Luteolysis in the pig: studies into the mechanism of action of prostaglandin F2[alpha]

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Luteolysis in the pig: Studies into the mechanism of action of prostaglandin F$_2\alpha$

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Iowa State University, 1994
Luteolysis in the pig: Studies into the mechanism of action of prostaglandin F$_{2\alpha}$

by

Lane Kevin Christenson

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the degree of DOCTOR OF PHILOSOPHY

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GENERAL INTRODUCTION

Statement of the Problem

The control and management of domestic livestock reproduction are critical components to all successful and efficient livestock production systems. In cattle, sheep and goats various protocols incorporating the use of the uterine luteolysin, prostaglandin $P_2\alpha$, have been developed to be used to control luteal function in these animals. However, in the pig, very few methods exist for estrus synchronization and/or controlling cyclicity. This limitation is due largely to the delayed susceptibility of the porcine corpora lutea to the uterine luteolysin, prostaglandin $P_2\alpha$, when compared to ruminants species (i.e., days 12-13 versus days 4-5 of the estrous cycle). Further, confounding the study of luteolysis in the pig is the fact that secretion of uterine prostaglandin $P_2\alpha$ occurs coincidently with luteal susceptibility. However, to overcome these problems, a unique model system (described below) was developed to further our understanding of the mechanism(s) of prostaglandin $P_2\alpha$-induced luteolysis in the pig.

Studies have been conducted in a variety of species in an attempt to determine how prostaglandin $P_2\alpha$ causes luteolysis. The pig, while having some disadvantages (described above), offers several critical advantages over other species in the design of studies to elucidate the luteolytic process. The first major advantage the pig offers is the number of corpora lutea found on the ovaries during each estrous cycle, which averages 10-20. Thus, superovulation regimes commonly employed in other species to attain sufficient numbers of corpora lutea are unnecessary. The second major advantage is the ability to use systemic estrogen-injections on days 11-13 of the porcine estrous cycle to prolong luteal function (i.e.,
pseudopregnant) through day 30. Corpora lutea on the ovaries of pseudopregnant pigs remain susceptible to prostaglandin F$_2$α through day 30 without the complication of a conceptus. Utilizing these attributes, a unique intraluteal implant system developed in our laboratory allowed us to investigate the luteolytic properties of prostaglandin F$_2$α on individual corpora lutea on an ovary in a very controlled manner.

Study one is a collection of three separate experiments. Experiment 1 was designed to evaluate the dose response effect of intraluteally administered prostaglandin F$_2$α on the function of individual corpora lutea in the estrogen-treated (pseudopregnant) pig. In experiment 2, we compared the effects of intraluteal prostaglandin F$_2$α administration in the pseudopregnant gilt with that observed in pregnant gilts. A final experiment evaluated the effect of day 13 porcine conceptus tissue homogenates on individual CL of pseudopregnant gilts treated simultaneously with prostaglandin F$_2$α.

Study two was designed to evaluate the sequence of events occurring during prostaglandin F$_2$α-induced luteal regression in the pseudopregnant pig. Ovaries were collected and luteal function (progesterone content) and structural changes (protein, DNA, weight, cell size) were evaluated at 3, 6, 12 and 24 hours after intraluteal prostaglandin F$_2$α treatment. Furthermore, both membrane bound and cytosolic protein kinase C activity were compared between prostaglandin F$_2$α-treated and vehicle-treated (silastic alone) CL from the same ovary. Lastly, morphometric evaluation of luteal tissue at the light microscope level was used to determine if changes in luteal cell size occurred.

The objective of the third study was to characterize and compare the patterns of uterine secretion of prostaglandin E$_2$ with those of prostaglandin F$_2$α during the estrous cycle and the corresponding days of early pregnancy in the pig. Additionally, a second experiment in study three was conducted
to determine if increased prostaglandin E$_2$ from the gravid horn could explain the locally elevated progesterone secretion by corpora lutea on the ipsilateral ovary of unilaterally pregnant pigs.

Explanation of Dissertation Format

The dissertation begins with a general introduction followed by the review of the literature, and three papers complete with their own reference sections and in the appropriate format for publication in each chosen journal. The general summary is followed by the literature citations for the review of the literature and general summary sections. The acknowledgement section completes this dissertation.
REVIEW OF THE LITERATURE

Introduction

The corpus luteum is among the most extensively studied endocrine structure in the reproductive field, yet it still remains poorly understood. Rothchild (1981) attributes much of this lack of understanding to the wide species variations in the extrinsic control (i.e., pituitary, uterine, conceptus) of its function. Further, Rothchild states that while extrinsically the control of luteal function is very complex, there are several universal characteristics of the corpora lutea of all mammalian species. First and foremost is its production and secretion of progesterone; second, and the most distinguishing characteristic of this endocrine gland, is its ephemeral nature; and last, is the universal role prostaglandin $F_2\alpha$ plays in its demise. The corpus luteum is truly a remarkable structure developing from the walls of the post-ovulatory follicle and maturing into a fully functional endocrine gland within several days. In polyestrous species such as the pig, the absence of conceptuses within the uterine lumen during a critical period predisposes the corpus luteum to regression. This allows another cohort of follicles to grow, mature and ovulate at the subsequent estrus. Following a fertile mating, the corpora lutea which have developed from these follicles will play a key role in the transport of the developing embryos to the site of implantation and maintenance of pregnancy to term via its secretion of progesterone. Although the site of prostaglandin $F_2\alpha$ synthesis is different (i.e., ovarian, uterine) across species it appears to be the universal luteolytic agent (Rothchild, 1981). It is therefore, likely, that the mechanism of prostaglandin-induced luteal regression of all species follows a similar path. The focus of the research in this dissertation relates to
increasing our understanding of the mechanism(s) through which prostaglandin F₂α induces regression of the porcine corpus luteum. In order to establish a coherent idea of how the porcine corpus luteum might regress in response to prostaglandin F₂α, it seems logical to start at the beginning and discuss the formation, cellular makeup, and function of the corpus luteum before delving into the possible mechanisms which lead to its death.

Historical Account of the Corpus Luteum

The discovery of the corpus luteum is credited to Volcherus Coiter in 1573 (Harrison, 1948), however, the first good description of this endocrine gland was given by Regner de Graaf in his monograph titled *De Mulierum Organis Generationi Inservientibus* published in 1672 and translated by G. W. Corner (1943). The term, corpus luteum, is generally reserved for the endocrine gland that forms from the ruptured follicle after release of the ovum during ovulation. However, early investigators commonly failed to obtain corpora lutea of known physiologic age based on an inadequate working knowledge of the ovarian cycle and as a result often examined and misclassified atretic follicles or dying corpora lutea as growing or fully functional corpora lutea (Sobotta, 1902). Consequently, the early literature describing the cellular makeup of the corpus luteum is indeed difficult to interpret.

Following the advice of his former mentor, L. Fraenkel determined that the presence of the corpora lutea on the ovary of the rabbit was necessary for maintenance of pregnancy (Fraenkel; see Niswender and Nett, 1988). Confirmation of the importance of the corpora lutea for maintenance of pregnancy in the pig was shown by du Mesnil du Buisson and Dauzier in 1957. Twenty two years after the realization that the ovary (i.e., corpus luteum) was essential for pregnancy, Allen and
Doisey (1923), first extracted and characterized sex steroids demonstrating that these compounds could duplicate the effects the ovary had on uterine histology. Final isolation of the progestational hormone "progesterone" from luteal tissue occurred a short 6 years later (Corner and Allen, 1929). These landmark discoveries in the reproductive biology field were made using the pig as the experimental model.

Development of the Porcine Corpus Luteum

**Origin and histologic development**

The origin and development of the corpus luteum within a variety of mammalian species have been the topic of many investigations throughout the nineteenth and twentieth centuries (reviewed by Sobotta, 1902; Marshall, 1905; Harrison, 1948; Browning, 1973). The most thorough and complete paper on the subject, however, was written in 1919 by George W. Corner, *On the Origin of the Corpus Luteum of the Sow from both Granulosa and Theca Interna*. In order to answer the question about the cellular origin of the porcine corpus luteum, Corner realized that a series of ovaries collected at known times after ovulation must be examined.

The rationale behind the design of his study, while simple in theory, allowed him to make great inroads into this complex problem. Included in Corner's study was a series of 171 sows slaughtered at known times throughout the estrous cycle and pregnancy. The first sows of the series were slaughtered during estrus (day 0 - day 2 of the estrous cycle) and those with preovulatory follicles served as the starting point for his description of the formation of the corpora lutea. The preovulatory follicles, which ranged in number from 1-16 on each pig ovary were 8-10 mm in diameter with the stratum granulosa cells forming a layer 6 to 9 cells thick around the antral fluid. Each granulosa cell was 8-10 μm in diameter
with a spherical nucleus 5 μm in diameter and a homogenous cytoplasm with very little evidence of lipid containing vacuoles. The granulosa cells were separated from the overlying theca interna cells and the vascular supply by a basement membrane. Within the theca interna, Corner noted regional cell differences, directly overlying the basement membrane, he describes a layer of spindle-shaped cells that lack evidence of cytoplasmic vacuoles. The characteristic theca interna cells of the preovulatory follicle formed a layer 3 to 5 cells thick and ranged from 10x17 to 12x17 μm in diameter and were either oval, rectangular, or spindle in shape. The cytoplasm of these cells contained an abundance of lipid vacuoles as evidenced by osmium tetroxide and Sudan III staining (Bloor et al., 1930). Interestingly, there appeared to be a gradient of cell size within the theca interna with the larger cells located the farthest from the granulosa cells (Corner, 1919). Additionally, he notes that the theca interna contains numerous capillaries which is characteristic of this highly vascular tissue. Surrounding the theca interna was the theca externa which consists of several layers of long spindle-shaped cells and a prominent connective tissue matrix. Prior to follicular rupture, mitotic figures appeared in theca externa with little evidence of similar activity in either the theca interna or granulosa cells.

Upon rupture, the ovulatory follicle is commonly referred to as a corpus hemorrhagicum (bloody body), this structure will then mature to form a corpus luteum (yellow body) which will be converted into a corpus albicans (white body) during the later stages of the luteal phase (Sorenson, 1979). Because of the difficulty in establishing a clear distinction morphologically and functionally between the end and beginning of these three stages (Sorenson, 1979), I will refer to all post-ovulatory structures as corpora lutea. The process of ovulation and expulsion of the ovum and follicular fluid
causes the follicular cavity to collapse. Evidence of smooth muscle like cells within the theca externa may elicit the slight eversion of the follicular walls noticed on day 2 and 3 of the estrous cycle. Because of the collapse and constriction of the theca externa, the inner walls of the follicular cavity were thrown into complex folds. However, microscopically, the granulosa and theca cell layers looked similar to their counterparts in the preovulatory follicle. Next, Corner noted the breakdown of the basement membrane separating the granulosa and theca interna cells. The breakdown of the basement membrane first appeared at the apices of the folds of the previous follicular wall and then continued to the bases of these folds throughout the entire corpus luteum. As the basement membrane disappeared, slender spindle-shaped cells appeared between the granulosa cells. Corner stated that these were likely endothelial cells, but had no direct proof. During this early migration of "endothelial cells," Corner saw neither evidence of thecal cell migration nor any evidence of conversion of thecal cells to a fibroblastic cell. During this same period, the granulosa cells throughout the corpus luteum began to hypertrophy with cells almost doubling in size, the nucleus became vesicular and the cytoplasm contained large round bodies resembling rings in section. These cells have been referred to as granulosa-lutein cells or the large luteal cells of the corpus luteum (Cavazos et al., 1969). Corner noted that in some locations within the developing corpus luteum the basement membrane separating the granulosa and theca was still present; however, the conversion of the granulosa cells to the luteal cell type had already occurred in these areas allowing him to conclude that the original large luteal cells of the corpus luteum were derived from the granulosa cells. The changes described by Corner to this point have occurred within the first 3-4 days after the onset
of estrus. Also during this period the capillary network spread throughout the corpus luteum and most large luteal cells were adjacent to a capillary. Over the next several days (5-8) of the estrous cycle the hypertrophied granulosa cells began to display some lipid inclusions and thecal-lutein cells migrated along the routes of invasion of capillaries into the parenchyma of the corpus luteum. Barker (1951) reported similar findings in his cytochemical investigation of lipids within the sow ovary, noting an almost identical pattern of theca-lutein cell invasion. These observations were based on the peculiar round bodies found within the granulosa-lutein cells, in combination with a greater abundance of lipid inclusions and a darker staining cytoplasm for theca cells. In the fully formed corpus luteum, Corner reported that the theca-lutein cells reside at the periphery and along the folds caused by follicular collapse and finally following the blood vessels in amongst the large luteal cells.

Corner further states that the corpus luteum may be considered to have completed its metamorphosis from a ovulatory follicle to a corpus luteum by the end of first week of the estrous cycle. He does, however, admit that it increases about 1-2 mm in diameter over the next week. Reports by Duncan et al. (1960), Masuda et al. (1967) and Cavazos et al. (1969) seem to confirm that the complete growth of the corpus luteum had occurred by day 8 of the estrous cycle as luteal weight was greatest on this day in their studies. However, a recent and in depth study demonstrated that while functional maturity of the pig corpus luteum (i.e., maximal progesterone production) may have been reached by day 8, similar to the studies of Masuda et al. (1967) and Cavazos et al. (1969), luteal weight increased through day 12 (Conley and Ford, 1991). More importantly these researchers reported that there was an approximate 50% increase in both DNA and protein content between days 8 and 12. The authors further
noted that the protein:DNA ratio did not change between days 8 and 12. Taking into account the increase in weight (29%), the greater increase in cell number (50%) as well as the lack of change in the protein:DNA ratio, the authors suggest that the extracellular matrix decreases and is replaced by cells (Conley and Ford, 1991). It is likely that these cells would be endothelial cells which have a low protein:DNA ratio, thereby increasing cell number (i.e., DNA content) while affecting luteal weight the least.

Morphologically speaking, the next step in understanding the corpus luteum came with the advent of the electron microscope by Ernst Ruska and Max Knoll during the 1930s (Bozzola and Russell, 1992). This brought about a resurgence in the anatomical description of tissues including the corpus luteum, only this time at the ultrastructural level. The ultrastructural description of the porcine corpus luteum followed the description of many others (armadillo, mink, rat, mouse, sheep and rabbit; Enders 1962, 1973). Bjersing (1967), Goodman et al. (1968) and Cavazos et al. (1969) published the only accounts describing the ultrastructure of the porcine corpus luteum during the estrous cycle.

Since, the manuscript prepared by Cavazos et al. (1969) followed Corner’s much earlier suggestion about the importance of obtaining specimens of a known age, I will limit my discussions to their findings. The granulosa-lutein cells within recently formed corpora lutea (day 1 of the estrous cycle) exhibited a gradient of changes progressing from the central cavity to the periphery of the forming luteal tissue. The cells adjacent to the basement membrane of the recently ovulated follicle resembled granulosa cells of the periovulatory follicle. These granulosa cells were cuboidal to columnar in shape and the cytoplasm was characterized by having long cisternae of rough endoplasmic reticulum and many polysomes, with a modestly developed Golgi apparatus. The
mitochondria of these cells had varied profiles and internally
the crista were transverse plate-like infolds with occasional
tubular profiles while the matrix showed a slight granular
density.

The "luteinized" granulosa cells of the early corpus
luteum located first in the central cavity of the ovulated
follicle had already doubled in size. These hypertrophied
cells (i.e., large luteal cells) appeared polygonal in shape,
the nucleus was eccentrically placed and the smooth
endoplasmic reticulum system was much more pronounced. Short-
strands of rough endoplasmic reticulum were present in the
cytoplasm, while the more developed Golgi complex consisted of
flattened membranous saccules and associated small secretory
vesicles, the mitochondria had changed little at this time.

On day 4 of the estrous cycle, luteinization of all
granulosa cells was essentially complete as all of these cells
had undergone a mass accumulation of smooth endoplasmic
reticulum and hypertrophied. The mitochondria of these large
luteal cells exhibited circular and oval profiles and a
moderately dense matrix, while the cristae consisted almost
entirely of twisted tubules. The remaining cell organelles
within the large luteal cells were relatively unchanged with
the exception of a build-up of vesicles near the Golgi. By
day 8 of the cycle, the presence of rough endoplasmic
reticulum was very limited and dense bodies became apparent.
These dense bodies were interpreted to be residual bodies and
they increased in number through day 12 of the cycle. The
limited presence of lipid droplets was also noted at this
time.

