The Relationship of the Porcine Corpus Luteum and the Pituitary Gland

Honors Thesis

Presented in Partial Fulfillment of the
Requirements for Graduation With Distinction

The College of Food, Agricultural, and
Environmental Sciences

The Ohio State University

Autumn 1996

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Introduction

In the swine industry, an understanding of the hormonal mechanisms of the estrous cycle and pregnancy is essential to increased reproductive success. The interaction of the corpus luteum (CL) and the pituitary gland is an integral element of pregnancy maintenance in the domestic species (Cupps, 1991). However, this relationship has not been established in the pig (Hunter, 1981).

In most domestic species, a surge of luteinizing hormone (LH) from the pituitary gland stimulates ovulation and the subsequent formation of a corpus luteum. The CL then proceeds to secrete high levels of progesterone, which, in turn, suppresses further LH secretion through negative feedback on the pituitary (Cupps, 1991). If the cycle continues to completion, the uterus secretes prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), causing CL regression and permitting another LH surge. However, in the event of a pregnancy, the CL and its ability to secrete progesterone are maintained (Frandson *et al.*, 1992). Although the mechanisms of CL maintenance are not yet fully understood, it appears that there is a positive effect of LH on the maintenance of the CL of most domestic species, including the cow (Snook *et al.*, 1969).

Attempts to confirm the stimulatory relationship between LH and the porcine CL have produced conflicting results, some of which favor the theory of an autonomous porcine CL. Early *in vivo* studies found a decreased CL weight among pregnant sows treated with ovine LH antiserum (Spies *et al.*, 1967), although others noted sows hypophysectomized after the LH surge showed no difference in CL weight from untreated animals (du Mesnil du Buisson *et al.*, 1963). Additional *in vivo* research indicated that high serum levels of LH corresponded to subsequently elevated levels of progesterone (Anderson *et al.*, 1974). However, in any of these cases, other uncontrolled physiological phenomena could have contributed to these findings. In *in vitro* studies using purified populations of large and small luteal cell types, researchers have found that increased progesterone is produced by both cell populations, especially the small luteal cells, upon LH treatment (Lemon *et al.*, 1977). However, this segregation may result in the loss of necessary cellular communications between the two cell types. Studies incorporating mixed cell cultures from cycling sows have conflicting results; both high (Mattioli *et al.*, 1985) and little or no (Hunter, 1981) progesterone production were observed in response to LH treatment at various points in the cycle.

Changes in the luteal cells of pregnant sows indicate conditions favorable to LH effects. Increased LH receptors are present on luteal cells of pregnant sows (Ziecik *et al.*, 1980), suggesting an increased opportunity for this hormone to affect the cells. Increased granular endoplasmic reticulum in the CL, used for protein production, is also noted (Belt *et al.*, 1970), perhaps indicating a greater receptor synthesis. However, little work has actually been done in the area of luteal response to LH in early pregnant sows.

Progesterone is a steroid hormone, indicating that its structure is a modification of a cholesterol molecule. The cholesterol substrate for progesterone production in the corpus luteum may come from either internal or exogenous cholesterol. Provision of cholesterol, in the form of lipoproteins, increased progesterone production by dispersed bovine luteal cells in culture (Pate and Condon, 1982) Luteinizing hormone has been theorized to have an effect on the uptake of low density lipoprotein cholesterol by luteal cells (Rajkumar *et al.*, 1985). Therefore, the inclusion of low density lipoprotein (LDL) may provide an appropriate substrate for progesterone production , thus amplifying the response of luteal cells to LH.

In other species, protein kinase C (PKC) and adenyl cyclase play a role in the mechanism by which LH stimulates progesterone production (Cupps, 1991). However, the role of PKC and adenyl cyclase in progesterone production by porcine luteal cells has not yet been defined. Diminished progesterone production by porcine luteal cells exposed to PKC inhibitors might provide a clue to the cellular pathway of LH stimulation.

Materials and Methods

Luteal tissue was collected from sows in early pregnancy at the time of slaughter. The stage of tissue was determined by the size of an associated fetus.

A mixed cell population, consisting of both large and small luteal cells, was obtained from the tissue by enzymatic dissociation. The pooled corpora lutea of each sow were dissociated by treatment with a collagenase solution (Cls 4, Worthington Biochemicals, Freehold, NJ). After the cells were dissociated, they were washed to remove the collagenase solution. The cells were counted using a

hemocytometer and dispersed in Medium 199 (Gibco Co., Grand Island, NY). Each experimental group, containing a final dilution of 50,000 cells/1 ml M199, then received further treatments.

