COMPARISON OF INDUCED CORPORAL LUTEA FROM PREPUBERAL GILTS AND SPONTANEOUS CORPORAL LUTEA FROM MATURE GILTS: IN VITRO PROGESTERONE PRODUCTION


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ABSTRACT

Prepuberal (P) gilts were induced to ovulate with pregnant mare serum gonadotropin followed 72 h later by human chorionic gonadotropin (hCG). Three P gilts and three mature (M) gilts each were ovariectomized on d 10, 14, 18, 22 and 26 (d 0 = day of hCG for P gilts and onset of estrus for M gilts). Gilts ovariectomized on d 14, 18, 22 and 26 were hysterectomized on d 6 to ensure maintenance of the corpora lutea (CL). Two to five grams of minced luteal tissue were dispersed using collagenase and hyaluronidase in HEPES buffered salt solution supplemented with glucose and bovine serum albumin. Dispersed cells were rinsed in Dulbecco's Modified Eagle Medium (DMEM), counted (ratio of large to total number of luteal cells determined) and then incubated for 1 h in DMEM. With aliquots standardized to 2.5 X 10^4 viable, large cells (>25 µm diameter) were incubated in 1 ml DMEM for 2 h in the presence of either 10, 50, 100 or 1,000 ng luteinizing hormone (LH); .1, 1, 10 or 100 ng hCG; 10, 100 or 1,000 ng norepinephrine (NE) or either .75, or 1.5 mM dibutyryl cyclic adenosine monophosphate (dbcAMP). Progesterone (P4) in the medium was quantified by radioimmunoassay. Basal P4 production (no P4 stimulator added to the medium) on d 10, 14, 18, 22 and 26 for P gilts was 246 ± 9, 66 ± 4, 64 ± 6, 41 ± 3 and 69 ± 6 ng/ml medium, respectively, and for M gilts was 281 ± 12, 128 ± 8, 53 ± 4, 82 ± 6, 101 ± 5 ng/ml medium, respectively. Luteal cells from M gilts were more responsive to LH, hCG and NE than those of P gilts on d 10 and 14 (P<.1), but there were no differences in large to total luteal cell ratios between induced and spontaneous CL. These results indicate that the induced CL of the prepuberal gilt are less sensitive to gonadotropins than the spontaneous CL of the mature gilt.

(Key Words: Pigs, Corpus Luteum, Progesterone.)

Introduction

Prepuberal gilts fail to maintain pregnancy to 25 d after induced ovulation (Shaw et al., 1971; Segal and Baker, 1973; Rampacek et al., 1976b). Administration of progesterone (P4) or gonadotropins following induced ovulation increased the percentage of prepuberal gilts that maintained pregnancy (Shaw et al., 1971; Ellicott et al., 1973; Segal and Baker, 1973; Rampacek et al., 1976b), indicating that luteotropic support was adequate. However, hysterectomy following induced ovulation resulted in luteal maintenance to at least d 30 (Segal and Baker, 1973; Rampacek et al., 1976a), indicating that luteotropic support was adequate if the uterine luteolysin was removed. In addition, induced corpora lutea (CL) of prepuberal gilts were more sensitive to prostaglandin F2α (PGF2α)-induced luteolysis than
spontaneous CL of mature gilts (Puglisi et al., 1978, 1979). Also, daily administration of human chorionic gonadotropin (hCG) caused luteal regression in hysterectomized prepuberal gilts induced to ovulate, but not in hysterectomized mature gilts (Rampacek et al., 1985). Therefore, the inability of the prepuberal gilt to maintain pregnancy after induced ovulation might involve an altered response of induced luteal cells to luteotropic and luteolytic agents.

This study was conducted to determine if induced CL from prepuberal gilts and spontaneous CL from mature gilts differed in their response to various stimulators of P₄ secretion, in vitro. In addition, the ratios of large to total luteal cells were compared between induced and spontaneous CL.

**Materials and Methods**

**Animals.** Fifteen crossbred prepuberal (P) gilts, 120 to 130 d of age were induced to ovulate with a single im injection of 1,500 IU pregnant mare serum gonadotropin (PMSG) followed 72 h later by an im injection of 500 IU hCG (day of hCG = d 0). Fifteen mature (M) gilts from the same population as the prepuberal gilts and which had displayed one or more estrous cycles of 18 to 22 d (onset of estrus = d 0) were used. Three P gilts and three M gilts were ovariectomized on d 10, 14, 18, 22 or 26. Gilts ovariectomized on d 14, 18, 22 and 26 were hysterectomized on d 6 to ensure luteal maintenance. On d 26, sufficient quantities of luteal tissue were recovered from only 2 P and 2 M gilts.