Cavazos et al. (1969) and Bjersing (1967) noted the
appearance of the theca-lutein (small) cells described by
Corner, while Goodman et al. (1968), found no evidence of
these cells. Bjersing stated that these cells were mainly
small perivasculart cells with uneven contours containing
several large lipid droplets and a less developed agranular (smooth) endoplasmic reticulum, than the granulosa-lutein cells. Cavazos et al. (1969) described them as spindle-shaped cells with an oval nucleus with considerable quantities of clumped chromatin, they also noted the highly variable size of the spherical mitochondria and the large lipid droplets.

Functional development

The functional state of a corpus luteum is commonly related to its steroidogenic capacity (i.e., progesterone production; Rothchild, 1981). Luteal progesterone production primarily results from conversion of cholesterol taken up via low- and high-density lipoproteins as opposed to de nova synthesis (Strauss et al., 1981). Low and high density lipoproteins, contain cholesterol esters, which can be stored within the cytoplasm and used as a source of free cholesterol for steroidogenesis. The two enzymes or enzyme complexes involved in conversion of free cholesterol to progesterone are cytochrome P450 side-chain cleavage, and 3-β-hydroxysteroid dehydrogenase. The transport of cholesterol to the inner membrane of the mitochondria is generally considered the rate limiting step in the P450 side-chain cleavage mediated conversion of cholesterol to pregnenolone (Jefcoate et al., 1992). Localized predominately within the smooth endoplasmic reticulum, 3-β-hydroxysteroid dehydrogenase converts pregnenolone to progesterone (Hanukoglu, 1992).

Studies with isolated mitochondrial membranes from corpora lutea suggest that only 2% of the total oxygen uptake by the corpus luteum is utilized by the mitochondrial enzyme, P450 side-chain cleavage, (Swann and Bruce, 1987). Based on this low metabolic cost, the majority of energy used by the corpus luteum seems to be directed into cellular activities, other than progesterone production. In addition to progesterone, the corpus luteum also produces prostaglandins
as well as several protein hormones (i.e., relaxin, prolactin and oxytocin) during the estrous cycle (Einspanier et al., 1986; Sherwood and Rutherford, 1981; Ali et al., 1986). However, based on the very low levels of these other secretory products, it would seem that the majority of cellular energy is likely utilized for the growth, differentiation, and general maintenance of the luteal cell.

Systemic progesterone concentrations progressively increase from a nadir at the time of ovulation to reach maximum levels by day 8 of the estrous cycle. Progesterone concentrations remain at those levels through days 12-14, before declining with the onset of luteolysis (Stabenfeldt et al., 1969; Hunter, 1981). In vitro culture experiments utilizing porcine luteal cells, however, have shown repeatedly that luteal cells isolated from pigs on days 3-6 of the estrous cycle produce greater amounts of progesterone than those isolated during the mid-luteal phase (days 7-14; Patek and Watson, 1983; Hunter, 1981; Duncan et al., 1960; and Mattioli et al., 1985). These contradictory in vivo and in vitro results may be the result of a less developed blood supply to the corpus luteum during the early luteal phase or a possibly greater dependence of the luteal cells during the late luteal phase on extrinsic (i.e., pituitary) hormonal support. As previously discussed, development of the corpus luteum (Corner, 1919; Cavazos et al., 1969; Masuda et al., 1967) as determined by gross histology and changes in luteal weight and luteal tissue progesterone concentrations appeared to be completed by day 8 of the estrous cycle. These observations suggest that the vast majority of the vascularization of this endocrine gland had already taken place by this day. In support of this conclusion, Magness et al. (1983) observed a substantial (=40%) increase in ovarian blood flow over the first 6 days of the estrous cycle, which then plateaued and remained high for the next 8 days before
declining with the onset of luteolysis. Changes in blood flow to the porcine ovary during the luteal phase can be attributed primarily to changes in luteal blood flow, because the corpora lutea receive ~90% of all ovarian blood flow, as shown by microsphere injection (Ford et al., 1982).

Subpopulations of luteal cells

Lemon and Loir (1977) were the first to separate porcine luteal cells into two subpopulations for in vitro culture. Utilizing corpora lutea from pregnant sows, they separated cells into two groups based on diameter differences. One population of cells ranged from 15-19 μm in diameter (small cells), while the other ranged from 30-50 μm in diameter (large cells). They were also the first to describe the marked difference in progesterone producing abilities exhibited by those two subpopulations of steroidogenic cells, noting that the large luteal cells produced 5 times the amount of progesterone that the small luteal cells did. Lemon and Loir (1977) also noted a transitory increase in progesterone secretion by both cell types following addition of luteinizing hormone to the medium, but noted that large luteal cells increased their secretion of progesterone less than 1-fold while the small luteal cells increased their secretion approximately 50-fold. Lastly, these authors demonstrated that on an ultrastructural basis the large and small luteal cells separated in their study were similar to those described by Cavazos et al. (1969).

Subsequent to this original study, however, virtually all of the characterization of these two luteal cell types in vivo as well as in vitro culture have been carried out in ruminant species, (see Niswender et al., 1986). I will discuss the general differences of these two luteal cell types as have been extensively determined for the ewe, followed by what is known for the pig. In the ewe, dissociated large luteal cells
collected during the mid- to late-luteal phase (day 9-13) were generally considered as cells greater than 22 μm in diameter, while small cells were 15-22 μm in diameter (Fitz et al., 1982). One of the potential flaws with dissociation and cell separation techniques may be the unequal loss of or damage to large versus small luteal cells (O'Shea, 1986). In support of this hypothesis, Hoyer et al. (1984) reported that following isolation and dissociation, large luteal cell viability is 10-20 percent less than that observed for small luteal cells.

O'Shea et al. (1986), Rodgers et al. (1984), and Farin et al. (1986) have carried out extensive ultrastructural classification and morphometric analysis of ovine corpora lutea. These researchers attempted to overcome some of the shortcomings of cell dissociation techniques, as well as describe changes, if any, in cell populations during the estrous cycle of the ewe. In these studies large luteal cells were found to be 30-32 μm in diameter, while small luteal cells were found to be 17-19 μm in diameter during the mid- to late-luteal phase. These two cell populations correspond well with those observed in the flow cytometric studies of Brannian et al. (1993). Brannian et al. (1993) dissociated ovine corpora lutea and then sorted cells based on size with a flow cytometer. Steroidogenically active ovine luteal cells (3-β-hydroxysteroid dehydrogenase positive) separated by cell size showed a bimodal distribution. In this study one cell population ranged at 19-20 μm while the other was at 30-40 μm (Brannian et al., 1993). Further, these researchers went on to demonstrate that the ultrastructure of these cells was similar to that previously described for the small and large luteal cell types, respectively (O'Shea et al., 1986).

Morphometric analysis of ovine corpora lutea illustrates that based on nuclei count, there were 4 to 5 small luteal cells to every 1 large luteal cell. Moreover, endothelial cells account for greater than 60% of all the cells within the
ovine corpus luteum. However, based upon percent volume, the large ovine luteal cells accounted for \( \approx 30\% \), small luteal cells \( \approx 22\% \), endothelial cells \( \approx 12\% \), and the remaining \( 36\% \) included fibroblasts, lymphocytes and unidentified cells \( (\approx 9\%) \), vascular space \( (\approx 12\%) \), and intracellular space \( (\approx 15\%; \) O'Shea et al., 1986). To my knowledge no morphometric analysis of porcine luteal tissue during the estrous cycle or pregnancy has been reported.

Following isolation of ovine small and large luteal cells, it has been repeatedly demonstrated that small luteal cells contained numerous luteinizing hormone receptors on their plasma membrane and respond in vitro to luteinizing hormone with a large increase in progesterone secretion (see review by Schwall et al., 1986). In contrast, large luteal cells contain few, if any, luteinizing hormone receptors and show little change in progesterone secretion in response to luteinizing hormone. Similar conclusions have been reached for porcine small and large luteal cells based on the responsiveness of the two luteal subpopulations to luteinizing hormone stimulated progesterone secretion (Lemon and Loir, 1977; Hunter, 1981).

Meduri et al. (1992) immunocytochemically localized the luteinizing hormone receptor within porcine antral and preovulatory follicles and early luteal (days 3-5) and mid-luteal (day 7-10) phase porcine corpora lutea. In large antral follicles (3-6 mm in diameter) both theca interna and granulosa cells were labeled, granulosa cells next to the basement membrane stained more intensely than those near the antrum. In preovulatory follicles the labeling intensity was greater for the granulosa cells, while in the theca interna two zones were noted. The layer next to the basement membrane contained no evidence of staining, while the outer layers of the theca interna were intensely stained. This observation lends credence to Corner's (1919) original suggestion that the
cells lying next to the basement membrane were not steroidogenic and appeared as fibroblastic cells. Further, it may be that these cells are undifferentiated theca interna cells which might undergo mitosis and differentiate later into luteal cells. Luteinizing hormone receptor immunolabeling of day 3-5 corpora lutea was restricted to small groups of cells located at the periphery of the corpora lutea. No staining was observed for the large cells in the central portion of the corpora lutea. In the mid-luteal phase corpora lutea, intensely stained cells were observed at the periphery of the corpora lutea, and along the fibrovascular septa penetrating into the corpora lutea (Meduri et al., 1992). The immunolabeling pattern observed for the luteinizing hormone receptor parallel the descriptive studies of Corner (1919) as previously discussed, suggesting that these positively-labeled cells were theca interna derived. Characterization of luteinizing hormone receptors on pig luteal cells (mixed population) by Scatchard analysis has shown that receptor numbers double from day 6 to 10 of the estrous cycle, show no change through day 12 before receptors numbers declined on day 14 to 16 of the estrous cycle (Zeicik et al., 1980).

In contrast to the localization of the luteinizing hormone receptors on ovine small luteal cells, prostaglandin $F_2\alpha$ and $E_2$ receptors reside preferentially on the plasma membrane of large luteal cells (Fitz et al., 1982; Balapure et al., 1989). The presence of high-affinity (20 nM) prostaglandin $F_2\alpha$ binding sites on large porcine luteal cells was later determined by Gadsby et al. (1990). They further noted the presence of a low-affinity (170 nM) receptor on both small and large luteal cells. Characterization of the prostaglandin $F_2\alpha$ binding sites on large luteal cells throughout the estrous cycle, demonstrated that high affinity binding sites increased slowly from day 6 through 12 of the estrous cycle, then increased rapidly (2.5 fold) on day 13
before declining on d 14-17 of the estrous cycle (Gadsby et al., 1990). In a subsequent experiment, Gadsby et al., (1993), did not observe an increase in prostaglandin F₂α receptors until day 14 of the estrous cycle. They attributed this to possible differences in detection of estrus.

Another major difference between the large and small luteal cells is the presence of rough endoplasmic reticulum within the large cells allowing these cells to produce protein secretory products (Rodgers et al., 1983). In the ewe, luteal production of oxytocin has been implicated in the mechanism of luteal regression (Silva et al., 1991). In the pig, peptide and protein hormone secretion by porcine corpora lutea during the estrous cycle has received little attention because tissue content of these hormones, with the exception of relaxin, is relatively low in comparison to ruminants (Eispanier et al., 1986; Choy and Watkins, 1988). Further, the role of these secretory proteins in relationship to reproductive function during the estrous cycle is unknown for the pig, and will not be considered further.

Although the specific contribution of the theca and granulosa cells to the corpus luteum is still a matter of controversy, it is almost universally accepted that both cells are involved in formation of the corpus luteum. Further, during the estrous cycle it is also widely accepted that small luteal cells are primarily derived from the theca while large luteal cells are primarily derived from the granulosa cells. However, in the case of pregnancy where the corpus luteum’s lifespan is greatly extended, there is substantial although circumstantial evidence that small luteal cells may in fact convert to large luteal cells (Alila and Hansel, 1984).

Factors involved in luteal development

In this section, I will describe several of the known regulators, both extrinsic and intrinsic, which influence the
function and development of the early porcine corpus luteum. Extrinsic factors known to influence luteal function during the estrous cycle in other species include, follicle stimulating hormone, luteinizing hormone and prolactin (Greenwald and Rothchild, 1968; Stormshak et al., 1987). The corpus luteum of the pig appears to differ considerably from the corpora lutea of most other mammals, in their ability to form and develop normally though day 10 of the estrous cycle without anterior pituitary support (Anderson, 1966).

Hypophysectomy and hypophysial stalk transection studies in the pig demonstrated that formation of functional corpora lutea could proceed without pituitary support (Anderson et al., 1967). These studies evaluated luteal function (i.e., tissue progesterone concentration) on day 11, 15, 19 of the estrous cycle or pregnancy following stalk transection on the day after the first exhibition of estrus (day 0). Comparison of corpora lutea from day 11 treated (stalk sectioned) and control cyclic and pregnant gilts demonstrated that luteal progesterone concentration was unaffected. However, there was a noticeable decrease in luteal weight in the day 11 stalk sectioned gilts suggesting that structural development may have been altered. Both day 15 and day 19 hypophyseal stalk sectioned pregnant gilts had reduced luteal function when compared to control (pregnant) gilts. Results following hypophysectomy were similar to those mentioned above for the stalk transected gilts. Confirming the need for hypophysial support, administration of anterior pituitary extracts on day 12 to 20 of the estrous cycle prevented luteal regression in hysterectomized hypophyseal stalk transected gilts until day 20. Additionally, twice daily administration of human chorionic gonadotrophin to pregnant or hysterectomized gilts that had also been hypophysectomized on day 1 prevented luteal regression suggesting that luteinizing hormone was a critical hormone for luteal maintenance. Sammelwitz and Nalbandov
(1958) administered exogenous progesterone to intact pigs during days 0 to 12 of the estrous cycle or pregnancy thereby inhibiting pituitary release of luteinizing hormone and follicle stimulating hormone. They observed no effect of exogenous progesterone on early luteal development (day 0-12) for either cyclic or pregnant pigs. However, if the pregnant gilts received progesterone past day 12, luteal regression ensued, whereas corpora lutea were maintained if progesterone treatment was terminated on day 12 of pregnancy. Luteal regression occurred in cyclic gilts following discontinuation of progesterone on day 12 as expected. These studies provide additional support for the gonadotrophin independent (day 0-12) and dependent (after day 12) stages of the porcine corpus luteum.

Prolactin’s role in luteal maintenance, while very important in rodents and carnivores, is questionable in domestic animals (Murphy and Rajkumar, 1985). Sammelwitz et al. (1961) and Duncan et al. (1961) found no evidence of luteotrophic activity of prolactin in intact cycling gilts. More recently, Dusza et al. (1986) administered prolactin to gilts on day 12 and 13 of the estrous cycle and observed no change in plasma progesterone concentrations when compared to saline injected control gilts. In vitro studies demonstrated no effect of prolactin on progesterone production by porcine luteal cells isolated from corpora lutea obtained on days 5-13 of the estrous cycle (Gregoraszczuk, 1983; Przala et al., 1984). In contrast, in vitro and in vivo evidence suggests that prolactin can stimulate luteal cell progesterone secretion from day 1-3 corpora lutea (Gregoraszczuk, 1983, 1990 and Ciereszko and Dusza, 1993). Gregoraszczuk (1990) further, showed that the large luteal cells were responsible for the increase in progesterone secretion. The role of prolactin in porcine luteal function remains unclear.

