. (1981). The high density lipoproteins (HDL) and low density lipoproteins (LDL) were dialyzed for a minimum of 72 h at 4°C in three changes of phosphate-buffered saline (PBS). They were then concentrated by dialyzing for 8 to 12 h against 30% polyethylene glycol (molecular weight = 20,000) in PBS. Protein concentration was determined according to Bradford (1976) using bovine gamma globulin as a standard. Lipoproteins were stored at 4°C for a maximum of 4 wk.

**Cell Preparation.** All steps were carried out at 4°C unless otherwise indicated. The incubation media was Ham's F-12 (Gibco) containing 100 U/ml polymyxin, 100 µg/ml streptomycin, 2.5 µg/ml metronidazole (Sigma) and supplemented with 2 µg/ml porcine insulin, 40 ng/ml hydrocortisone and 5 µg/ml transferrin. On excision, ovaries were placed in sterile incubation media on ice. The CL were removed from the ovary, stripped of outer connective tissue and weighed. Dissociation was carried out in 5 ml of media per g of CL. The CL were diced and incubated in a shaking water bath (37°C, 120 oscillations/min, 10 min), the tissue was allowed to settle for 5 min on ice, and the supernatant was discarded. Three further incubations (2 x 45 min, 1 x 30 min) were carried out with media containing 2 mg/ml collagenase (Type V, Sigma) and the supernatant was retained. After each incubation, aprotinin (Sigma, 1 µg/ml) was added to the aspirated supernatant. After the last incubation, any undissociated tissue was subjected to vigorous pipetting prior to removal of the supernatant. All supernatants were centrifuged (500 x g, 10 min) and pellets gently were resuspended and incubated 10 min in media containing collagenase and 1 mM EGTA. This final incubate was poured through 295-µm nylon mesh, aprotinin was added and the cells were washed three times in plain media (500 x g, 10 min). Cells were suspended in 30 ml of media and layered on a discontinuous Ficoll gradient (modified from Ursely and Leymarie, 1979) for 45 min at room temperature. The higher concentration of Ficoll containing the large cell-enriched fraction was filtered through 73-µm nylon mesh, the lower concentration containing small cells was filtered through 25-µm nylon mesh and the interface (approximately 100 to 150 ml) was discarded. Cells were pelleted and washed three times in media. Cells were counted, viability was estimated by trypan blue exclusion and concentrations were adjusted to 1 x 10³ large cells per ml in the large cell-enriched fraction and 1 x 10² small cells per ml in the small cell fraction. One-milliliter aliquots were plated in 24-well plastic culture plates and incubated overnight (37°C, 95% air/5% CO₂, 100% humidity).

**Incubation.** After overnight incubation (13 to 16 h), media was removed (0 h) and replaced with 1.0 ml of media containing 0, 10, 50 or 100 µg LH (USDA pLH-B-1, AFP-5400, NIH) or 0, 10, 50 or 100 µg of either LDL or HDL protein. In addition, each dose of lipoprotein was combined with each dose of LH. All incubations were done in duplicate. After 2 h, 500 µl was removed, replaced with identical, fresh test substances and incubated for a further 22 h (total incubation time, 24 h). All samples were stored at −20°C until assayed for P₄ content.

**Progesterone.** Samples were analyzed for P₄ content (Yuthasastrakosol et al., 1974) without ether extraction. Standards containing high, medium and low concentrations of P₄ in media gave inter-assay coefficients of variation of 11.3, 12.2 and 12.6% and intra-assay coefficients of variation of 5.9, 8.1 and 7.7%, respectively.

**Statistical Analysis.** Progesterone was expressed as µg P₄·10³ cells⁻¹·h⁻¹. Values were log-transformed to stabilize the variance and analyzed by the General Linear Models procedure (SAS, 1985). Differences were evaluated using the Bonferroni inequality (SAS, 1985) to establish an experiment-wise error rate of .05. Differences between cell types were assessed at each incubation time on each of d 10, 15 and 18 using a randomized complete block design with individual pigs as blocks. Differences among days of the cycle were assessed for each cell type at each incubation time, with individual pigs within each day as the error term. Treatment effects were assessed by factorial analysis for each cell type at each incubation time of each day. Numbers of large and small cells per g CL were analyzed by analysis of variance.
Results

The cell isolation procedure produced very pure small-cell preparations, but the large-cell populations varied in their ratio of large: small from 1:3 to 1:13.1 (mean ± standard error, 1:4.2 ± .7). Values for large cells thus represent production by populations enriched in large cells. Large cells typically exceeded 30 μm in diameter; small cells were under 20 μm diameter. Erythrocytes were a very minor contaminant in the small cell fraction. Statistical analysis detected no consistent variation or interaction of purity of cell preparation on P4 content. All data are given as least-squares means.