**Tissue Preparation and Incubation.** Corpora lutea from each gilt were minced and 2 to 5 g of tissue were transported to the laboratory in 20 ml of .1 M HEPES buffered salt solution supplemented with 120 mM NaCl, 5 mM KCl, 5 mM glucose, 1.5% bovine serum albumin and 1 mM CaCl₂, pH 7.4 at 4 C. The medium was decanted, 20 ml of fresh medium were added and tissue was incubated for 20 min in a Dubnoff metabolic shaker. This and all subsequent incubations were carried out at 37 C. Medium was decanted, and 20 ml of the .1 M HEPES buffered salt solution containing .3% collagenase, type 1A and .2% hyaluronidase were added to the tissue. The incubation flask was capped and incubated for 15 min. Medium and liberated cells were discarded and 20 ml of .3% collagenase in HEPES solution were added and incubated for 45 min. At the end of the 45-min incubation, the tissue suspension was gently drawn in and out of a glass pipette to facilitate tissue disruption. The supernatant was decanted into a 50-ml tube and centrifuged for 5 min at 120 × g, 4 C. The supernatant was decanted and the pellet was washed 3 times in 10 ml Dulbecco's Modified Eagle Medium (DMEM) at pH 7.4, 4 C. The pellet was resuspended in 10 ml DMEM, gassed with 95% O₂:5% CO₂ (95:5), capped and stored at 4 C until additional cells were liberated. The tissue remaining after the first enzymatic digestion with .3% collagenase was resuspended in .3% collagenase in HEPES solution, and incubated for an additional 45 min. The supernatant was treated, as was the supernatant from the first enzymatic digestion.

Liberated cells were pooled from the first and second enzymatic dissociations. The pooled suspension was filtered through a 240-µm and then a 64-µm nylon mesh filter, in series, using a Swinnex 22-mm filter holder and a 20-ml plastic syringe. An additional 10 ml DMEM at 4 C were washed through the filters, leaving a final cell suspension volume of 30 ml. A 200-µl aliquot of the cell suspension was mixed with 200 µl of .2% trypan blue stain, placed on a hemocytometer and examined under 1000x magnification to determine the number of viable large luteal cells (25 to 50 µm). Viable cells excluded the stain. Another 200-µl aliquot was mixed with 400 µl of Rappaport stain, placed on a hemocytometer and examined under 450x magnification to count viable small luteal cells (10 to 20 µm). Viable cells had clear cytoplasm and dark staining nuclei. Ratios of viable, large luteal cells to the total number of viable cells (large and small) were calculated. While cell number and viability being determined, the cell suspension was gassed (95:5), capped and incubated for 1 h. The suspension was then centrifuged for 5 min at 120 × g; the supernatant was discarded. The pellet was resuspended in DMEM to an appropriate volume which would result in 2.5 × 10⁴ viable, large luteal cells/100 µl. Aliquots of 100 µl were placed in 16-× 100-mm glass culture tubes. Four tubes each were incubated with DMEM containing .38 µg lipoprotein or

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* Research Organics, Inc., Cleveland, OH.
* Sigma, St. Louis, MO.
* Gibco, Grand Island, NY.
* Millipore, Bedford, MA.
* Miles Laboratory, Elkhart, IN.
DMEM containing .38 μg lipoprotein and 10, 50, 100 or 1,000 ng porcine luteinizing hormone (USDA, pLHB1); .1, 1, 10 or 100 ng hCG (CR121; NIH); 10, 100 or 1,000 ng norepinephrine (NE); .75, or 1.5 mM dibutyryl cyclic adenosine monophosphate (dbcAMP). Each tube was brought to a final incubation volume of 1 ml with DMEM. Tubes were gassed (95:5), capped and incubated for 2 h. Culture tubes were then centrifuged at 1,800 x g for 10 min at 4°C; supernatant was decanted into culture tubes and frozen at 20°C until hormone analysis.