In addition to pituitary derived hormones, those of
conceptus/uterine origin have also been evaluated extensively in the pig for luteotrophic activity (Geisert et al., 1990; Ford and Christenson, 1991). Estrogen administration on days 12-13 post-estrus prevented luteal regression in cyclic gilts (Kidder et al., 1955). Later, Perry et al. (1973) determined that porcine conceptuses produced estrogen on day 11-13 of pregnancy. This finding resulted in the formation of the endocrine/exocrine theory of uterine prostaglandin F2α secretion to help explain luteal maintenance or maternal recognition of pregnancy in the pig (Bazer and Thatcher, 1977). The studies of Frank et al. (1977) and Moeljono et al. (1977) suggested that uterine exposure to estrogen prevented secretion of prostaglandin F2α into the vasculature (endocrine), while increasing its secretion in the uterine lumen (exocrine). However, while estrogen's antiluteolytic role via suppression of uterine prostaglandin F2α secretion was well established, a significant amount of evidence also suggested that estrogen or another conceptus derived product might have direct luteotrophic effects. These included the observed high incidence of prolonged function (Anderson, 1966; Niswender et al., 1970; Christenson and Day, 1971) and high progesterone concentrations (Ford and Christenson, 1979) of corpora lutea adjacent to the gravid uterine horn of unilaterally pregnant pigs. Ball and Day (1982) also demonstrated a direct effect of the porcine conceptus on luteal function. In their study, luteal implants containing day 30 porcine conceptus homogenates, prostaglandin F2α or the combination of the two, were placed directly into individual corpora lutea of day 30-35 pregnant gilts. They observed that corpora lutea containing the combination of conceptus homogenate and prostaglandin F2α were heavier and contained more progesterone than the corpora lutea containing prostaglandin F2α alone, when evaluated five days after implantation. Furthermore, these investigators also
demonstrated that charcoal extraction of the conceptus homogenate removed its luteotrophic activity suggesting that the active component was of low molecular weight, possibly an estrogen or prostaglandin. Conley and Ford (1989) and Conley et al. (1989) established and utilized a unique intraluteal silastic implant procedure to evaluate the direct effects of estradiol-17β on luteal function. This technique offered the opportunity to compare corpora lutea treated with estradiol-17β or vehicle (silastic alone) with unimplanted corpora lutea on the same ovary. All corpora lutea were exposed equally to all other extrinsic factors of pituitary or uterine origin within the pig. They found that intraluteal estrogen could maintain all corpora lutea through day 19 of the estrous cycle, but only if sufficient estrogen was administered into an individual corpus luteum to prevent endogenous uterine prostaglandin F₂α secretion. The results of these studies suggest an all or none effect of estradiol-17β on luteal function. In a more recent study, Ford and Christenson, (1991) administered estradiol-17β plus a known luteolytic dose of prostaglandin F₂α into individual corpora lutea and demonstrated that estradiol could not directly prevent prostaglandin F₂α-induced luteolysis at the level of the corpora lutea.

In contrast to estrogen's inability to prevent luteolysis directly at the level of the corpus luteum, prostaglandin E₂ when simultaneously administrated with prostaglandin F₂α at a 4 to 1 ratio into an individual corpus luteum on day 11 of the estrous cycle was capable of preventing luteolysis through day 19 (Ford and Christenson, 1991). Uterine luminal infusions of prostaglandin E₂ have had inconsistent effects on luteal function in the pig. Sander et al. (1982) and Schneider et al. (1983), and Okrasa et al. (1985) were unable to maintain luteal function or stimulate progesterone secretion following intrauterine infusion of prostaglandin E₂. In contrast,
Akinlosotu et al. (1986, 1988) demonstrated that pharmacologic doses of prostaglandin E\textsubscript{2} infused into the uterine lumen every 4 h or continuously from day 7 to day 23, completely maintained luteal function until day 23 when prostaglandin E\textsubscript{2} infusions were terminated. These conflicting results are not surprising, considering that concentrations of prostaglandin E\textsubscript{2} sufficient to overcome the high levels of prostaglandin F\textsubscript{2\alpha} secreted into the uterine vasculature on days 13 to 16 of the estrous cycle, must be extremely high (Moeljono et al., 1977).

Conceptus estrogen may change the ratio of prostaglandin E\textsubscript{2} to prostaglandin F\textsubscript{2\alpha} emanating from the uterus by changing the contribution of prostaglandins from stromal or glandular components of the endometrium. Prostaglandin secretion from stromal and glandular cells isolated from day 13 nonpregnant and pregnant gilts demonstrated that the prostaglandin E\textsubscript{2}:prostaglandin F\textsubscript{2\alpha} ratio was markedly increased for stromal cells from pregnant gilts, when compared with the nonpregnant gilts (Zhang and Davis, 1991). The glandular portion of the endometrium while also increasing secretion of both prostaglandin F\textsubscript{2\alpha} and E\textsubscript{2} on day 13 demonstrated no preferential shift in the ratio of these two hormones. Additionally, Geisert et al. (1982) observed a preferential increase in uterine lumen prostaglandin E\textsubscript{2} concentrations following systemic estrogen secretion on days 11-13 of the estrous cycle. Conceptus produced prostaglandins may also contribute to a shift in the prostaglandin E\textsubscript{2} to prostaglandin F\textsubscript{2\alpha} ratio emanating from the uterus on days 11-13 of pregnancy. Davis et al. (1983) demonstrated that the porcine conceptus increases prostaglandin synthesis markedly on day 11 and produces greater concentrations of prostaglandin E\textsubscript{2} than prostaglandin F\textsubscript{2\alpha}.

Prostaglandins produced by the porcine corpus luteum itself have also been well studied (Patek and Watson, 1983; Guthrie et al., 1979; Jones and Gadsby, 1993). Also cyclic
changes in growth factors and cytokines within the porcine corpus luteum suggest that these compounds are important to luteal function, especially as it may relate to luteinization, vascularization and regression of the corpus luteum. However, as these functions are yet unclear, the role of these compounds will not be discussed in any further detail.

Patek and Watson (1983) found that prostaglandin F$_2$α and prostaglandin E$_2$ production from luteal slices obtained from early (day 3-6) and late (day 15-19) porcine corpora lutea exceeded those from mid-luteal phase corpora lutea. However, they further showed that inhibition of luteal prostaglandin production with indomethacin had no effect on in vitro progesterone production from corpora lutea collected at any stage. Mattioli et al. (1985) found that dispersed porcine luteal cells produced prostaglandin F$_2$α as early as day 3 and found no significant change for the remaining six days (day 5, 7, 9, 11, 13, 15) of the estrous cycle tested.

Luteal Regression in the Pig

Uterine influence

The importance of the uterus in controlling estrous cycle length was first recognized by Loeb (1923) who hysterectomized cyclic guinea pigs and noted that luteal regression did not occur. Later, he suggested that a substance originating from the uterine mucosa controlled the lifespan of the corpus luteum (Loeb, 1927). Similarly, the corpora lutea of the pig have also been shown to be maintained following hysterectomy during the luteal phase of the estrous cycle (Anderson, 1973). In 1969, a fatty acid derivative (prostaglandin F$_2$α) found in high concentration in uterine homogenates was shown to possess potent luteolytic activity (reviewed by Horton and Poyser, 1976). Further, prostaglandin F$_2$α was shown to be elevated in uterine venous plasma of several species coincident with the
functional demise of the corpus luteum (Horton and Poyser, 1976).

Gleeson and Thorburn (1973) were the first to measure prostaglandin F\(_2\alpha\) secretion emanating from the uterus of the nonpregnant pig. These researchers found a high correlation between the increase in uterine venous prostaglandin concentration and the decline in luteal progesterone secretion at the end of the estrous cycle. Later Gleeson et al. (1974), Moeljono et al. (1977), and Shille et al. (1979) demonstrated the pulsatile nature of uterine prostaglandin F\(_2\alpha\) secretion in the nonpregnant pig and confirmed the temporal association between increasing uterine prostaglandin F\(_2\alpha\) secretion and the declining luteal progesterone secretion on days 13-16 of the estrous cycle. Interestingly, single systemic injections or infusions of prostaglandin F\(_2\alpha\) into the ovarian artery of the nonpregnant pig prior to day 12-13 of the estrous cycle failed to initiate luteolysis (Diehl and Day, 1974; Conner et al., 1976; Conley and Ford, 1991). In contrast, exogenous prostaglandin F\(_2\alpha\) effectively initiated luteolysis in nonpregnant gilts when administered after day 12 of the estrous cycle. However, the role of the exogenously administered prostaglandin F\(_2\alpha\) as the sole impetus for luteolysis was established using pregnant and hysterectomized gilts (i.e., both are physiologic conditions where uterine prostaglandin F\(_2\alpha\) secretion is effectively reduced). When these animals were given systemic injections of prostaglandin F\(_2\alpha\) similar to the that required to initiate luteolysis in nonpregnant pigs, prostaglandin F\(_2\alpha\) was shown to induce luteolysis but not until 12 days after the onset of estrus or mating (Moeljono et al., 1976).

The most plausible reason that prostaglandin F\(_2\alpha\) was unable to initiate luteolysis prior to day 12 was the relative low numbers of prostaglandin F\(_2\alpha\) receptors (binding sites) on luteal cells until day 13 or 14 of the estrous cycle (Gadsby
et al., 1990, 1993). In a recent study, Estill et al., (1993) demonstrated that repeated injections (every 12 hours) of prostaglandin F\(_2\alpha\) on days 6 through 12 of the estrous cycle initiated early luteal regression, as evidenced by the early return to estrus by day 13. Based on the relative lack of prostaglandin\(_2\alpha\) receptors until day 13 of the estrous cycle and the ability of pulsatile prostaglandin \(F_2\alpha\) administration to initiate premature luteolysis, these authors speculated that prostaglandin \(F_2\alpha\) could cause up-regulation of its own receptors on luteal cells. More recently, this same laboratory (Estill and Britt, 1993) attempted to examine if exogenous prostaglandin \(F_2\alpha\) administration on day 6 to 12 could elevate luteal cell prostaglandin \(F_2\alpha\) binding sites. They observed no increase in binding sites in the prostaglandin \(F_2\alpha\)-treated gilts. However, their experimental design was seriously flawed, based on the results of their original paper (Gadsby et al., 1990). In that previous study, prostaglandin \(F_2\alpha\) receptors declined to values similar to those observed on day 10-12 immediately after the transient increase on day 13 of the estrous cycle. Additionally, in that earlier study, Gadsby et al. (1990) stated that this decline in receptor numbers (down-regulation) was likely in response to endogenous prostaglandin \(F_2\alpha\) receptor binding. This apparent down-regulation of receptors after uterine prostaglandin \(F_2\alpha\) secretion argues directly against an up-regulation of prostaglandin \(F_2\alpha\) receptors in response to its agonist. Furthermore, if prostaglandin \(F_2\alpha\) did upregulate receptor numbers during the early estrous cycle (day 6-12), the protocol used would not detect this change as each subsequent dose of prostaglandin \(F_2\alpha\) administered at 12 hour intervals might be expected to effectively decrease prostaglandin \(F_2\alpha\) receptor numbers to pre-prostaglandin \(F_2\alpha\) injection levels. Evidence for heterogeneity in the large luteal cell population was presented by Taylor and Clark.
These researchers observed that several secretagogues including prostaglandin F\(_{2\alpha}\) elicited differential responses in large luteal cells suggesting the existence of two or more types of these cells in the pig corpus luteum. It might be speculated that prostaglandin F\(_{2\alpha}\) could differentially enhance receptor formation in one large luteal cell while causing down-regulation in another. Additional evidence that prostaglandin F\(_{2\alpha}\) may upregulate its own receptors on luteal cells is the apparent lack of an increase in prostaglandin F\(_{2\alpha}\) receptors in pregnant and nonpregnant estrogen-treated gilts on days 13 and 14 (Gadsby et al., 1993).

While it is quite accepted that prostaglandin F\(_{2\alpha}\) is the hormone which causes luteolysis in the pig and most other species studied to date (reviewed by Horton and Poyser, 1976); the mechanism through which prostaglandin F\(_{2\alpha}\) causes luteolysis is still unknown (Knickerbocker et al., 1988; Stormshak et al., 1987). The following section will provide a general discussion of the known morphological changes as they occur during natural luteolysis compared to those of prostaglandin F\(_{2\alpha}\)-induced luteolysis. This will be followed by a biochemical and biophysical discussion of what is known about the process of luteolysis in the pig. Where appropriate, information from other species will also be included in this review.

**Morphological regression**

The first descriptions of luteal regression completed at the light microscope level were given by Loeb (1911; guinea pig) and Corner (1921; pig). The general observations of the early study in the pig demonstrated that luteal regression in this animal began on days 14 to 15 of the estrous cycle (Corner, 1921). Microscopically, Corner (1921) observed a large increase in cytoplasmic vacuoles (lipid droplets) within the granulosa-lutein cells during the early stages of luteal
regression. Over the next several days he noted an almost complete disappearance of the granulosa-lutein cells (large luteal cells as described by the author), collapse of the capillaries, and degeneration of the nuclei of endothelial cells. Many of the theca-lutein cells described by Corner (1919) appeared to still be present long after the loss of the granulosa-lutein cells (Corner, 1921).

Natural luteolysis Deane et al. (1966) and Bjersing et al. (1970) described in detail the morphologic changes within the ovine corpus luteum as it regressed. Further, these morphologic changes were related to functional changes such as progesterone content, as well as histochemical localization of lipids and 3-β-hydroxysteroid dehydrogenase activity. At the light microscope level, the first obvious sign of luteal regression was the disintegration of the nuclei of large luteal cells (Deane et al., 1966). They also noted a reduction in large luteal cell size and the appearance of more cytoplasmic vacuoles within these cells. Bjersing et al. (1970) observed dark chromatin granules and the clumping of chromatin along the nuclear membrane of luteal cells on day 15 of the ovine estrous cycle in association with decreased luteal progesterone concentrations. These nuclear changes as well as the increased presence of cytoplasmic vacuoles within luteal cells were the first morphologic signs of luteal regression. Histochemical localization techniques demonstrated that the cytoplasmic vacuoles previously noted at the light microscope level were in fact artifacts of the paraffin dehydration-embedding procedure and were remnants of lipid droplets (Deane et al., 1966; Bjersing et al., 1970). Histochemical localization of 3-β-hydroxysteroid dehydrogenase activity in luteal tissue illustrated that decreased luteal activity of this enzyme was associated with decreased luteal progesterone content (Deane et al., 1966; Bjersing et al.,
1970).

On the ultrastructural level, Deane et al. (1966) observed that on days 12 and 13 of the ovine estrous cycle, prior to a decline in luteal function (i.e., decreased luteal tissue progesterone concentration), the number of lipid droplets increased as did mitochondrial size in the steroidogenic cells of the corpus luteum. In addition to these two changes, over the next two days of the estrous cycle (day 14-15), they noted that the electron density of the mitochondrial matrix increased, the nuclear membrane exhibited a more irregular outline and the number of dense bodies within the large luteal cells decreased. Formation of myelin like figures from the smooth endoplasmic reticulum (i.e., site of progesterone synthesis) was prevalent in the ovine large luteal cells, but only after luteal progesterone concentrations had already declined (Deane et al., 1966).

Observations of Bjersing et al. (1970) on ovine luteal regression at the ultrastructural level, while similar to those of Deane et al. (1966) were much more detailed. Lipid vesicles (droplets) were present in low numbers in the large luteal cells on day 12 and 13 of the estrous cycle, then began to increase in number on day 14 and continued to increase in number and size through day 15. In this study, corpora lutea with the lowest progesterone concentrations exhibited the greatest numbers of lipid vacuoles within large luteal cells. The remaining changes observed by Bjersing et al. (1970) refer to corpora lutea collected on days 14 or 15 of the estrous cycle, which had low progesterone levels (i.e., were functionally regressed). The nuclei of large luteal cells undergoing regression were irregular in shape, the two layers of the nuclear envelope were widely separated, and clumping of chromatin along the inner nuclear membrane was noticeably increased. They also observed an increase in mitochondria size, a denser matrix and the presence of granules within the
mitochondria. Additionally, the amount of rough endoplasmic reticulum was extremely reduced in comparison to the levels seen in day 10 corpora lutea. The smooth endoplasmic reticular membranes became more vesicular (less tubular) and less distinct after the decrease in luteal progesterone concentration. They further noted the disappearance of the small dense bodies (cytosomes) within the large luteal cells.

Christensen and Gilliam (1969) reported on the wide variety of cytoplasmic inclusions (cytosomes, dense bodies, small bodies, lysosomes, lipid droplets, microbodies) within luteal cells. They suggested that these inclusions might be secretory vesicles and or primary or secondary lysosomes based on their size and the presence or absence of acid phosphatase activity. The small dense granules 0.2 μm in diameter seen in large luteal cells by Bjersing et al. (1970) and many others since, were the focus of a study by Gemmell et al. (1974). These structures appeared to form from the Golgi and then accumulated at the cell membrane where they appeared to fuse with the plasma membrane and release their contents into the intercellular space (Gemmell et al., 1974). These authors went on to show that these structures were not lysosomes (absence of acid phosphatase). Both Bjersing et al. (1970) and Gemmell et al. (1974) noted the disappearance of these smaller dense granules during the early stages of luteal regression in the ovine. Later studies indicated that these cytoplasmic dense bodies contained oxytocin in the ewe (Rodgers et al., 1983), and relaxin in the pig (Belt et al., 1971).

Investigation of lysosomal function in the ovine corpus luteum revealed that total acid phosphatase activity did not increase during luteal regression (Dingle et al., 1968). However, the fragility of lysosomes (i.e., their ability to withstand the isolation procedure) increased after day 15 of the estrous cycle in association with the decline in luteal
progesterone production. This increase in fragility was thought to precipitate the release of the lysosome's contents within the cell causing intracellular destruction.