The number of large cells per g of CL did not differ among d 10, 15 and 18 (14,200 ± 540, 5,700 ± 150 and 400 ± 300, respectively). It should be noted that, despite combining CL from four ovaries for each d 18 preparation, three of five preparations had no large cells. The number of small cells per g of CL declined after d 15 (d 10 with 94,400 ± 25,400 equaled d 15 with 84,800 ± 20,300, which were less than 18 with 16,000 ± 4,900; P<.01). At all stages, the number of small cells per g exceeded the number of large.

The 0-h concentration of P4 represents unstimulated, or basal P4 production. This basal P4 production by large cells exceeded that of small cells on all days (figure 1, P<.0001). Basal production by large cells was greatest on d 10 (d 10 > d 15, P<.0001; d 15 = d 18; values for d 18 were generated from the limited number of cells found in two preparations). Basal production of P4 by small cells declined over the cycle (d 10 > d 15, P<.0001; d 15 > d 18, P<.0004).

Secretion of P4 by d 10 large cells was stimulated by LDL in a dose-dependent fashion and inhibited by HDL at 2 h (figure 2). At 24 h, LDL was stimulatory but HDL had no significant effect. No treatment affected P4 release by d 10 small cells at either 2 or 24 h. By d 15 (figure 3) both LDL and HDL stimulated P4 secretion by large cells, with LDL being more stimulatory than HDL at 24 h. The large cells were significantly stimulated by the highest dose of LH at 2 h only (figure 4) and there was no LH × lipoprotein interaction (P=.067). Day 15 small cells also were stimulated by both lipoproteins at 2 and 24 h, with LDL being more stimulatory at both times (figure 3). The 50 μg dose of LDL provided maximum stimulation to small cells. Small cells were not stimulated by LH. The few large cells

![Figure 1](image1.png)  
**Figure 1.** Basal (0 h) progesterone production at three stages of the estrous cycle. Columns that do not have a common letter differ (P<.05).

![Figure 2](image2.png)  
**Figure 2.** Effect of homologous lipoproteins on large cell-enriched fraction isolated on d 10. Within each incubation time, columns that do not have a common letter differ (P<.05).
found on d 18 appeared to be stimulated by 100 µg LDL (14.3 ± 9.5 vs 188.8 ± 9.5), but the small numbers of cells available for incubation renders the data unreliable. The d 18 small cells were stimulated equally by 100 µg of LDL or HDL at 2 h, but at 24 h, LDL was more stimulatory (figure 5).

Discussion

Response of each cell type to each of the potential stimulators tested was dependent on stage of the estrous cycle. Large cells always produced greater quantities than small cells under both basal and stimulatory conditions. There was large animal-to-animal variability in P4 production, similar to that noted by Lemon and Mauléon (1982). The statistical model included individual animals to prevent this variability from obscuring treatment responses. Luteinizing hormone had very little effect on P4 production by either cell type, a finding unique to this study. Mixed porcine luteal cells

![Figure 3. Effect of homologous lipoproteins on large cell-enriched (L) and small cell (S) fractions isolated on d 15. Within each incubation time and cell fraction, columns that do not have a common letter differ (P<.05).](image-url)
in short-term culture were stimulated by 1 μg/ml bLH (Hunter, 1981), or a range of 1 nM to 1 μM bLH, with 1 μM being maximally effective (Mattioli et al., 1985). Large cells and small cells from pregnant sows were stimulated when superfused by 10 μg pLH either over 18 min (Lemon and Loir, 1977) or when the 10 μg was given as a pulse (Lemon and Mauléon, 1982). All these studies differed in either cell preparatory techniques, incubation conditions and/or type and dose of LH than those employed in the current study. The large and small cells in the present study were in medium-term culture and were exposed to homologous LH at lower, and presumably more physiological, doses. These same levels were capable of stimulating P4 production by ovine (Fitz et al., 1982) and bovine (Pate and Condon, 1982) luteal cells. In other species (Fitz et al., 1982; Koos and Hansel, 1981) and in swine (Lemon and Mauléon, 1982), small cells were more responsive than large cells to LH. Possibly small cells require high levels of LH for stimulation, or LH induces release of preformed P4. Such release could have occurred in the pre-treatment incubation period utilized here. If, however, LH stimulated release of P4, an additive effect of LH and lipoproteins could be expected, and this did not occur. The minimal stimulatory effect of LH is consistent with the theory of an autonomous CL (Hunter, 1981).