After a six hour incubation at 37° C under a 5% CO_2/95% atmosphere , the cells were separated from the incubation medium by centrifugation. The medium was stored at -20 C until analyzed for progesterone concentration using radioimmunoassay (RIA).

Progesterone concentration, as determined by RIA, was compared between treated luteal cells and untreated controls. Significant differences were determined using analysis of variance for a randomized complete block design with the individual pig as the block.

Experiment 1

This portion of the study was designed to determine if LH plays a role in progesterone production by porcine corpora lutea. The washed cells were divided into four groups: a control and three treated groups. Luteinizing hormone was added to the treated groups in graded increments of 10 ng, 100 ng, and 1 μg/ml.

Basal progesterone production by the population of mixed luteal cells tended to be stimulated by luteinizing hormone in a dose-dependent fashion. Progesterone production was significantly elevated by the highest dose of LH, 1μg /ml (Figure 1).

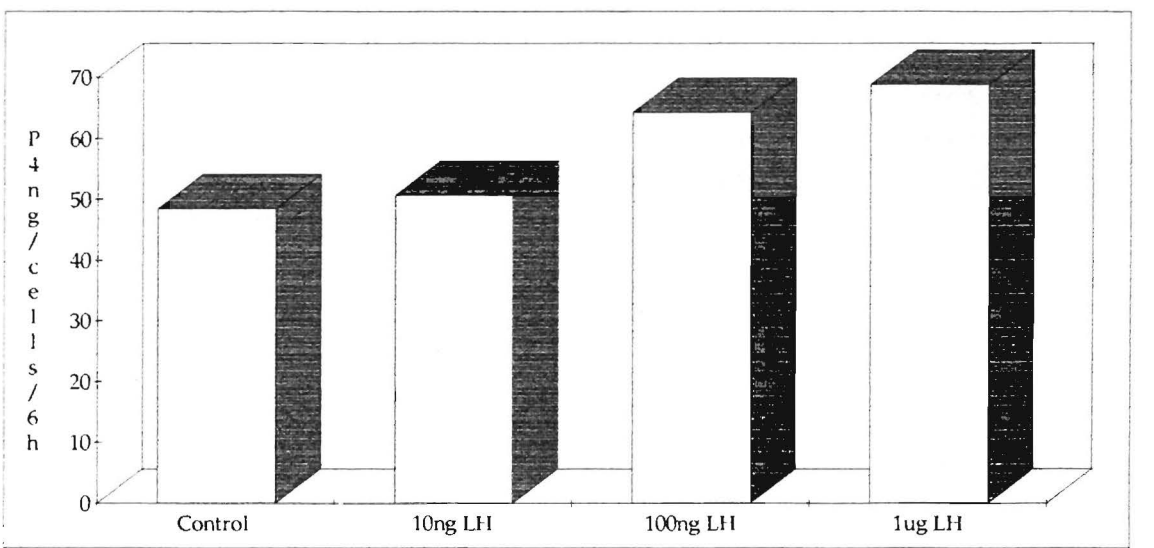

Figure 1: Progesterone production (ng/50,000 cells/6 hours) in mixed luteal cell populations exposed to luteinizing hormone (n=7 pigs).

Experiment 2

Having determined in Experiment 1 that LH does seem to play a role in progesterone production by porcine leuteal cells, Experiment 2 was designed to assess the effect of LH on LDL-stimulated progesterone production. The cells were divided into five groups: a control group, a group receiving only LDL, and three experimental groups that received a set amount of LDL (140 μg cholesterol/ml) and increasing levels of LH (10 ng, 100 ng, and 1 μg/ml).

When low density lipoproteins were present, the progesterone production of the mixed cell population was amplified (Figure 2). Again, the cells seemed to respond in a dose-dependent manner to the levels of LH.