Hormone Assay. Progesterone in the medium was quantitated by a single antibody radioimmunoassay using the procedure of Kraeling et al. (1981), with the modification that the medium was not extracted. Addition of 2, 4, 6, 8, 10, 16, 20, 40 and 80 ng of P4 to 1 ml of medium resulted in the recovery of 1.9, 4.8, 5.9, 7.3, 8.7, 14.7, 18.9, 44.0 and 90.4 ng, respectively, measured in the assay. A dose-response curve for 25 to 200 μl of medium containing 10 ng/ml P4 was parallel (P>.2) to the P4 standard curve. Intra- and inter-assay coefficients of variation were 6.5% (n = 24) and 17.2% (n = 26), respectively.

Statistical Analysis. Data were subjected to analysis of variance using the General Linear Models procedure of Statistical Analysis System (SAS, 1982). The statistical method used to assess differences in response to P4 stimulators between ages (P vs M) within a given day was a split-plot regression across dose levels using pigs within age as error for testing age. The dose response was evaluated using P4 production of cells in medium containing only lipoprotein as the zero dose (control); slope comparisons among doses for each age within day were made. To evaluate trends throughout the days of the study for basal (lipoprotein but no P4 stimulators present) P4 production and luteal cell ratios, a regression analysis across time, as above, was applied.

Results
Basal in vitro P4 production (mean ± SE) on d 10, 14, 18, 22 and 26 for luteal cells of P gilts was 246 ± 9, 66 ± 4, 64 ± 6, 41 ± 3 and 69 ± 6 ng/ml of medium, respectively, and for M gilts was 281 ± 12, 128 ± 8, 53 ± 4, 82 ± 6 and 101 ± 5 ng/ml of medium, respectively. Basal P4 production was not different between P and M luteal cell preparations (P>.1), but there was a day effect (P<.05). Progesterone production decreased from d 10 to 14 and from d 14 to 18, remained low on d 22, and increased on d 26 to values similar to those on d 14.

Figure 1. Progesterone secretion of cells from induced corpora lutea (CL) of prepuberal (P) gilts or spontaneous CL of mature (M) gilts at various days following induced ovulation or estrus in the presence of varying doses of luteinizing hormone (LH). The pooled SE for d 10, 14, 18, 22 and 26 was 13.9, 10.9, 4.2, 5.2 and 10.6, respectively.
Response of luteal cells from P and M gilts to LH is shown in figure 1. The M luteal cell response to increasing doses of LH was greater than that of the P luteal cells on d 10 (P<.1), 14 (P<.1) and 22 (P<.05). However, the response on d 18 (P<.05) and d 26 (P<.1) was similar for both P and M luteal cells.

Progesterone response of P and M luteal cells to hCG is shown in figure 2. The M luteal cell response to hCG was greater (P<.05) than that of the P luteal cells on d 10 and 14. On d 18, the response of P luteal cells to hCG was greater (P<.1) than the M luteal cell response, yet overall the response on d 18 was small com-
Figure 4. Progesterone secretion of cells from induced corpora luteal (CL) of prepuberal (P) gilts or spontaneous CL of mature (M) gilts at various days following induced ovulation or estrus in the presence of varying doses of dibutyryl cyclic adenosine monophosphate (dbcAMP). The pooled SE for d 10, 14, 18, 22 and 26 was 8.2, 7.3, 9.2, 7.5 and 10.7, respectively.

Paired with the response on d 10 and 14. There was no response (P>.1) on d 22 or d 26.

The P₄ response of luteal cells from P and M gilts to NE is illustrated in figure 3. On d 10 (P<.05) and 14 (P<.1), the M luteal cell response was greater than the P luteal cell response. On d 18, P₄ production was not affected by the presence of NE (P>.1). On d 22 and 26, NE caused an increase (P<.05) in P₄ output, yet the response was similar (P>.1) for P and M luteal cells.

Response of luteal cells to varying doses of dbcAMP is shown in figure 4. On d 10 and 14, the M luteal cell response to dbcAMP was greater (P<.05) than the P luteal cell response. Neither P nor M luteal cells responded to dbcAMP on d 18 (P>.1), while both P and M luteal cells responded (P<.05) similarly to increasing doses of dbcAMP on d 22 and 26.