O'Shea et al. (1977) studied in detail the natural regression of the ovine luteal vasculature during the estrous cycle. They saw no evidence of degenerative changes in the endothelium of ovine corpora lutea obtained prior to day 14 of the estrous cycle. However, by day 15, the regressive changes in the capillary wall were marked. Endothelial cells exhibited areas of localized degeneration and detachment from the basement membrane. A general increase in cellular debris within the capillary lumen was also observed. Further, O'Shea et al. (1977) were the first to recognize that the corpus luteum exhibited a phenomenon called apoptosis as described by Kerr et al. (1972) for other tissues. These apoptotic bodies or cellular remnants were similar to the autophagic bodies and large dense bodies described by other authors (Cavazos et al., 1969; Deane et al., 1966; Bjersing et al., 1970; Koering and Kirton, 1973).

Cavazos et al. (1969) published the only ultrastructural description of luteal regression in the pig. These authors compared corpora lutea collected during the early luteal phase (days 4, 8 and 12) with those collected on days 14, 16, and 18 of the estrous cycle. They noted the presence of lipid vesicles in some granulosa-lutein cells on day 12, their presence increased in number and size through day 18 of the estrous cycle. The smooth endoplasmic reticulum within the porcine large luteal cells became more dilated and whorls of the membrane were more prevalent as luteolysis progressed. Additionally, on day 16 and 18 of the estrous cycle, intracellular collagen content increased as did the presence of large cytoplasmic dense bodies often containing recognizable cell organelles (apoptotic bodies). These authors also noted that during the later stage (day 18) of the
estrous cycle, that the last viable cells present within the corpus luteum appeared to be those described as theca-lutein cells (small luteal cells). No mention of changes in the mitochondria or nucleus of the steroidogenic cells were made by these authors.

Recently, Yuan et al. (1993) examined porcine luteal tissue collected on day 10, 15 and 18 of the estrous cycle at the ultrastructural level with both the scanning electron microscope (SEM; day 10 and 15) and the transmission electron microscope (TEM; days 10, 15 and 18). The surface morphology of dispersed and isolated day 10 large luteal cells (>25 μm) were characterized by the numerous amounts of microvilli. In contrast, day 10 small luteal cells (<20 μm) had a much smoother surface membrane. These investigators reported that day 15 large luteal cells appeared shrunken when compared to those collected on day 10 of the estrous cycle (changes in small cells were not reported). Similar to the SEM observations described above, TEM revealed that on day 10 the cell membranes of large luteal cells were convoluted with many microvilli, while those of small cells were less convoluted with little evidence of microvilli (Yuan et al., 1993). The small luteal cells observed in day 10 porcine corpora lutea had vesicles of smooth endoplasmic reticulum and numerous lipid droplets.

Further, these investigators also reported on the appearance of two different types of large luteal cells; based on the amount and appearance of the smooth endoplasmic reticulum. The large luteal cell accounting for approximately 65% of all large luteal cells contained a small amount of non-tubular type of smooth endoplasmic reticulum. The other large luteal cell type had large, fingerprint, tubular smooth endoplasmic reticulum. Consistent with the presence of two large luteal cell types, the bovine corpus luteum has also been reported to have two large luteal cell types in a
preliminary report by Fields et al. (1991). One of the bovine large luteal cell types (beta) ranged in size from 40-46 μm and contained numerous mitochondria and a more prominent smooth endoplasmic reticulum, than the other large luteal cell type (alpha) which was 32-38 μm in diameter. Additionally, Fields et al. (1991) reported that the beta type cells were more responsive to the lytic action of prostaglandin F₂α, as evidenced by their loss of secretory granules and swelling of the endoplasmic reticulum and mitochondria. Interestingly, on day 15 of the porcine estrous cycle, the large porcine luteal cells appeared to be of decreased size, and cells containing the abundant fingerprint type of endoplasmic reticulum were not seen (Yuan et al., 1993). Small luteal cell observed on day 15 had little if any evidence of mitochondria, vesicles of smooth endoplasmic reticulum, or lipid droplets. According to the authors, very few luteal cells of either type were intact on day 18 and therefore no description was given. This study of the porcine corpus luteum while providing some baseline information can be criticized for the actual number of cells that were evaluated (i.e., 20 small and 25 large luteal cells). More significantly these authors provided no information on the morphologic basis used to distinguish between small and large luteal cells at the TEM level.

Prostaglandin F₂α-induced regression Upon the discovery of prostaglandin F₂α as a natural luteolysin, the mechanism of luteolysis became one of the most studied phenomenon in the reproductive field (see review by Horton and Poyser, 1976). Okamura et al. (1972) published the first ultrastructural account of prostaglandin F₂α-induced regression. These researchers used the pregnant rat as their experimental animal and noted many of the same characteristic cell changes already mentioned above for natural luteolysis. However, even though no measure of luteal function was
determined in the study by Okamura et al. (1972), the authors suggested that prostaglandin F₉α-induced regression might differ from natural luteolysis, especially as it relates to the time course of events.

Koering and Kirton (1973) using the pregnant rabbit as a model animal, administered one dose of prostaglandin F₉α on three consecutive days prior to sacrifice on days 8, 14 and 21 of pregnancy. Comparisons were made to control (saline injected) rabbits killed on the same days. These authors measured and observed signs of functional and structural regression following prostaglandin F₉α administration. By the third day of prostaglandin F₉α treatment, all three groups (day 8, 14 and 21) exhibited signs of luteal regression as described above. The most dramatic change observed in the luteal tissue was the presence of small membrane bound structures within the parenchyma of the corpus luteum. These structures contained a dense matrix and various cell organelles, which are similar to the apoptotic bodies (cell fragments) already described by Kerr et al. (1972) for other tissues.

The process of luteolysis or sequence of events however required that earlier time points after prostaglandin F₉α be evaluated. Bagwell et al. (1975) examined the time course of morphologic events occurring in response to prostaglandin F₉α administration. In their study, guinea pigs were hysterectomized on day 5 of the estrous cycle, to eliminate endogenous prostaglandin F₉α as well as other possible confounding effects of uterine luteotrophic/luteolytic factors. Beginning on the tenth day after hysterectomy, guinea pigs were administered (125 μg) prostaglandin F₉α intraperitoneally twice daily. Corpora lutea were collected from 5 guinea pigs on day 1, 2, 3, 4, and 5 after prostaglandin F₉α administration, control guinea pigs received saline injections. No changes in luteal cell ultrastructure
were observed in the corpora lutea 1 day after prostaglandin F$_{2\alpha}$. By day 2, however, lipid vesicle numbers were increasing, as was the intercellular space, suggesting a decrease in cell size. Further, clusters of degenerating luteal cells (4-5 cells) appeared in tissue cross sections (focal degeneration). Changes seen on the third day were similar to those on day 2 but more pronounced. By the fourth day of treatment, patches of degeneration were numerous throughout the corpus luteum, myelin figures appeared within the cytoplasm of luteal cells, and collagen filled the intercellular spaces. Ultrastructural observations on day 5 were similar but more pronounced than those of day 4.

Van der Walt (1978) examined the rapid (1-3 hour) effects of prostaglandin F$_{2\alpha}$ on ovine luteal tissue ultrastructure. Ewes were administered 300 u of prostaglandin F$_{2\alpha}$ or saline (control) via the abdominal aorta on day 10 of the estrous cycle and corpora lutea were collected 1, 2 and 3 hours after treatment. Control tissues (day 10) prior to and after saline injection were not different. Steroidogenic cells exhibited elongated mitochondria with tubular cristae, numerous microvillar projections and very little lipid. Within 1 hour of prostaglandin F$_{2\alpha}$ treatment, the mitochondrial appearance was more spherical and the presence of vesicles became more prevalent in the luteal cells. At 2 hours, almost all of the mitochondria were spherical, the matrix was dense and glycogen granules were observed in the cytoplasm. The microvillar projections observed in control tissues had disappeared by 2 hours, while the number of vesicles was increased compared to the 1 hour treatment group. Three hours following prostaglandin F$_{2\alpha}$ treatment, many mitochondria were ruptured and the number of lipid droplets had increased as had the presence of degenerative areas within the luteal tissue. Van der Walt (1978) made neither any mention of changes in nuclear composition of luteal cells nor the mention of endothelial,
fibroblastic or immune cells at all.

Umo (1975) compared ultrastructural and functional changes in the ovine corpus luteum during natural and prostaglandin F$_2$α-induced regression in the ewe. Umo (1975) injected 10 mg prostaglandin F$_2$α intramuscularly into two ewes on day 9 of the estrous cycle and collected the corpora lutea twelve hours later; these corpora lutea were compared to two saline injected (control) ewes and two ewes on day 15 of the estrous cycle (natural luteolysis). Corpora lutea obtained during natural luteolysis exhibited the changes as described previously (Bjersing et al., 1970). Prostaglandin F$_2$α-treated ewes showed no difference in luteal tissue morphology when compared to the natural regressing corpora lutea. It appears that prostaglandin F$_2$α-induced luteolysis exhibits a progression of morphologic events similar to those observed during natural luteolysis, no evidence to the contrary has been published that I am aware of.

As mentioned in a previous section of this literature review, the corpus luteum is composed predominately of endothelial cells. Azmi and O’Shea (1984) carried out a very extensive study of the mechanism of loss of luteal endothelial cells during both natural and prostaglandin F$_2$α-induced luteolysis in the guinea pig. Natural luteolysis was evaluated by comparing day 9 corpora lutea (fully functional) and day 16 corpora lutea (regressed). Intraperitoneal administration of a single dose of cloprostenol, a synthetic prostaglandin F$_2$α analog, to twenty-five (day 9) cyclic guinea pigs was followed by collection of corpora lutea at 1, 3, 12, 24, and 48 hours later for evaluation of endothelial cell changes. Intraperitoneal saline injected controls were also included and were not different from day 9 controls.

The capillaries of the day 9 control corpora lutea appeared normal (i.e., vessels were lined by a single uninterrupted layer of endothelial cells attached to a well
developed basement membrane, the cytoplasm of the endothelial cells was thin and only near the nucleus did the endothelial cells bulge into the capillary lumen; Hossain and O'Shea, 1981). The most common changes seen during both natural and prostaglandin F2α-induced luteolysis were the protrusion of endothelial cells into the capillary lumen, nuclear and cytoplasmic shrinkage and lobulation, as well as fragmentation, loss of cytoplasmic density, fusing of plasma membranes among degenerating cells, and the presence of degenerating cell fragments within cytoplasmic vesicles of otherwise normal endothelial cells.

Below is a short synopsis of the time course of events observed during the loss of luteal endothelial cells (Azmi and O'Shea, 1984). Within one hour of prostaglandin F2α treatment, no definitive morphologic changes had occurred in the corpus luteum. Three hours after prostaglandin F2α administration, capillary fenestrations could no longer be seen and small numbers of capillaries contained protruding endothelial cells. The capillaries still showed the same changes observed at 3 hours by twelve hours, plus a greater number of endothelial cells exhibited clumping of chromatin along the nuclear membrane. At twenty-four hours, condensed dead cells were observed both on the walls and within the lumen of the capillaries. The changes observed at 48-hours were most like those seen on day 16 of the estrous cycle with widespread degeneration of the capillaries and capillary lumens filled with cellular debris (i.e., apoptotic bodies). On day 16 of natural luteolysis as well as during prostaglandin F2α-induced luteolysis, capillary degeneration appeared to be randomly distributed throughout the corpus luteum as degenerate and healthy endothelial cells could be seen side by side.

To obtain objective measures of the process of luteolysis from a morphological standpoint, investigators turned to
stereologic techniques. Azmi et al. (1984) were the first to stereologically examine changes in corpora lutea during the estrous cycle. In their study, guinea pig corpora lutea from day 9, 12 and 16 of the estrous cycle were evaluated. I will relate the compositional changes seen between day 12 and 16, as this represents a fully functional corpus luteum, to a regressed one. On day 12 of the estrous cycle, there were $204.4 \times 10^3$ steroidogenic (luteal) cells, $539.9 \times 10^3$ endothelial cells and $79 \times 10^3$ other cell types. By day 16 the number of endothelial cells ($144.4 \times 10^3$) had dropped almost 4-fold. In contrast, numbers of luteal cells ($171.3 \times 10^3$) obtained from day 16 corpora lutea did not decrease significantly. However, while luteal cell numbers did not change significantly, luteal cell volume decreased approximately 2.5-fold. Morphologically, this decrease in luteal cell size was evident as an increased cytoplasmic density, irregularity of the cell outline, and cellular degeneration and condensation. Luteal vascular space ($\text{mm}^2$) also decreased between day 12 and 16 as would be expected following the large loss of endothelial cells. The removal of endothelial cells and the shrinking of luteal cells caused the intercellular space (% of total) to increase from 1.5% on day 12 to 18.5% on day 16 of the estrous cycle.

Many reports (Farin et al., 1986; O'Shea et al., 1986; Rodgers et al., 1984; O'Shea et al., 1990) have evaluated luteal composition via morphometric analysis during the estrous cycle of the ewe and cow. These studies in general were designed to look at luteal tissue composition during the growth of the corpus luteum and not during the regression of this tissue. Briefly, Farin et al. (1986) distinguished between functionally (progesterone concentrations) active and inactive ovine corpora lutea obtained on day 16 of the estrous cycle. They observed a sharp reduction in endothelial cell numbers with no change in the numbers of small or large luteal
cells or fibroblasts cells. Further, no significant
difference in cell size was observed between the regressed and
nonregressed corpora lutea.

The importance of the mitochondria to steroid production
was the impetus for the morphometric quantification of these
structures in the large and small ovine luteal cells (Kenny et
al., 1989). Early studies in the ewe suggested that during
luteolysis the mitochondria enlarge (Deane et al., 1966;
Bjersing et al., 1970). This study evaluated corpora lutea
obtained on days 4, 8, 12, and 16 of the estrous cycle; those
on day 16 were divided into two subsets (nonregressed and
regressed) based on functionality as described by Farin et al.
(1986). The large luteal cell mitochondrial volume density
(volume density is the calculated volume of a cell or cellular
organelle based on their two dimensional areas) increased
progressively from 13% on day 4 to 24% on day 16, while the
small luteal cell increased from 11% on day 4 to 13% on day 8
where it remained. No difference in mitochondrial volume
density was observed between the regressed and nonregressed
day 16 corpora lutea. These investigators interpreted this
lack of change in mitochondrial volume density as evidence
that the mitochondria size was not changed. This
interpretation is not accurate as no indication of the number
of mitochondria within individual cells was given. Therefore,
it is possible that fewer larger mitochondria could account
for the equal mitochondrial volume densities observed in the
regressed and nonregressed day 16 corpora lutea.
Additionally, a measure of mitochondrial activity would have
allowed these investigators to make possible correlations
between form and function of the mitochondria.

Braden et al. (1988) combined the use of exogenous
prostaglandin F₂α administration and morphometric analysis at
the ultrastructural level to quantitate changes occurring in
ovine luteal cell populations during regression. In this
study, prostaglandin F₂α was administered to ewes on day 10 of the estrous cycle and corpora lutea were recovered at 12, 24, and 36 hours after treatment. Controls included both day 10 and 12 cyclic ewes. Upon collection, a portion of the luteal tissue was fixed for electron microscopy and morphometric analysis. A portion of the remaining corpus luteum was dissociated into a single cell suspension for quantitation and classification by size (small <22 μm or large >22 μm cells) of steroidogenic (3-β-hydroxysteroid dehydrogenase positive) cells via hemocytometer counts and image analysis. Prostaglandin F₂α caused a significant decrease in systemic serum progesterone levels by 12 hours which remained low through 36 hours. Luteal weight was unchanged at 12 hours, but was significantly less at both 24 and 36 hours after prostaglandin F₂α treatment. Quantitation of steroidogenic cells following tissue dissociation demonstrated that large luteal cells were fewer at 12 hours when compared to control day 10 corpora lutea. No change in number of small luteal cells was observed at this time. Twenty-four hours after prostaglandin F₂α treatment, both small and large cell numbers had declined and this reduction in cell numbers continued through 36 hours. Results obtained from morphometric analysis of these same tissues did not agree with those following tissue dissociation. Morphometric analysis suggested that no change in volume density or number of large luteal cells occurred in the corpus luteum until 36 hours. At this time the large luteal cell size decreased as indicated by a lower volume density and equal numbers of cells within the corpus luteum. However, the small cell volume density and number were significantly lower at 24 hours, similar to the tissue dissociation results, indicating that the loss of small cells accounted for the decreased volume density of this particular cell type. Braden et al. (1988) observed a decrease in endothelial cell numbers at 36 hours after prostaglandin F₂α
treatment. The results of this experiment reiterate the inappropriate use of tissue dissociation techniques to quantitate luteal cell numbers (O’Shea et al., 1986; Rodgers et al., 1984). Further, based on the earlier loss of small luteal cells observed by these authors and the fact that high affinity prostaglandin F$_2$α receptors are known to reside preferentially on large luteal cells it would seem that intracellular communication between these cell types might be critical for normal luteal regression.