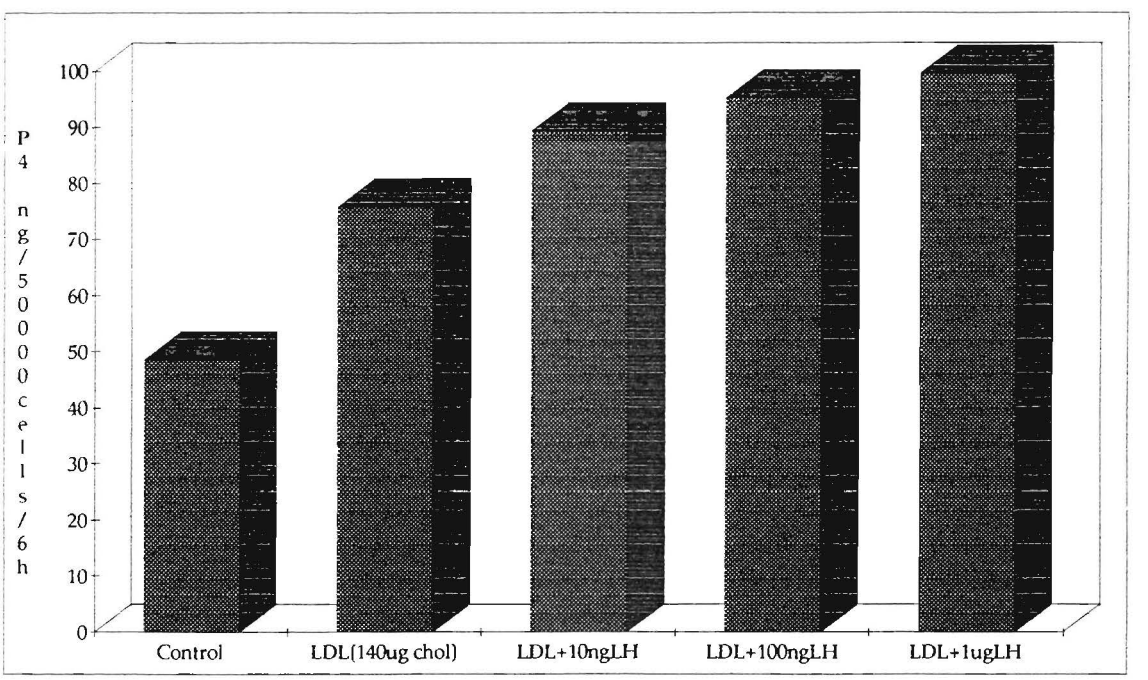

Figure 2: Progesterone production (ng/50,000 cells/6 hours) in mixed luteal cell populations (n=7 pigs) exposed to luteinizing hormone and low density lipoproteins (140 ug cholesterol).

Experiments 3 and 4

After determining in Experiments 1 and 2 that LH does seem to play a role in progesterone production by porcine leuteal cells of early pregnancy, Experiments 3 and 4 were designed to study the possible pathways within the cell by which LH acts. Specifically, the mechanism of a PKC pathway was tested by using 2 separate inhibitors of this cellular factor.

Experiment 3 focused on the PKC inhibitor staurosporine (STS). The washed cells were broken down into seven separate groups: a control group, groups receiving graded amounts of LH (10 ng, 100 ng, and 1μg/ml), and groups receiving graded amount of LH in addition to a standard amount of LDL (140 μg/ml). The groups were further subdivided; half of the cells received 100nM STS/ml in addition to other treatments.

The focus of Experiment 4 was the PKC inhibitor H-7. The washed cells were divided into seven separate groups as defined in Experiment 3. In this case, the groups were subdivided so that half of the cells received 100 μg H-7/ml in addition to other treatments.

Incubation of the mixed luteal cell populations in combination with one of the PKC inhibitors did decrease the progesterone production by those cells. In both cases, the treated groups, containing either STS or H-7, experienced a dose-dependent increases in the production of P4 in the presence of LH. However, in each case, the presence of a PKC inhibitor decreased the overall P4 production by the porcine luteal cells, as compared to the basal P4 (Figure 3 and Figure 4).