Absolute amounts of P4 at 2 h always exceeded 24 h. Others have noted that P4 production by porcine and primate luteal cells declines after 4 h (Stouffer et al., 1976; Mattioli et al., 1985). Luteal cell viability in the current study was approximately 70% at 24 h, so reduction was not due to cell death. Interestingly, response to lipoproteins was proportionally greater at 24 h than at 2 h. Lower hourly P4 secretion rates may reflect product inhibition of the 3β-hydroxysteroid-dehydrogenase-isomerase complex (Caffrey et al., 1979; Sawyer et al., 1979). Alternatively, the response of porcine luteal cells to lipoproteins could suggest that substrate is limiting by 24 h.

Specific differences in P4 production varied among days of the estrous cycle for both large cells and small cells. Although d 15 cells released more P4 than d 18 cells, the qualitatively similar responses of cells at these ages suggest that d 15 cells were regressing. Small cells were, however, more responsive to lipoprotein stimulation than d 18 cells (0 h, d 15 = d 18; 2 h, d 15 > d 18). While this was true for both
cell types, large cells were so infrequently obtained on d 18 as to suggest that those found originated from sows with naturally longer cycles. Lemon and Loir (1977) noted that large cells were essentially absent from d 18 CL but were found in CL at d 30 of pregnancy; Kineman et al. (1987) found large cells in increasing frequency from d 14 to d 26 after hysterectomy. This hints at a correlation of the presence of large cells with luteal function. Alila and Hansel (1984) found large cells of the early bovine CL to be derived from granulosa cells, while large cells in older CL may have developed from theca or stem cells. The granulosa-derived large cells disappeared through the cycle and no large cells survived past d 215 of pregnancy. The ratio of large to small cells in the ovine CL increased through the estrous cycle due to an overall increase in cell size, although this size increase was not seen in CL harvested near the onset of anestrus (Schwall et al., 1986). Porcine CL may not perpetuate a large cell population in the presence of a non-gravid uterus.

There were pronounced differences in lipoprotein utilization between d 10 and 15, which represent stages before and after the porcine CL is committed to regression. Granulosa cells in the human (Carr et al., 1982) and pig (Veldhuis et al., 1984) are highly responsive to LDL despite absence of this lipoprotein in follicular fluid. The latter authors suggested that granulosa cells develop an ability to utilize LDL to facilitate P4 synthesis at luteinization. The current study supports this, since at the earliest luteal stage examined only the large cells increased P4 release when supplied with LDL. Although small cells may have been utilizing stored cholesterol to support steroidogenesis at a maximal rate, such stores should have been at least somewhat depleted during the dispersion and pre-incubation in cholesterol- and serum-free media. The lack of response suggests that d 10 small cells lack receptors for lipoproteins. Low density lipoprotein may be the preferred lipoprotein for P4 synthesis for porcine CL because HDL was not only less stimulatory to both cell types at d 15, but was inhibitory for d 10 large cells. Cultured porcine granulosa cells, the presumptive precursors for large cells, show a similar preference for LDL (Veldhuis et al., 1984).

This study has demonstrated a cell-specific change in luteal P4 production over the period of commitment to luteolysis and supports the suggestion (Hunter, 1981) that the porcine CL is autonomous with respect to LH through much of the luteal phase. Substrate utilization was a potent effector of P4 production and homologous LDL was the preferred substrate. Large cells may be more closely correlated with luteal function than small cells since large cells responded to pLH, were more sensitive to lipoproteins and had virtually disappeared by d 18. However, response of each cell type to LDL changed before and after commitment to luteolysis and either or both of these cell types may yet prove to be involved in onset of luteal regression. The effect of luteolysins on each cell type is currently being examined to determine if luteolysis is correlated with a cell-specific increase in sensitivity to inhibitors, in addition to the changes in P4 synthesizing capacities documented here.

Literature Cited


LUTEAL CELL PROGESTERONE IN THE PORCINE CYCLE