**Effect of Prostaglandin F$_2$α on Luteal Blood Flow**

Prostaglandin F$_2$α is a known vasoconstrictive agent, and it was originally proposed that this activity was the mechanism through which it caused luteolysis (Pharriss et al., 1970). Further, many laboratories have reported temporally associated decreases in both blood flow and progesterone secretion of corpora lutea during luteolysis at the end of the estrous cycle (Niswender et al., 1973; Ford and Chenault, 1981; Magness et al., 1983). Studies in the ewe have demonstrated that luteal blood flow is decreased markedly within 2 hours of the administration of a luteolytic dose of prostaglandin F$_2$α (Nett and Niswender, 1981). In contrast, these same investigators reported (Nett et al., 1976) that total ovarian blood flow had not declined until 4 hours after a luteolytic dose of prostaglandin F$_2$α. Systemic progesterone levels, however, did not decline until 6 hours after prostaglandin F$_2$α administration (Nett et al., 1976). The earlier decline in luteal blood flow than total ovarian blood flow is likely due to the presence of ovarian arterio-venous anastomoses which shunt blood away from the corpus luteum towards the ovarian stroma during the early stages of luteolysis (Mattner et al., 1981). These observations suggested that the rapid decline in luteal blood flow induced
by prostaglandin F₂α treatment might be the initial trigger for luteolysis, however, the cause and effect relationship has been difficult to establish (Einer-Jensen and McCracken, 1981).

Moore (1977) demonstrated that an experimentally induced reduction of total ovarian blood flow on day 8 of the ovine estrous cycle to 23-60% of its previous level had no appreciable effect on subsequent luteal progesterone secretion or lifespan. Azmi and O'Shea (1984) observed no significant effect of total occlusion of the ovarian artery for 15-120 minutes in guinea pigs on subsequent luteal cell morphology. In contrast, these investigators did note that there was a significant degeneration of endothelial cells as the length of occlusion increased from 15 to 120 minutes. The pattern of endothelial cell degeneration after ovarian arterial occlusion was similar to that previously described following cloprostenol administration or during natural luteolysis in the guinea pig (see page 37). The degeneration of endothelial cells following vascular occlusion may be the result of a phenomenon called ischemia-reperfusion injury (to be discussed in next section).

In the pig, decreased ovarian blood flow has also been closely linked to decreases in luteal progesterone output (Magness et al., 1983; Rathmacher and Anderson, 1968). Further, ovarian arterial sensitivity to vasoconstrictive agents is increased during the luteal phase (days 11-13) versus the follicular phase (day 20-21) of the estrous cycle in the pig (Reynolds and Ford, 1984). Moreover, the vasoconstrictor sensitivity of the ovarian artery remained low during early pregnancy (day 13) in the pig and was similar to that seen during the follicular phase. This effect of pregnancy on reducing the contractile properties of the ovarian artery is consistent with an observed transient increase in ovarian blood flow during early pregnancy (day 13-
14) in the pig (Magness et al., 1983), possibly via estrogen or prostaglandin E₂. These hormones, both produced by the conceptus and/or uterus during the period of maternal recognition of pregnancy, are known to increase blood flow through the utero-ovarian vasculature (Ford, 1985).

In conclusion, it is clear that blood flow to the corpus luteum decreases markedly following its exposure to prostaglandin F₂α, but its role in the luteolytic process is unclear. The inability of vascular occlusion alone to initiate luteolysis suggests that prostaglandin F₂α must also have additional effects, possibly a direct cytotoxic effect on luteal cells. The importance of vascular changes in prostaglandin F₂α-induced luteolysis, however, is suggested by the failure of this luteolytic agent to kill luteal cells in vitro (Hoyer et al., 1984; Hunter et al., 1981; Jones and Gadsby, 1993; Mattioli et al., 1985; Wegner et al., 1991). A possible mechanism through prostaglandin F₂α could initiate luteolysis is via the phenomenon called ischemia-reperfusion injury (discussed below).

Ischemia Induced Changes in Luteal Cell Function

In addition to the obvious metabolic effects that a reduction in blood flow can elicit (i.e., reduced oxygen and nutrient uptake, gonadotrophin delivery), a sharp reduction in blood flow followed by reperfusion can elicit a phenomenon called ischemia-reperfusion injury. This type of tissue injury relies on the production of reactive oxygen species, such as hydrogen peroxide, superoxide radicals and lipid peroxides. Normally, a cascade of enzymes protects the cell from the normal physiologic production of these reactive oxygen molecules. However, following, an ischemic insult, it is thought that one or more of the enzymes (superoxide dismutase, catalase and glutathione peroxidase) involved in
the reduction of these molecules is damaged or overwhelmed following the reperfusion of oxygenated blood. Since, endothelial cells separate oxygen-rich blood and tissue parenchymal cells, essentially acting as a buffer, it might be logically assumed that following an ischemic-reperfusion episode the greatest damage would occur in the endothelial cells. The vascular occlusion study of Azmi and O’Shea (1984) describing endothelial cell loss in the guinea pig corpus luteum as previously described supports this assumption. To reiterate, Azmi and O’Shea (1984) found that occlusion of the vascular supply to the ovary for 15 to 120 minutes followed by reperfusion (30 minutes to 24 hours) caused degeneration of endothelial cells specific to the corpus luteum with no apparent effects on morphology of the steroidogenic cells.

In a recent study, the effects of ovarian ischemia-reperfusion on luteal function was tested in pregnant rats (Sugino et al., 1993). In this study, the ovarian artery was completely occluded for 30 minutes, followed by a reperfusion period of 90 or 210 minutes before collection of serum for progesterone analysis and luteal tissue for superoxide dismutase and glutathione peroxidase activity as well as lipid peroxide levels. Ischemia-reperfusion caused a significant drop in serum progesterone concentrations by 90 minutes, which returned to basal levels by 210 minutes. Decreased serum progesterone levels were temporally associated with reduced superoxide dismutase activity and increased luteal lipid peroxide levels. No change in glutathione peroxidase activity was detected in luteal tissue during this experiment. To evaluate if the decreased superoxide dismutase might be the cause of the elevated lipid peroxide levels, superoxide dismutase (converts superoxide radicals to H₂O₂) plus catalase (converts H₂O₂ to H₂O and O₂) were administered immediately prior to and after vascular occlusion (Sugino et al., 1993). Exogenous superoxide dismutase plus catalase decreased the
level of luteal tissue lipid peroxidation to near basal levels and maintained plasma progesterone levels at preischemic concentrations. While ischemic-reperfusion injury can cause a decrease in progesterone production similar to prostaglandin 
$F_2\alpha$ treatment, the return of serum progesterone concentrations to preischemic control levels within 210 minutes of reperfusion supports the conclusions of other investigators that vascular occlusion alone is insufficient to induce luteolysis.

Free radicals and peroxides are known to cause a multitude of potential cellular problems, they oxidize protein sulfhydryl groups, induce lipid peroxidation, cause breaks in DNA strands, activate poly (ADP-ribose) polymerase, alter protein production and microtubule arrangement and decrease membrane fluidity (Schilling and Elliott, 1992). However, while the exact mechanism of oxidant induced injury is not known, substantial evidence points to an alteration in the ability of the cell to maintain homeostatic control of its intracellular Ca$^{++}$ concentrations (Schilling and Elliott, 1992).

Interestingly, several of the effects attributed to oxidative damage have been observed to occur within regressing corpora lutea. Of the oxidative changes observed to occur in luteal tissue, changes in membrane fluidity, lipid peroxidation and oxygen radical formation have been observed to occur in regressing luteal tissue (Carlson et al., 1984; Sugino et al., 1993). Both lipid peroxidation and oxygen radical generation are known to influence plasma membrane fluidity and as a result it has received considerable attention. Further, it is easy to hypothesize that changes in plasma membrane fluidity may interfere with membrane protein-mediated processes, such as gonadotrophin binding, transduction (G-protein) of receptor binding to second messenger systems, facilitated transport mechanisms as well as
ion pumps which maintain normal cell homeostasis (Buhr et al., 1979). During luteal regression in the rat and cow, decreases in microsomal membrane (endoplasmic and plasma membrane and associated proteins) fluidity have been observed to occur within 24 hours of prostaglandin F$_2$α administration (Carlson et al., 1981, 1982, 1984; Buhr et al., 1979; Goodsaid-Zalduondo et al., 1982). Alterations in membrane fluidity are characterized as a change from a fluid (liquid-crystalline) phase lipid to a gel-phase lipid as measured by x-ray diffraction at normal physiological temperature. Further, analysis and comparison of microsomal membrane and liposomal (multilamellar vesicles prepared from lipid extracts of microsomal membranes) fluidity from regressing rat corpora lutea suggested that membrane protein changes were critical for the loss of membrane fluidity (Carlson et al., 1981). In contrast, changes in membrane cholesterol content, ratio of unsaturated to saturated fatty acids, and fatty acid chain length were not related to membrane fluidity (Carlson et al., 1981). Additional study demonstrated that changes in membrane fluidity were dependent on Ca$^{2+}$ and phospholipase A$_2$ activity (Carlson et al., 1981). Unfortunately, these previous studies utilized entire homogenized corpora lutea and therefore changes in membrane fluidity cannot be attributed to a particular cell type. In contrast, a preliminary study by Buhr et al. (1986) observed no association of luteal tissue (endothelial, luteal cells) membrane fluidity changes with stage of the estrous cycle or following prostaglandin F$_2$α treatment in the pig.

Superoxide radical formation has also been shown to be elevated in plasma membrane samples of rat corpora lutea undergoing regression (Sawada and Carlson, 1989). A more recent study by Sawada and Carlson (1991) evaluated a battery of changes associated with superoxide radical formation in rat luteal tissue following prostaglandin F$_2$α or saline (control)
treatment. This study compared treated and control luteal tissue plasma membrane levels of superoxide radicals, lipid peroxide, ATP-dependent Ca\(^{2+}\) transport, phospholipase A\(_2\) activity and membrane fluidity at 0, 5, 10, 20, 40, 60 and 120 minutes after prostaglandin F\(_2\alpha\) or saline treatment. Additionally, plasma progesterone concentrations were determined as were superoxide dismutase, catalase and glutathione peroxidase activities in ovarian homogenates. Plasma progesterone levels in prostaglandin F\(_2\alpha\)-treated rats were significantly lower within 20 minutes and continued to progressively decline to low levels over the first 60 minutes. Progesterone concentrations remained low through completion of this study at 120 minutes. The apparent rapid decrease in luteal function in this study, may be the result of the superphysiological dose of prostaglandin F\(_2\alpha\) administered to these rats. These investigators observed that within 10 minutes of injection of prostaglandin F\(_2\alpha\) there was a substantial increase in superoxide radical formation, which preceded the decline in serum progesterone levels by 10 minutes. Formation of superoxide radicals returned to basal levels within 40 minutes of treatment. Changes in plasma membrane fluidity paralleled those of superoxide formation, while plasma membrane lipid peroxidation was not observed until 120 minutes after prostaglandin F\(_2\alpha\) administration. Plasma membrane ATP-dependent Ca\(^{2+}\) transport was increased at 60 and 120 minutes in prostaglandin F\(_2\alpha\)-treated rats, while phospholipase A\(_2\) levels were elevated at twenty and 60 minutes. Changes in the free radical scavenging enzyme activities (superoxide dismutase, catalase and glutathione peroxidase) were not different between control and prostaglandin F\(_2\alpha\)-treated rats within the two treatment periods. The results of this study clearly illustrate that superoxide radical formation is an early event in prostaglandin F\(_2\alpha\)-induced luteolysis. Further, changes
associated with superoxide radicals (i.e., decreased membrane fluidity, increased lipid peroxidation and phospholipase A₂ activity) followed changes in superoxide radical formation as expected. Moreover, the ATP-dependent Ca²⁺ pump in the plasma membrane was fully activated at 60 minutes, suggesting that homeostatic mechanisms were attempting to reduce cytosolic Ca²⁺ concentrations by this time. Interestingly, changes in free radical scavenging enzymes do not appear to play a role in earlier processes of luteolysis. This study also illustrates the rapidness with which luteal progesterone production declines, suggesting that the initial events of luteolysis are primarily directed at interrupting progesterone secretion.

Generation of hydrogen peroxide has also been shown to increase during luteolysis in the rat (Riley and Behrman, 1991). Moreover, exogenous hydrogen peroxide has been shown to cause decreases in luteinizing hormone stimulated cAMP accumulation and progesterone production in isolated and cultured luteal cells from pseudopregnant rats (Behrman and Preston, 1989; Gatzuli et al., 1991; Behrman and Aten, 1991). The reduction in progesterone production caused by H₂O₂ appears to result from a blockade of mitochondrial uptake of cholesterol (Behrman and Aten, 1991) the supposed rate limiting step in progesterone production.

In conclusion, the role of superoxide radical formation in prostaglandin F₂α-induced luteolysis is supported solely by their temporal association reported in these rodents studies. However, while several of the terminal events or results of superoxide formation (i.e., lipid peroxidation, decreased membrane fluidity) have been shown to increase in luteal tissue of domestic livestock, their final role in the luteolytic process remains to be determined.
Direct Effects of Prostaglandin \( F_{2\alpha} \) on Luteal Cells

High affinity membrane receptors for prostaglandin \( F_{2\alpha} \) have been localized on large luteal cells of most species including the pig (Knickerbocker et al., 1988; Gadsby et al., 1990), supporting a direct effect of prostaglandin \( F_{2\alpha} \) on these cells. Furthermore, evidence of a membrane receptor suggests that prostaglandin \( F_{2\alpha} \) regulation of luteal cell function might be mediated by one of the two major second messenger systems, cAMP and/or the phosphatidylinositol pathways. Conversion of ATP to cAMP via the receptor mediated activation of adenylate cyclase allows the subsequent activation of cAMP-dependent protein kinases which will, in turn, cause the phosphorylation of specific proteins, thereby altering cell function (Gennis, 1989). In contrast to the single second messenger generated in the cAMP pathway, the phosphatidylinositol pathway generates two molecules, inositol 1,4,5-triphosphate (IP\(_3\)) and diacylglycerol via the phospholipase C catabolism of phosphatidylinositol 4,5-bisphosphate (PIP\(_2\); Gennis, 1989). Subsequently, IP\(_3\) can cause an increase in intracellular Ca\(^{++}\) concentrations, by causing the release of endoplasmic reticular Ca\(^{++}\) stores. This increase in free cytosolic Ca\(^{++}\) in turn binds to and causes the movement of protein kinase C to the plasma membrane where it binds to diacylglycerol and to the plasma membrane phospholipid, phosphatidylserine, thereby activating protein kinase C and allowing it to phosphorylate other proteins. In addition, increases in free cytosolic Ca\(^{++}\) can activate calmodulin, as well as many other Ca\(^{++}\) dependent-enzymes (Gennis, 1989).

Early studies in the rat demonstrated that prostaglandin \( F_{2\alpha} \) prevented luteinizing hormone-induced cyclic AMP accumulation in isolated luteal tissue and luteal cell suspensions (reviewed by Lahav et al., 1989). Moreover,
prostaglandin \( \text{F}_2\alpha \) was shown to inhibit adenylate cyclase activity; however, this effect was not direct, as prostaglandin \( \text{F}_2\alpha \) added directly to isolated luteal adenylate cyclase had no effect on its activity (Lahav et al., 1989). Instead, incubation of rat luteal cells in the presence of the \( \text{Ca}^{++} \) ionophore (A23187) was shown to inhibit luteinizing-hormone induced accumulation of cAMP similar to prostaglandin \( \text{F}_2\alpha \) (Dorflinger et al., 1984). Additionally, this study determined that prostaglandin \( \text{F}_2\alpha \) could inhibit cAMP accumulation in the presence of a \( \text{Ca}^{++} \) channel blocker and without the benefit of extracellular \( \text{Ca}^{++} \) in the culture medium suggesting that prostaglandin \( \text{F}_2\alpha \) utilizes an intracellular source of \( \text{Ca}^{++} \) (Dorflinger et al., 1984). Coincident with these observations in luteal tissue it was shown that prostaglandin \( \text{F}_2\alpha \) also modulated phosphatidylinositol metabolism in luteal cell membranes of several species (see Houmard et al., 1992). The results of these early experiments suggested that prostaglandin \( \text{F}_2\alpha \) might well mediate its effects on luteal cells via the phosphatidylinositol second messenger system possibly by increasing free cytosolic \( \text{Ca}^{++} \) concentration via \( \text{IP}_3 \). Since then numerous in vitro culture studies have evaluated phosphatidylinositol modulation of ovine, bovine and rodent corpora luteal function (Houmard et al., 1992; Davis et al., 1986; Lahav et al., 1988). A limited sampling of the experiments carried out in this area is given below in order to familiarize the reader with this subject matter; studies utilizing porcine luteal tissue are emphasized.