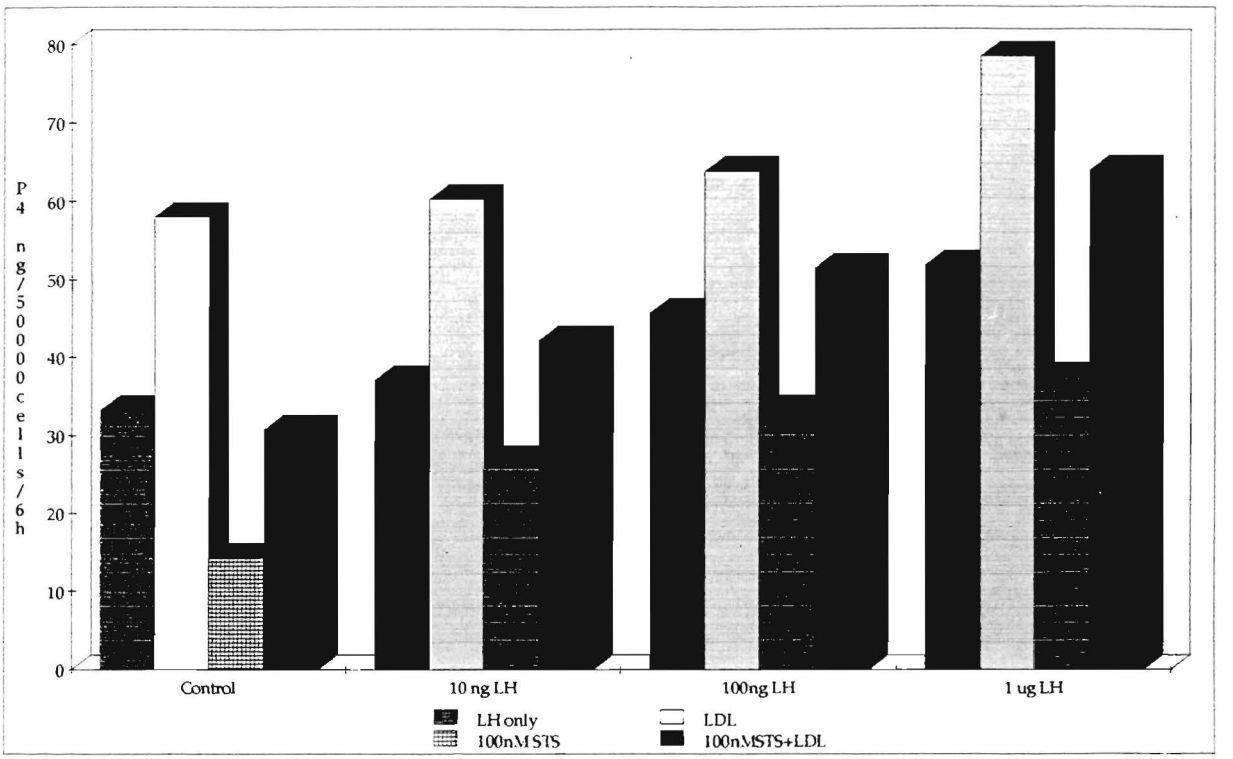

Figure 3: Progesterone production (ng/50,000 cells/6 hours) in mixed luteal cell populations (n=4 pigs) exposed to luteinizing hormone and low density lipoprotein (140 ug cholesterol) in conjunction with STS (100nM).