The ratios of large cells to the total number of cells (large and small) are presented in table 1. There were no differences (P>.1) between P and M gilts on any of the days studied; however, there was a day effect (P<.05). The cell ratios were similar on d 10 and 14, began to increase on d 18, and increased further on d 22 and 26. The cell ratios on d 22 and 26 were greater (P<.05) than the ratios on d 10 and 14.

Discussion

The temporal pattern of basal P₄ production by porcine luteal cells, in vitro, reported in this study was similar to in vivo data reported by

| TABLE 1. RATIO OF LARGE LUTEAL CELLS TO TOTAL NUMBER OF LUTEAL CELLS (LARGE AND SMALL) OF CORPORA LUTEA FROM PREPUBERAL (P) GILTS INDUCED TO OVULATE AND FROM MATURE (M) GILTS |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Days after estrus or induced ovulation |
| **Age** | 10 | 14 | 18 | 22 | 26 |
| **P** | .070 | .057 | .062 | .161 | .120 |
| **M** | .043 | .046 | .095 | .106 | .169 |
| **Total** | .057 ± .015b | .051 ± .015b | .078 ± .015bc | .133 ± .015c | .144 ± .019c |

a Mean ± pooled SE.
b,c Means that do not have a common superscript letter differ (P<.05).
Masuda et al. (1967). Ovarian-venous plasma P₄ concentrations in gilts hysterectomized on d 5 to 7 were high on d 14, decreased on d 18, and then increased by d 25. There was no difference in the ratio of luteal cells between P and M gilts, but large cells relative to total cells increased throughout the days of the study. This is consistent with data reported by Fitz et al. (1981) in the cycling ewe. Large luteal cell numbers increased in the ovine CL as the luteal phase advanced (d 8 to 12).

Luteinizing hormone (LH) stimulated P₄ production of luteal cells from P and M gilts on d 10, 14, 18, 22 and 26; however, LH-stimulated P₄ production in P luteal cells was less than that in M luteal cells on d 10, 14 and 22. If the luteal cell response on d 10 and 14 was expressed on a per cell basis (approximately 1.2 × 10⁻³ ng/cell), these results are in agreement with those previously reported for the pig (Gregoraszczuk, 1983; Rajkumar et al., 1984). Direct comparisons between these results and those of other investigators must be made with caution due to varying incubation (or culture) times, amount of stimulator used, content of P₄ assayed (media vs cellular) and species differences.

Human chorionic gonadotropin stimulated P₄ production of M and P luteal cells on d 10, 14 and 18. Progesterone response to increasing doses of hCG was less in P luteal cells than in M luteal cells on d 10, 14 and 22. The apparent negative response on d 10 was attenuated with increasing dose. This response on d 10 was less in P cells compared with M cells on d 10 and 14, which was similar to the pattern of response observed with LH and hCG. There was no response of P or M luteal cells to NE in the medium on d 18.

Addition of dbcAMP, to bypass the receptor complex, resulted in increased P₄ production of P and M luteal cells on d 10, 14, 22 and 26. Again the response of M luteal cells was greater than that of P luteal cells on d 10 and 14. Similar to the other P₄ stimulators,dbcAMP failed to cause an increase in P₄ production on d 18. The failure of luteotropins to stimulate luteal cells on d 18 is concomitant with the low basal release of P₄ on that day.

Induced CL of P gilts are more sensitive to PGF₂α luteolysis than spontaneous CL of M gilts (Puglisi et al., 1978, 1979), and these data indicate that the induced CL of P gilts are less sensitive to gonadotropin stimulation than spontaneous CL of M gilts. This imbalance in the sensitivity of luteotropic and luteolytic agents on d 10 and 14 could alter P₄ synthesis of induced CL.

The M luteal cell responses to LH, hCG, NE and dbcAMP were greater than those of P gilts on d 10 and 14. These results indicate that the induced CL of the P gilt were less sensitive to gonadotropins and other luteal cell stimulators than the spontaneous CL of the M gilt. Prepuberal gilts induced to ovulate may not have sufficient steroid or gonadotropin priming prior to induced ovulation, resulting in deficient steroid production of the CL. These differences between induced CL of P gilts and spontaneous CL of M gilts in sensitivity to gonadotropins and PGF₂α (Puglisi et al., 1978, 1979) could explain pregnancy failure in the prepuberal gilts induced to ovulate.

**Literature Cited**


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