**Progesterone production**

Studies of prostaglandin \( \text{F}_2\alpha \) effects on in vitro progesterone production have resulted in very inconsistent results, with reports of stimulatory effects (Speroff and Ramwell, 1970), inhibitory effects (Fitz et al., 1984) and no
activity (Litch and Condon, 1988). Prostaglandin $P_4\alpha$ has been shown to decrease ovine large luteal cell basal progesterone secretion, while not affecting small luteal cell production levels (Wegner et al., 1991). Further, Conley and Ford (1989) were unable to demonstrate an inhibitory effect of prostaglandin $P_4\alpha$ on luteinizing hormone-stimulated progesterone synthesis by dispersed ovine luteal cells. These findings are consistent with the observation that luteinizing hormone receptors are found predominately on small luteal cells, while prostaglandin $P_4\alpha$ receptors are found predominately on large luteal cells (Schwall et al., 1986).

Studies evaluating prostaglandin $P_4\alpha$ effects on in vitro porcine luteal tissue have shown stimulatory (Mattioli et al., 1985) and inhibitory effects (Yuan et al., 1993). The studies of Mattioli used dispersed porcine luteal tissue obtained on day 3, 5, 7, 9, 11, 13 and 15 of the estrous cycle. Luteal cell cultures were exposed to prostaglandin $P_4\alpha$ and medium was drawn off at 15, 30, 60, 120, 180, 240 and 300 minutes. Prostaglandin $P_4\alpha$ stimulated progesterone secretion in luteal tissue homogenates obtained on day 11, 13 and 15 of the cycle, while not affecting those on days 3 through 9 (Mattioli et al., 1985).

In contrast, dispersed and isolated small ($<20 \mu m$) and large ($>25 \mu m$) porcine luteal cells exhibited a reduction in basal progesterone secretion in response to prostaglandin $P_4\alpha$ treatment (Yuan et al., 1993). In this study, luteal cells were cultured overnight prior to treatment of cells with prostaglandin $P_4\alpha$ (3 doses) in the presence of low density lipoprotein. Two and 24 hours after addition of prostaglandin $P_4\alpha$ to the culture medium, an aliquot was drawn off for analysis of progesterone production. Prostaglandin $P_4\alpha$ had no effect on progesterone levels from either day 10 small or large luteal cells. In contrast, small luteal cells (day 15) exhibited a dose-dependent decrease in progesterone levels in
response to prostaglandin F₂α both at 2 and 24 hours. However, it should be noted that control small luteal cells exhibited a 6-fold decrease in basal progesterone production and a 14% loss in cell viability between the initial plating and the completion of the experiment 24 hours later. Porcine large luteal cells exposed to only the intermediate dose of prostaglandin F₂α (10³ pg) exhibited a significant decrease in progesterone production at 2 hour that returned to control levels by 24 hours. The authors provided no explanation for the transitory response or why only the intermediate dose worked. In summary, the results of this study are inconclusive.

Cytosolic calcium

Similar to progesterone secretory responses, free cytosolic Ca²⁺ levels in cultured ovine large luteal cells increase in response to prostaglandin F₂α exposure, while no such effect was seen in small luteal cells (Wegner et al., 1991). Furthermore, conditions where free cytosolic Ca²⁺ levels are increased experimentally [i.e., use of the Ca²⁺ ionophore (A23187)] only large ovine luteal cells exhibited a decrease in progesterone secretion (Wegner et al., 1991), while no effect of the elevated free cytosolic Ca²⁺ was seen in the small luteal cells. Additional studies (Hoyer et al., 1989; Wegner et al., 1990) using various methods (i.e., Ca²⁺ free extracellular medium, Ca²⁺ channel blockers) suggested that prostaglandin F₂α utilizes an intracellular source of Ca²⁺ to increase free cytosolic levels.

Recently, it was shown that the increase in free intracellular Ca²⁺ induced by prostaglandin F₂α in ovine large luteal cells was of endoplasmic reticular origin (Wegner et al., 1993). In that study, prostaglandin F₂α, thapsigargin and A23187 (i.e., Ca²⁺ ionophore) were used in specific treatment combinations to evaluate the role of luteal cell
endoplasmic reticular Ca\(^{++}\) stores. Thapsigargin is a sesquiterpene lactone tumor promotor, which specifically inhibits the Ca\(^{++}\) ATPase pump associated with replenishing endoplasmic reticulum Ca\(^{++}\) stores. Blockade of the endoplasmic reticular Ca\(^{++}\) ATPase pump results in the leakage of this Ca\(^{++}\) store into the cytosol (i.e., increasing free cytosolic Ca\(^{++}\) levels; Wagner et al., 1993). Treatment of large and small luteal cells with thapsigargin alone caused an immediate increase in free cytosolic Ca\(^{++}\) levels. This increase in free cytosolic Ca\(^{++}\) in response to thapsigargin, indicates the loss of the endoplasmic reticular Ca\(^{++}\) stores. Incubation of large, but not small luteal cells with prostaglandin F\(_{2\alpha}\) elicited a similar increase in free cytosolic Ca\(^{++}\) levels as seen with thapsigargin. However, preincubation of large luteal cells in medium containing thapsigargin failed to elicit an increase in free cytosolic Ca\(^{++}\) in response to subsequent prostaglandin F\(_{2\alpha}\) exposure, confirming that Ca\(^{++}\) utilized by the prostaglandin F\(_{2\alpha}\) was of endoplasmic reticular origin. Since, prostaglandin F\(_{2\alpha}\) was unable to affect the Ca\(^{++}\) ATPase pump directly in small cells, it would appear that the release of endoplasmic reticular Ca\(^{++}\) stores in large luteal cells is likely not the result of an inhibition of the Ca\(^{++}\) ATPase pump but is, instead, the result of another intracellular messenger, most likely IP\(_3\). In addition to the effects elicited by thapsigargin, prostaglandin F\(_{2\alpha}\) and A23187 on free cytosolic Ca\(^{++}\) levels, changes in progesterone production for both large and small ovine luteal cells were also analyzed (Wegner et al., 1993). In large ovine luteal cells, an increase in free intracellular Ca\(^{++}\) concentrations caused by prostaglandin F\(_{2\alpha}\), thapsigargin or the Ca\(^{++}\) ionophore (A23187) or combinations of these compounds was always associated with a reduced progesterone level in the medium. In contrast, these same three compounds had no effect on small luteal cell progesterone production, even though the latter two compounds
caused a significant increase in free intracellular Ca^{++} concentrations (Wegner et al., 1993). Consistent with other in vitro studies, neither large nor small ovine luteal cells exhibited any decrease in cell viability following prostaglandin F_{2\alpha}, thapsigargin or A23187 treatment.

**Protein kinase C**

In the pig, few studies have been conducted evaluating prostaglandin F_{2\alpha} effects on phosphatidylinositol metabolism. These studies were conducted in granulosa cells obtained from small (1-5 mm) follicles of prepuberal gilts (Veldhuis, 1987). In those studies, isolated granulosa cells were preincubated with tritiated myoinositol and ^{14}C labeled arachidonic acid for 18 hours to allow for the sufficient incorporation of these labels into PIP_{2}. Cells were then washed to remove excess and nonspecific label, prior to exposure of cells to medium with and without (control) prostaglandin F_{2\alpha}. They reported an immediate increase in IP_{3} and diacylglycerol concentrations in medium from prostaglandin F_{2\alpha} treated cells. These data support a role for the phosphatidylinositol pathway in porcine ovarian function.

Since, it is known that prostaglandin F_{2\alpha} increases luteal cell PIP_{2} hydrolysis (i.e., increasing IP_{3} and diacylglycerol) many studies have evaluated protein kinase C activity in luteal tissue. The effects of protein kinase C activation on both small and large luteal cell steroidogenesis have been well studied in rodents, sheep and cattle (Behrman et al., 1989; Wiltbank et al., 1989; Davis, 1992). However, in each of these species, protein kinase C activation has been shown to have slightly different roles in luteal steroidogenic function (Davis, 1992). Investigations into how protein kinase C may alter luteal function, commonly incorporates the in vitro use of phorbol esters (PMA, phorbol 12-myristate 13-acetate; TPA, 12-O-tetradecanoylphorbol 13-acetate) and
synthetic diacylglycerols (1,2-diolein) both of which are protein kinase C activators (Musicki et al., 1990). Additionally, specific protein kinase C inhibitors (staurosporine) have been used in in vitro luteal cell culture experiments (Musicki et al., 1990). To my knowledge no studies have evaluated protein kinase C activity changes in luteal tissue following in vivo prostaglandin F\(_2\alpha\) treatment.

In rat luteal cells, prostaglandin F\(_2\alpha\) has been shown to inhibit gonadotrophin-stimulated cAMP and progesterone synthesis (Thomas et al., 1978). Further, Baum and Rosberg (1987) demonstrated that cultured rat luteal cells exposed to phorbol ester and the Ca\(^{++}\) ionophore (A23187) mimicked the effects prostaglandin F\(_2\alpha\) had on luteal cells (i.e., decreased gonadotrophin-stimulated cAMP and progesterone production).

A more through study of protein kinase C activity by Musicki et al. (1990), however, demonstrated that the antigonadotrophic effects of prostaglandin F\(_2\alpha\) and phorbol ester were mediated by separate processes. In that study, cytosolic and membrane protein kinase C activity was quantified by measuring the incorporation of \(^{32}\)P labeled ATP into acceptor histone proteins in the presence of excess phosphatidylserine, Ca\(^{++}\) and diacylglycerol (1,2-diolein) following exposure of luteal cells to the phorbol ester (PMA) and prostaglandin F\(_2\alpha\). Unfortunately, this study did not measure progesterone production, as cAMP was used as the sole measure of rat luteal cell gonadotrophic function. Treatment of dispersed rat luteal cells with a maximal inhibitory dose of PMA caused a 26% decline in gonadotrophin-stimulated cAMP accumulation when compared to control luteal cells; no effect of PMA on basal cAMP was observed. Treatment of luteal cells with the maximal inhibitory dose of prostaglandin F\(_2\alpha\) caused a much greater decrease in cAMP levels (60% decline).

Furthermore, an additive inhibitory effect on gonadotrophin-induced cAMP levels was observed following simultaneous
addition of PMA and prostaglandin F$_2$$\alpha$ to luteal cell cultures. Moreover, staurosporine, a specific protein kinase C inhibitor, was shown to prevent the PMA induced decrease in gonadotrophin-stimulated CAMP levels without affecting the inhibition induced by prostaglandin F$_2$$\alpha$ treatment. Lastly, prostaglandin F$_2$$\alpha$ failed to cause a significant increase in membrane bound protein kinase C activity in rat luteal cells. PMA caused a seven-fold increase in membrane associated protein kinase C activity within 3 minutes of treatment, with a concomitant 80% decrease in cytosolic protein kinase C activity levels. Therefore in the rat, it appears that prostaglandin F$_2$$\alpha$ may not initiate its antigonadotrophic effects through the protein kinase C pathway. However, in evaluating studies conducted on rat luteal tissue, it is critical to recognize the source of luteal tissue (i.e., gonadotrophin-stimulated immature rats) when interpreting the results. Further, tissue dissociation and luteal culture systems may also induce artifactual changes in the second messenger systems.

Studies in the sheep demonstrate that the phorbol ester (PMA) can inhibit basal progesterone secretion by cultured large luteal cells and dispersed luteal (mixed) cells (Wiltbank et al., 1989; Conley and Ford, 1989). Additionally, direct activation of protein kinase C by PMA was able to decrease gonadotrophin-stimulated progesterone production from small luteal cells (Wiltbank et al., 1989). This result is in contrast to previous observations demonstrating a lack of an effect of prostaglandin F$_2$$\alpha$ and increased free cytosolic Ca$^{++}$ on reducing gonadotrophin-induced progesterone secretion from small ovine luteal cells (Wegner et al., 1991). Further, long-term (12-18 hr) treatment of cells with PMA was shown to deplete cultured large and small luteal cells of total protein kinase C activity. Interestingly, following protein kinase C depletion, both large and small luteal cells tended to produce
greater levels of progesterone. This increase in progesterone secretion was postulated by Wiltbank et al. (1989) to be due to the elimination of the inhibitory protein kinase C activity. Since prostaglandin F2α appears to have no effect on small luteal cell progesterone production or cell viability in culture, the physiologic role of protein kinase C activation in these cells is presently unclear.

Pate and Nephew (1988) provided evidence that prostaglandin F3α could inhibit in vitro cultured bovine luteal cell steroidogenesis, but only when lipoproteins were present in the culture medium. These results suggest that prostaglandin F3α might interfere with cholesterol transport, since most of the cholesterol for steroid synthesis by luteal cells is derived from either low or high density lipoproteins (Grummer and Carroll, 1988). Further, the transport of cholesterol to the inner mitochondrial membrane has been proposed to be the rate limiting step in progesterone production (Jefcoate et al., 1992).

In a subsequent study in the ewe, Wiltbank et al. (1990) set out to determine if protein kinase C might mediate the antisteroidogenic effects of prostaglandin F3α in the presence and absence of lipoproteins. In the first of a series of three experiments, Wiltbank et al. (1990) determined that high density lipoproteins increased large luteal cell and luteinizing hormone-stimulated small luteal cell progesterone secretion. In the second experiment, prostaglandin F3α effects on large and small luteal cell progesterone secretion in the presence and absence of lipoproteins was evaluated in an in vitro culture system. Prostaglandin F3α was shown to decrease progesterone production of large luteal cells in the presence but not in the absence of lipoproteins. In contrast, prostaglandin F3α had no effect on either basal or luteinizing hormone-stimulated progesterone secretion by small luteal cells in the presence or absence of lipoproteins. The third
experiment evaluated the role protein kinase C may play in prostaglandin F₂α-induced decreases in large luteal cell progesterone production again in the presence and absence of lipoproteins. To accomplish this goal, these investigators treated cultured large luteal cells with PMA for 18 hours, creating protein kinase C deficient cells as previously described (Wiltbank et al., 1989). This was followed by subsequent treatment with lipoproteins and prostaglandin F₂α. They observed that large luteal cell progesterone production was increased by lipoprotein in both normal and protein kinase C deficient cells. However, the stimulatory effects of lipoproteins were inhibited by prostaglandin F₂α in normal but not in protein kinase C-deficient large ovine luteal cells. These results provide very convincing evidence that protein kinase C is involved in the antisteroidogenic activity of prostaglandin F₂α. However, it once again should be pointed out that luteal cell viability was not affected by in vitro prostaglandin F₂α exposure, suggesting that other mechanisms are necessary to cause the death of luteal cells.

In luteal cells isolated from the bovine corpus luteum, it appears that the small luteal cell function is altered preferentially by the phospholipase C-protein kinase C second messenger system (Benhaim et al., 1990; Dowd et al., 1990). This finding is further supported by the presence of high affinity phorbol ester receptor sites in small luteal cells and not endothelial or large luteal cells (Dowd et al., 1990). Furthermore, in contrast to what was observed in the sheep and rat, protein kinase C activation via phorbol esters, synthetic diacylglycerol and exogenous phospholipase C treatment has been shown to stimulate basal progesterone production by dispersed luteal cells (Brunswig et al., 1986; Benhaim et al., 1990; Davis et al., 1987). Interestingly, in the bovine, treatment of dispersed luteal cells with luteinizing hormone causes an increase in IP₃ and intracellular Ca²⁺ concentrations
(Alila et al., 1989; Davis, 1992). Furthermore, treatment with a phorbol ester in the presence of luteinizing hormone has been shown to markedly decrease luteinizing hormone-induced increases in IP$_3$, without affecting luteinizing hormone-stimulated cAMP or progesterone secretion (Davis, 1992).