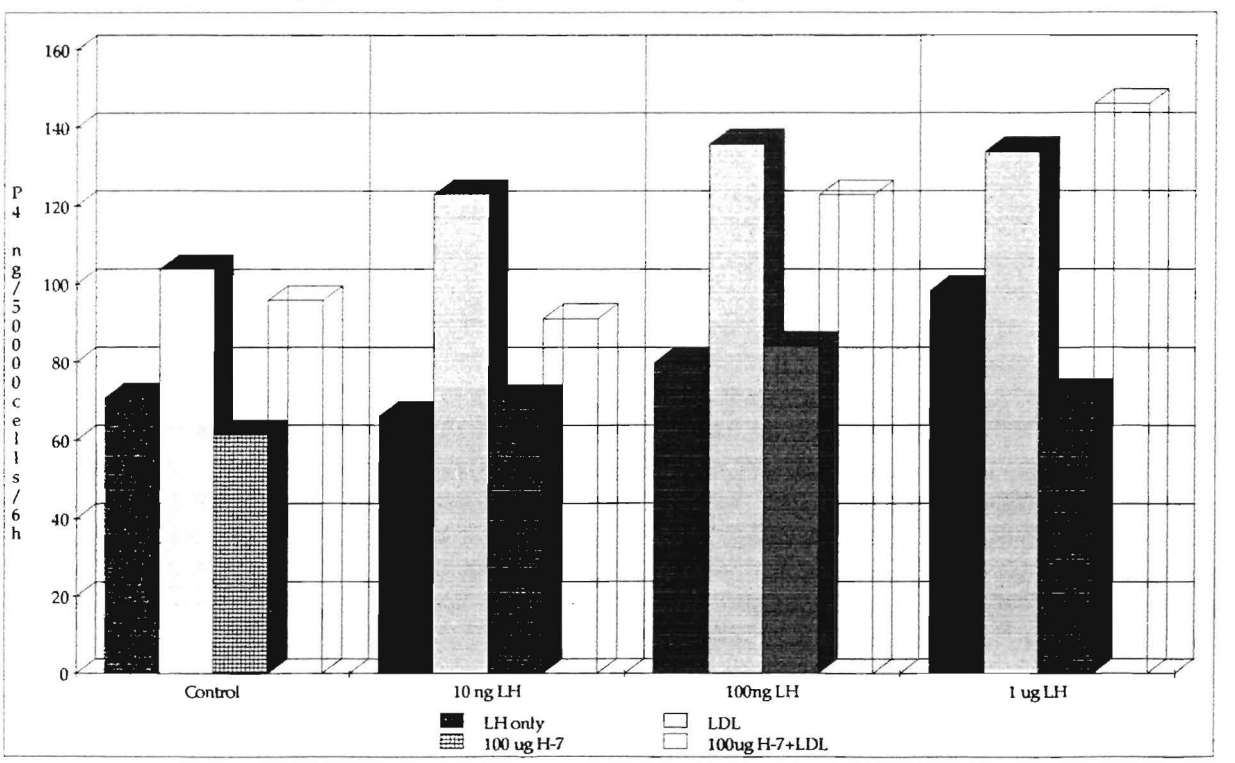

Figure 4: Progesterone production (ng/50,000 cells/6 hours) in mixed luteal cell populations (n=4 pigs) exposed to luteinizing hormone and low density lipoprotein (140ng cholesterol) in conjunction with H-7 (100ug).

Discussion

The results of these experiments seem to indicate that LH stimulates P4 production by porcine luteal cells of early pregnancy. Such evidence of increasing P4 production with increasing LH stimulation is further bolstered by the evidence of LDL stimulation. .

The trend of LH stimulation at various dosages indicates a pattern of response which is not entirely linear. Dosages of LH including 0 ng, 10 ng, and 100 ng produce an increasing response in progesterone production. However, 1 μg of LH seems to result in a lower progesterone concentration than anticipated. Each of the replicates reflects such a trend. This leveling off of responsiveness may reflect some somatic threshold of depleted substrate or maximal enzyme function. Perhaps further *in vivo* research could determine the cause of such an alteration.

The addition of LDL results in an amplification of the progesterone production by the mixed population of porcine luteal cells. This is likely due to the provision of cells with an appropriate substrate for hormone production. The amplification is seen in both basal and LH-treated cell populations. .

The continued progesterone production by the porcine luteal cells in the presence of PKC inhibitors seems to indicate that PKC does not act as a second messenger of LH. Having ruled out this substance, it is likely that adenyl cyclase, which is known to be present in porcine luteal cells, or another unknown substance fills the role of second messenger in the porcine luteal cells. However, further research is needed to draw any conclusions in this area.

While each of the experiments reflected the trend of LH to stimulate progesterone production by the porcine mixed cell populations, the interactions of the various treatments can also be considered. Although the simultaneous use of LH and LDL to stimulate progesterone production by the cells seems to result in a greater production than either individual stimulus, there does not appear to be any further interaction between the stimuli. Only in experiment four is there a significant response to LH and LDL beyond what would be expected from either of the two alone. The reason for the interaction observed in this case and not the others is unclear; it may be attributable to H-7 interaction with the mechanisms at

work inside the cell or may be a chance occurrence. Further research is required to determine the cause and true significance, if any, of this interaction.

The use of the early pregnant sow in this study is of great importance. Very few previous works have considered this population, overlooking a crucial element in luteal research. Corpora lutea are, in fact, the dominant force responsible for the maintenance of early pregnancy. In the absence of the corpora lutea the pregnancy will be lost.

Progesterone production in porcine luteal cells of early pregnancy is influenced by LH stimulation. Comparison of the basal progesterone production of the control group with experimental cell populations indicates a progesterone production that increases commesurate an increasing dosage of LH. Low density lipoproteins do appear to be an important substrate for progesterone production, as seen by amplified levels of progesterone in its presence.

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Acknowledgments

I would like to thank everyone for all of their help and support in completing this project. I am especially grateful to Dr. and Mrs. Ottobre and all of the staff of the Ottobre lab for their assistance over the course of this study. I am also very grateful to Brian Petroff, who helped a great deal. I would like to thank my honors committee members for taking the time to evaluate my project, and all of the wonderful professors who have helped me understand and put the information together. Lastly, I would like to thank my husband for his support and criticism.