Most of the studies in the pig which have evaluated protein kinase C activity, have been carried out on granulosa cells (see Flores et al., 1993). Studies into the role protein kinase C may play in porcine luteal regression are very limited (Rajkumar et al., 1991; Wheeler and Veldhuis, 1987, 1989; Yuan and Connor, 1992). Noland and Dimino (1986) characterized the distribution of protein kinase C activity in porcine ovarian tissue. They compared microsomal, mitochondrial and cytosolic protein kinase C-specific activities from medium sized (3-7 mm) follicles and corpora lutea of unknown age or function. They observed that basal protein kinase C activities were similar for crude homogenates of follicles and corpora lutea. In contrast, crude luteal homogenate protein kinase C activity was markedly enhanced (3-fold) over that seen in follicular tissue when assayed in the presence of phosphatidylycerine, diacylglycerol and Ca$^{2+}$. The subcellular localization of luteal protein kinase C activity demonstrated that 91% of the total protein kinase C activity was found in the cytosolic fraction with the remainder associated in mitochondrial and microsomal membranes. The greater levels of protein kinase C seen in the porcine corpus luteum are consistent with a possible role for this kinase in luteal function. Wheeler and Veldhuis (1987) evaluated the catalytic and receptor-binding properties of protein kinase C isolated from mid-luteal phase porcine corpora lutea. In their study, they evaluated the protein kinase C mediated phosphorylation of the acceptor histone III-s protein by $^{32}$P labeled ATP, in the presence and absence of various levels of
Ca++, diacylglycerol and different phospholipids. Additionally, they evaluated several phorbol esters for binding characteristics and ability to activate isolated porcine luteal cell protein kinase C. They observed that porcine luteal protein kinase C activity was strictly dependent on the presence of Ca++, diacylglycerol, and phosphatidylinserine. Further, TPA was shown to exhibit the highest affinity and greatest stimulation of protein kinase C activity of the several phorbol esters evaluated. In two subsequent studies, Wheeler and Veldhuis (1988, 1989) evaluated the ability of protein kinase C (i.e., TPA treatment) to influence both receptor mediated (i.e., luteinizing hormone) and non-receptor mediated (i.e., cholera toxin, forskolin) cAMP production by dispersed and cultured porcine luteal cells. They observed no effect of any dose of TPA on basal cAMP production. In contrast, TPA caused a dose dependent increase in luteinizing hormone mediated and all non-receptor mediated increases in cAMP. Porcine luteal steroidogenesis was not evaluated in association with cAMP changes in these studies of protein kinase C activity and therefore a functional role for protein kinase C in luteal regression can not be determined.

Yuan and Connor (1992) in a recent study reported protein kinase C activity in large (>25 μm) and small (<20 μm) luteal cells on days 10 and 15 of the porcine estrous cycle. Total protein kinase activity was unchanged for large luteal cells on day 10 and 15, while small luteal cells had 18- and 32-fold lower levels for those two days, respectively. Cytosolic protein kinase C accounted for 60-70% of the total protein kinase C activity of large luteal cells, while in small luteal cells cytosolic protein kinase C exceeded the total by 71% and 139% on day 10 and 15, respectively. This decrease in total protein kinase C appeared to result from the degradation of small luteal cell cytosolic and membrane bound protein kinase
C activity in response to triton X-100 which was used to solubilize membrane bound protein kinase C for total protein kinase C determinations (Yuan and Connor, 1992).

Additionally, Yuan and Connor (1992) evaluated the effects of the phorbol ester (PMA), Ca\textsuperscript{2+} ionophore A23187, 1,2-dioleoyl-sn-glycerol (DAG), phosphatidylserine and dibutyryl cAMP on luteal cell protein kinase C activity. Large luteal cells responded to the PMA treatment with an increase in cytosolic protein kinase C activity and the translocation of 57% and 70% of protein kinase C to the plasma membrane. Incubation of luteal cells with A23187 or DAG had no effect on protein kinase C activity, whereas phosphatidylserine + A23187 or phosphatidylserine + A23187 + DAG elevated protein kinase C activity. Lastly, dibutyryl cAMP caused an increase in both large and small luteal cell protein kinase C activity.

The different endogenous levels of protein kinase C in porcine large and small luteal cells as well as differential responses to various cellular stimulants of protein kinase C activity may help explain the previously reported differential effects of luteolytic and luteotropic hormones. Again, as mentioned for the previous experiment, since luteal steroidogenesis was not evaluated, a functional role for protein kinase C in luteal regression cannot be determined.

Inhibition of steroidogenesis induced by protein kinase C was recently evaluated in porcine granulosa cells (Flores et al., 1993). Similar to the observations of Pate and Nephew (1988), Wiltbank et al. (1990), and Fitz et al. (1993) all three of which used luteal cells, Flores et al. (1993) reported that protein kinase C activation in porcine ovarian tissue had its major inhibitory effects on lipoprotein metabolism and cholesterol utilization. These in vitro results in the pig suggest that the inhibition by the phorbol ester, TPA, may be similar to other hormonal mechanisms that work through the protein kinase C, such as prostaglandin F\textsubscript{2}\alpha.
PAPER 1

COMPARISON OF PGF$_2$\alpha-INDUCED LUTEOLYSIS IN EARLY PREGNANT AND ESTROGEN-TREATED "PSEUDOPREGNANT" GILTS.
Comparison of PGF₂α-induced luteolysis in early pregnant and estrogen-treated "pseudopregnant" gilts

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RUNNING HEAD: PORCINE LUTEAL SENSITIVITY TO PROSTAGLANDIN F₂α

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Conceptus estrogen clearly plays a major role in luteal maintenance in the pig, however, other conceptus-derived substances or conceptus-induced uterine secretory products appear to have a local luteotrophic/antiluteolytic effect on the corpora lutea (CL) and likely may play a key role in maternal recognition of pregnancy in the pig. The objective of these studies was to compare PGF₂α-induced luteolysis in estrogen-treated "pseudopregnant" gilts versus pregnant gilts during the period of maternal recognition of pregnancy. In Experiment 1, doses of PGF₂α ranging from 1-100 µg were administered intraluteally to pseudopregnant gilts to determine the dose necessary to cause functional (progesterone) and structural (weight) luteal regression similar to that observed during the natural estrous cycle. Luteal sensitivity to this minimally effective luteolytic dose of PGF₂α was then determined for both pseudopregnant and pregnant gilts in Experiment 2. Experiment 3 investigated whether day 13 porcine conceptus tissue could directly prevent PGF₂α-induced luteolysis at the level of the CL. The minimally effective luteolytic dose of PGF₂α (100 µg) determined in the pseudopregnant pig caused a similar decline in progesterone concentration and weight of CL in pregnant gilts, suggesting that the susceptibility of CL of pregnant and pseudopregnant pigs to PGF₂α is similar. However, luteal weight was greater (P < 0.05) for the pregnant gilts than for pseudopregnant gilts, suggesting that estrogen treatment alone cannot mimic the conceptus effects on CL growth and development. Experiment 3 demonstrated that day 13 conceptus-
tissue implanted directly into individual CL could partially inhibit PGF\textsubscript{2}\alpha-induced luteolysis, providing for the first time direct evidence that porcine conceptuses as early as day 13 contain factors which can directly (i.e., at the level of the CL) prevent luteal regression. These data also provide strong evidence for the presence of conceptus-induced uterine secretory factor(s) which may be required for complete luteal maintenance during early pregnancy.
INTRODUCTION

During the estrous cycle, the pig corpus luteum (CL) remains refractory to the luteolytic effects of exogenously administered prostaglandin F₂α (PGF₂α) until days 12-13, a time corresponding to uterine PGF₂α secretion (Diehl and Day, 1974; Connor et al., 1976). Therefore, studies investigating the luteolytic effects of an exogenously administered dose of PGF₂α have typically utilized pregnant and hysterectomized pigs (Moeljono et al., 1976; Ball and Day, 1982). Additionally, "pseudopregnant" gilts, produced by administering estrogen systemically on days 11-15 of the estrous cycle, have been used for studies into PGF₂α-induced luteolysis (Kraeling et al. 1975). Estrogen treatment of gilts on days 11-15 of the estrous cycle will result in the extension of the estrous cycle for a period greater than 60 days as a result of prolonged luteal function (Geisert et al., 1990). Further, CL of pseudopregnant pigs remain responsive to the luteolytic effects of exogenously administered PGF₂α (Kraeling et al., 1975).

Previously, we demonstrated that intraluteal implantation of 10 mg of estradiol-17β into each of four randomly selected CL on day 11 of the porcine estrous cycle maintains the function of all CL through day 19 (Ford and Christenson, 1991). Using this "pseudopregnant" model, we have further shown that 100 μg of PGF₂α administered intraluteally to individual CL caused complete regression of the implanted CL, while adjacent CL on the same ovary remained fully functional (Ford and Christenson, 1991).

In the first experiment of the present study, we utilized the "pseudopregnant model" to determine if intraluteal administration of decreasing amounts of PGF₂α had a dose dependent effect on CL progesterone production and/or weight. In the second experiment we compared the effects of the
minimally effective luteolytic dose of PGF\(_{2}\alpha\) determined in Experiment 1 on CL of pregnant and pseudopregnant gilts. In the third experiment, we evaluated the effects of implantation of day 13 porcine conceptus tissue homogenates on response of individual CL of pseudopregnant pigs to PGF\(_{2}\alpha\).
MATERIALS AND METHODS

Animals

Twenty-seven Yorkshire gilts ranging from 6-8 months in age and weighing =175 kg were checked for estrus once daily (0800) with a boar. All gilts utilized in these experiments exhibited an estrous cycle of normal duration (18-21 days) immediately prior to assignment to an experiment.

Experiment 1

On day 11 of the estrous cycle (day 0 = first day of estrus), eight gilts were given thiamyal sodium to induce anesthesia and were maintained on a 3-5% halothane/oxygen mixture in a closed circuit system (Magness and Ford, 1982). The ovaries were exteriorized via a midventral laparotomy, and the number of CL determined prior to injection of intraluteal implants (Ford and Christenson, 1991).

Briefly, pairs of CL were marked by placing different numbers of silk ligatures in the ovarian stroma between the individual CL of a pair to allow for latter identification of CL. On each ovary of the treated gilts (n=4), 2 CL (one pair) were each injected with 50 µl of a quick curing silastic implant material containing 10 mg of estradiol-17β. This resulted in a total of 40 mg estradiol-17β/gilt, which was previously shown to maintain progesterone concentration and weight of all CL through day 19 of the estrous cycle (Ford and Christenson, 1991). An additional three pairs of CL on the ovary having the greatest number of CL and two pairs of CL on the other ovary were randomly assigned to receive intraluteal implants containing 1, 5, 10, 50 or 100 µg of PGF₂α/implant. All remaining CL on each ovary served as unimplanted controls. Due to the large number of CL (n=14) required for this treatment protocol, no vehicle (silastic alone) implants were placed in these treated gilts; however, previous experiments
have shown no effect of vehicle implants on luteal progesterone concentration or weight (Conley and Ford, 1989; Ford and Christenson, 1991). Control gilts (n=4) received vehicle (silastic alone) implants in two CL on each ovary. All remaining CL in these gilts served as unimplanted controls.

Eight days after the implantation procedure (day 19 of the estrous cycle) all gilts were ovariectomized and CL were carefully dissected away from the ovarian stroma, individually weighed, and frozen on dry ice and stored at -20°C until assayed for progesterone.

Experiment 2

Ten gilts were assigned to this experiment, 6 gilts were bred at the onset of estrous activity (day 0) and again 24 hrs later, while the remaining 4 gilts were not mated. On day 11 of the estrous cycle or pregnancy, all gilts were laparotomized as described for Exp. 1. Corpora lutea were counted on both ovaries, and one or two pairs of CL on each ovary were marked with silk ligatures for identification at the completion of the experiment.

In order to compare the effects of pregnancy versus that of pseudopregnancy on the sensitivity of CL to PGF$_2$α-induced luteolysis, two CL on each ovary of the nonpregnant gilts (n=4) received intraluteal implants containing 10 mg estradiol-17β. Additionally, two CL on one ovary of these pseudopregnant gilts were selected to receive implants containing 100 μg PGF$_2$α. Each ovary of the pregnant gilts received two intraluteal vehicle (silastic alone) implants and two intraluteal implants containing 100 μg of PGF$_2$α. All remaining CL on both ovaries of the pseudopregnant and pregnant gilts served as unimplanted controls. On day 19 of pregnancy or pseudopregnancy, gilts were ovariectomized and CL were processed as described for Exp. 1. The data for one gilt
in the pregnant group was excluded from this data set as she was determined to be nonpregnant as evidenced by her return to estrus on day 19 and the presence of corpora albicilians on her ovaries.

Experiment 3

In preparation for this experiment, 4 gilts were bred at the onset of estrus (day 0) and again 24 hrs later. On day 13 of pregnancy, embryos were collected as previously described (Pusateri et al., 1990). Immediately after collection, embryos were separated from the medium (phosphate buffered saline, pH 7.4), lyophilized, and pooled across litters before storage at -90°C until needed for intraluteal implantation.

On day 11 of the estrous cycle, five experimental gilts received intraluteal implants. Five pairs of CL distributed across both ovaries were randomly selected to receive one of five different treatments. The five intraluteal implants received by each gilt contained: 1) 100 μg PGF₂α, 2) 10 mg estradiol-17β, 3) 100 μg PGF₂α + 10 mg estradiol-17β, 4) 0.5 embryo equivalents of day 13 conceptus tissue and 5) 100 μg PGF₂α + 0.5 embryo equivalents of day 13 conceptus tissue. An embryonic equivalent is defined as the weight of an individual lyophilized pig embryo which was calculated by dividing the weight of the lyophilized embryonic tissue pool by the total number of CL for the four donor gilts. Eight days after implantation, CL were collected and processed as in Exp. 1.

Progesterone Assay

Luteal tissue was homogenized, and an aliquot of the luteal homogenate was extracted and assayed as described previously (Conley and Ford, 1989). All of the samples within an experiment were assayed together. The sensitivity of the assay (0.05 ng) was defined as the amount of steroid that yielded 95% of the counts per minute in the buffer control
tubes. A CL pool was included in each assay to calculate intraassay CV. The intraassay CV for experiments 1, 2 and 3 were 20.5%, 16.7% and 18.3%, respectively.

Statistical Analysis

For each experiment, effect of treatment (i.e., type of implant) on luteal tissue progesterone concentration (ng/mg luteal tissue) and luteal weight (mg) were evaluated by the GLM procedure of SAS, (1985) as for a completely randomized block (pig) design. In Exp. 2, the effect of treatment, reproductive status (pseudopregnant versus pregnant) and the treatment*status interaction were analyzed for PGF₂α-implanted and unimplanted CL only. Duncan's multiple range test was used to determine differences between treatment means if a significant (P < 0.05) F-test was observed.
RESULTS

Experiment 1
Intraluteal implantation of 10 mg of estradiol-17β into 4 CL on day 11 of the estrous cycle maintained progesterone concentration and weight of all CL through day 19 of the estrous cycle (Fig. 1A). In contrast, intraluteal implantation of PGF₂α caused a dose-dependent decrease (P < 0.05) in luteal tissue progesterone concentration (Fig. 1A) and weight (Fig. 1B) in CL implanted with 5 to 100 μg PGF₂α. The CL collected from the nonpregnant day 19 control gilts had progesterone concentrations of 3.73 ± 0.6 and 3.70 ± 0.4 ng/mg of luteal tissue for the vehicle-implanted and unimplanted, respectively; these CL were similar (P < 0.05) to the 50 μg and 100 μg PGF₂α-implanted CL in the pseudopregnant gilts. Further, the vehicle-implanted and unimplanted CL from the control gilts had weights of 121.7 ± 10.2 mg and 103.7 ± 8.2 mg, respectively, which were not different from the 100 μg PGF₂α-implanted CL.

Experiment 2
Progesterone concentrations and weights were not different for the vehicle-implanted and unimplanted CL in the pregnant gilts (Fig. 2A and 2B). However, intraluteal implantation of PGF₂α caused a marked decrease (P < 0.05) in luteal progesterone concentration and weight (Fig. 2A and 2B). In the pseudopregnant gilts, the unimplanted and estradiol-17β treated CL had similar progesterone concentrations and weights (Fig. 2A and 2B). Similar to the results in the pregnant gilts, intraluteal PGF₂α treatments in the pseudopregnant gilts caused a similar reduction in progesterone concentrations and weights for those CL (Fig. 2A and 2B). An effect of reproductive status on CL weight was observed as the CL of pregnant gilts were heavier (P < 0.001) than those
Figure 1. Progesterone concentrations (ng/mg luteal tissue wet weight; panel A) and luteal weights (mg; panel B) for unimplanted (UNI) corpora lutea and those treated with implants containing 10 mg of estradiol-17β (E2β) and 1-100 μg doses of PGF₂α. a,b,cMeans ± SEM with different superscripts differ (P < 0.05).
Figure 2. Progesterone concentrations (ng/mg luteal tissue wet weight; panel A) and luteal weights (mg; panel B) for pregnant (n=5) and pseudopregnant (n=4) pig corpora lutea. Treatment groups included unimplanted (UNI), vehicle-treated (VEH) and 100 µg PGF₂α-treated (PGF) corpora lutea for the pregnant gilts; and unimplanted, 10 mg estradiol-17β (E₂β) and 100 µg PGF₂α-treated corpora lutea for the pseudopregnant gilts. a,bMeans ± SEM within reproductive status with different superscripts differ (P < 0.05).
recovered from the pseudopregnant gilts. However, pregnancy status had no effect on luteal progesterone concentrations ($P > 0.40$).

Experiment 3

Intraluteal implantation of 40 mg of estradiol-17β maintained luteal tissue progesterone concentration and weight in these gilts. As reported in Exp. 1 and 2, no difference was observed in luteal measures between the unimplanted, vehicle-treated and estradiol-17β treated CL (Fig. 3A and 3B). Moreover, intraluteal implantation of a conceptus homogenate had no effect on either weight or progesterone concentration. Intraluteal implantation of 100 µg of PGF$_2\alpha$ in the presence of estradiol-17β caused a significant decrease in both luteal tissue progesterone concentration and luteal weight, similar to that observed in Exp. 1 and 2 where intraluteal PGF$_2\alpha$ was administered alone. In contrast, the addition of the conceptus tissue homogenate to the 100 µg dose of PGF$_2\alpha$ partially prevented luteal regression of those CL.
Figure 3. Progesterone concentrations (ng/mg luteal tissue wet weight; panel A) and luteal weights (mg; panel B) for pseudopregnant pig corpora lutea. Intraluteal treatments included: unimplanted (UNI), vehicle (VEH), 10 mg estradiol-17β (E2β), conceptus (CON), conceptus + 100 μg PGF₂α (CON + PGF) and 10 mg estradiol-17β + 100 μg PGF₂α (E2β + PGF). a,b,cMeans ± SEM with different superscripts differ (P < 0.05).
DISCUSSION

The results of Experiment 1 confirmed the previously observed local luteolytic effects of PGF₂α on an implanted CL while other CL on the same ovary were unaffected (Ford and Christenson, 1991). Experiment 1 also illustrated that luteal steroidogenesis was inhibited at a lower dose of PGF₂α (5 μg) than that required to induce structural regression (10 μg). These data are consistent with previous research which demonstrated that systemic administration of a subluteolytic dose of PGF₂α transiently decreased progesterone secretion by porcine CL without inducing structural regression (Conley and Ford, 1991). Experiment 1 furthermore, demonstrated that 100 μg of PGF₂α was the lowest dose which effectively induced both functional (progesterone synthesis) and structural (weight) regression, equivalent to that found in corpora albicantis on day 19 of the estrous cycle.

Experiment 2 clearly illustrated that the sensitivity of CL to PGF₂α was similar in pseudopregnant and pregnant pigs. Interestingly, however, CL of pregnant pigs were heavier than those of pseudopregnant pigs on day 19. The simplest explanation for this difference in CL weight is that exogenous estrogen treatment alone did not mimic all of the conceptus-mediated effects on CL growth and development. These data suggest the presence of additional conceptus-produced luteotrophic agent(s) or possibly conceptus-induced uterine secretory products. Evidence for a localized luteotrophic/antiluteolytic effect of the porcine conceptus/gravid uterus on luteal function is also provided by the observed increase in weight and progesterone content of CL adjacent to the gravid horn of unilaterally pregnant pigs when compared with CL adjacent to the nonpregnant horn (Niswender et al., 1970; Christenson and Day, 1971; Ford and Christenson, 1979; Christenson et al., 1994). Previous studies in the pig
have shown that if embryos are flushed from the uterus on day 11 of gestation, a normal 21 day interestrus interval is seen. However, if embryos are allowed to remain within the uterine lumen until day 13 of pregnancy prior to removal, luteal function will be extended five days beyond normal (i.e., 25 to 26 day interestrus interval; Dhindsa and Dziuk, 1968; Ford et al., 1982).

These data demonstrate that the day 12-13 porcine conceptus must protect CL from PGF$_2$-induced luteolysis. The fact that the day 13 porcine conceptus tissue (Exp. 3) could only partially inhibit PGF$_2$-induced luteolysis provides evidence that both conceptus-derived factors and conceptus-induced uterine secretory products are likely involved in luteal maintenance during early pregnancy. This hypothesis is supported by the fact that intraluteal implantation of additional conceptus tissue (up to two embryonic equivalents/CL) failed to yield results different from those observed following implantation of 0.5 embryo equivalents/CL (unpublished observations). The porcine conceptus produces, in addition to estrogen, a wide variety of biologically active compounds (i.e., catechol estrogens, prostaglandins, and polypeptides) which may interact with conceptus estrogen to maintain luteal function (Geisert et al., 1990). Ball and Day (1982) demonstrated that day 16-25 conceptus tissue also contained factor(s) that could inhibit PGF$_2$-induced luteolysis. Further, these authors demonstrated that the factor(s) responsible for inhibiting PGF$_2$-induced luteolysis was charcoal-absorbable suggesting a small molecular weight compound such as a prostaglandin or steroid.

Previous studies in our laboratory have evaluated the direct effects of estrogen and PGE$_2$, two porcine conceptus secretory products, in preventing both natural and PGF$_2$-induced luteolysis (Conley et al., 1989; Ford and Christenson, 1991). The results indicated that intraluteal estrogen
treatment had no direct luteotrophic/antiluteolytic effects on the CL, while confirming that estrogen's major antiluteolytic activity appeared to be mediated via decreased release of PGF$_2\alpha$ into the uterine venous drainage (Frank et al., 1977; Conley et al., 1989; Bazer, 1989). More importantly, while conceptus estrogen-induced uterine sequestration of PGF$_2\alpha$ is critical for luteal maintenance, this phenomenon does not occur until day 13-14 of pregnancy, one to two days after maternal recognition of pregnancy in the pig on day 12 (Frank et al., 1977; Shille et al., 1979; Bazer, 1989; Christenson et al., 1994). In contrast to estrogen, PGE$_2$ has been shown to locally prevent PGF$_2\alpha$-induced luteal regression in the pig (Ford and Christenson, 1991). Interestingly, PGE$_2$ could only partially prevent luteal regression, similar to that observed for the conceptus-implanted CL in the present studies suggesting that PGE$_2$ might be the local luteoprotective signal. In support of this hypothesis, Akinlosotu et al. (1986) demonstrated that intrauterine infusions of PGE$_2$ prevented luteal regression (i.e., declining progesterone production) in the nonpregnant pig through day 23 of the estrous cycle at which time infusions were terminated. Recently, we demonstrated a transient increase in PGE$_2$ emanating from the gravid uterus coincident with maternal recognition of pregnancy, an increase not observed during the porcine estrous cycle (Christenson et al., 1994). Moreover, unilaterally pregnant pigs exhibited elevated utero-ovarian venous PGE$_2$ concentrations emanating from the gravid versus the nongravid horn, while PGF$_2\alpha$ levels were not different across horns (Christenson et al., 1994).

The results of the present studies validate the use of the pseudopregnant pig as a viable model for in vivo investigations of PGF$_2\alpha$-induced luteolysis. These results also provide direct evidence that the early porcine conceptus (day 12-13) produces factors that can directly influence
luteal function and structure. In addition, these data provide strong evidence for a conceptus-induced uterine secretory factor(s) with luteotrophic activity.
REFERENCES


PAPER 2

EVALUATION OF BIOCHEMICAL AND STRUCTURAL CHANGES
IN INDIVIDUAL PORCINE CORPORA LUTEA DURING
PGF$_2\alpha$-INDUCED LUTEOLYSIS UTILIZING AN IN VIVO IMPLANT SYSTEM
EVALUATION OF BIOCHEMICAL AND STRUCTURAL CHANGES IN INDIVIDUAL PORCINE CORPORA LUTEA DURING PGF₂α-INDUCED LUTEOLYSIS UTILIZING AN IN VIVO IMPLANT SYSTEM


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RUNNING HEAD: Biochemical and Structural Changes During Luteolysis

Key Words: Corpus luteum, Protein kinase C, luteolysis, pig
ABSTRACT

To date, no in vitro system has been devised to allow the study of both functional and structural regression of luteal cells in response to PGF₂α. This study describes the use of a novel intraluteal implant system which results in the death of individual CL on an ovary in response to PGF₂α, while surrounding CL remain fully functional. With this technique it was possible to study both functional and structural regression of individual CL in vivo, without the confounding effects resulting from systemic injection of PGF₂α. Biochemical measurements of individual CL included progesterone concentration, protein kinase C activity and diacylglycerol levels. Structural measurements included luteal weight and the protein:DNA ratio which was used to estimate cell size. Further, stereologic determination of large luteal cell size was accomplished. Nonpregnant gilts were injected with estradiol benzoate (5 mg/injection) every 12 hr from 0800 on day 11 to 0800 on day 13 to prevent uterine PGF₂α secretion. At 0700 on day 13, CL on one ovary were selected at random to receive PGF₂α-implants (n=4) or implant material only (n=4), while the remaining CL on that ovary served as unimplanted controls. The other ovary was removed at that point and CL on that ovary served as 0 hr controls. Gilts were re-laparotomized at 3, 6, 12 and 24 hr after CL implantation, the remaining ovary was removed, and individual CL evaluated. PGF₂α-implanted CL exhibited a decline (P < .05) in progesterone concentrations at 12 and 24 h, and a decline (P < .05) in weight at 24 hr when compared to control CL (implant-only, unimplanted and 0 hr control CL). Furthermore, the protein:DNA ratio was reduced (P < .10) in the PGF₂α-treated CL at 12 and 24 hr. Moreover, this change in the protein:DNA ratio (cell size) was consistent with the reduced diameter (P < .05) of the large luteal cell in the
PGF$_2$α-treated CL. Protein kinase C activity and diacylglycerol concentrations did not change (P > .10) and therefore appear to be unassociated with either functional or structural changes in the PGF$_2$α-treated CL. Contrary to in vitro culture studies, the results of our in vivo study demonstrate no clear role for protein kinase C in the PGF$_2$α-induced luteolytic process. In contrast, our study does temporally link a decline in luteal progesterone concentrations with a decrease in the size of large luteal cells.
INTRODUCTION

PGF₂α is recognized as the natural luteolysin in many species, including the pig (1). However, the biochemical and structural mechanism(s) through which PGF₂α induces luteolysis remain unclear (2,3). Recently, the role of the phosphatidylinositol second messenger system, and in particular protein kinase C activity has been implicated in PGF₂α-induced decreases in steroidogenesis in cultured luteal cells of the ewe, cow and primate (4,5,6,7,8). However, PGF₂α does not induce cytotoxic effects on cultured luteal cells thus preventing simultaneous investigations into the biochemical (i.e., steroidogenesis) and structural changes occurring during luteolysis. The luteolytic effects obtained from systemic administration of PGF₂α are also difficult to interpret as many extrinsic factors (i.e., hypophyseal, uterine, etc.) often confound such experiments. To circumvent these difficulties, we (9) established a unique intraluteal implant system where individual porcine corpora lutea (CL) on an ovary could be injected with a quick-curing silastic implant containing PGF₂α. In those studies, PGF₂α-implanted CL exhibited both functional and structural luteolysis while adjacent CL on the same ovary injected with silastic alone remained fully functional. The objective of the present study was to evaluate the time course of functional (i.e., tissue levels of progesterone, protein kinase C activity and diacylglycerol) as well as structural (i.e., DNA, protein and cell size) changes over a 24 hr period after implantation of a luteolytic dose of PGF₂α into individual CL.
MATERIALS AND METHODS

Animals

Twenty Yorkshire gilts (6-8 months of age) maintained outside were exposed to a boar once daily (0700) and observed for estrous activity (day 0 = first day of estrus). All gilts exhibited at least one estrous cycle of normal duration (18-21 days) immediately prior to assignment to this experiment. Since, the porcine CL is not susceptible to the luteolytic effects of PGF$_2$α until day 12 of the estrous cycle, a time coincident with endogenous uterine PGF$_2$α release (10,11). Gilts were injected (i.m.) with 5 mg of estradiol benzoate in 2.5 ml sesame seed oil every 12 hr beginning at 0800 on day 11 and continuing until 0800 on day 13 of the treatment estrous cycle. This treatment protocol has been shown to prolong luteal function =6-10 days by preventing uterine PGF$_2$α secretion (12). On the morning of day 13 (0700 h), gilts were anesthetized and laparotomized (13). The ovaries were exteriorized and number of CL determined, the ovary with the greatest number of CL was selected to receive eight intraluteal implants using previously established methods (9).

Briefly, four pairs of CL on the selected ovary were marked with different numbers of silk ligatures in the ovarian stroma between individual CL of a pair. Two pair of CL were randomly assigned to be injected with the quick-curing silastic implant material containing 100 μg of PGF$_2$α, and the other two pair of CL received vehicle implants (silastic alone). All remaining CL on the treated ovary served as unimplanted controls. Immediately, after completion of the implantation procedure, the other ovary from each gilt was removed and those CL served as controls (time 0). Gilts were then returned to a recovery pen. At 3, 6, 12 and 24 hr after implantation, 5 gilts/time period were relaparotomized and the treated ovary was removed.
Implant Preparation

Implants were made by adding 250 μl of Medical Grade Elastomer #382 and 250 μl of Medical Fluid #360 (Dow Corning Corporation, Midland, MI) to a 12x75 mm tube containing 1 mg of PGF₂α, 10 times the desired intraluteal dosage for this hormone. The compounds and PGF₂α were thoroughly mixed before adding a small amount of the catalyst, stannous octoate, (Sigma Chemical Company, St. Louis, MO) followed by a brief mixing (30 sec). The silastic hormone mixture was then immediately drawn into a 1.0 ml tuberculin syringe (Becton-Dickinson, Franklin Lakes, NJ). An 18 gauge needle was then tightly placed on the syringe, and 50 μl of the silastic implant material was injected into the center of each of two individual CL. The implant material sets up completely within 3 minutes, forming a solid intraluteal implant. When the needle is withdrawn, the silastic material also plugs the injection site thereby preventing any hemorrhaging (14).

Intraluteal implants for the vehicle and PGF₂α-treated CL were not different in weight averaging 55.6 ± 2.3 mg.

Luteal Tissue Processing

 Corpora lutea from the control (time 0) and treated ovary (3, 6, 12 or 24 hr post-treatment) were dissected away from the ovarian stroma and individually weighed. Three CL from each treatment group (i.e., control, unimplanted, vehicle-implanted and PGF₂α-implanted) were then immediately frozen on dry ice and individually stored at -90°C for later analysis. One of the 3 CL/treatment group was homogenized in 10 ml of the DNA assay buffer (19.3mM NaH₂PO₄, 30.7mM Na₂HPO₄, 2M NaCl, 2mM EDTA) prior to determination of progesterone, DNA and protein concentrations. The remaining 2 CL in the vehicle-implanted and PGF₂α-implanted CL groups for 4 of the 5 gilts at each time point were evaluated for diacylglycerol and PKC activity, respectively. In addition, the fourth CL from the
vehicle and PGF, treatment groups were fixed in a 4.0% paraformaldehyde and 5.5% glutaraldehyde (Karnovskys) fixative and then dehydrated through a graded series of ethanol, immersed in two changes of a transitional medium (xylene) prior to paraffin embedding.

DNA Assay

Luteal tissue DNA concentration was determined by the method of Labarca and Paigen (15) as modified by Conley and Ford (16). Serially diluted calf thymus DNA (1.0, .75, .5, .125, .025, .005, and 0 µg/ml) was used as the standard curve. Equal aliquots of tissue homogenates or standards were combined with the DNA-specific dye Hoechst 33258 (Calbiochem, San Diego, CA). Fluorescence was measured at both excitation (358 nm) and emission (456 nm) wavelengths in a Gilford fluorometer. The sensitivity of the assay was .005 µg DNA/ml. An aliquot of a luteal homogenate pool was included at the beginning and end of each assay for determination of inter-assay CV. To determine intraassay CV several aliquots (n=5) of the same luteal homogenate pool were included in one assay. The inter- and intra-assay CV were 12.6% and 5.0%, respectively.

Protein Assay

Protein concentration in luteal tissue homogenates was determined by the Bio-Rad Protein Assay (Bio-Rad Chemical Division, Richmond, CA) as described by Conley and Ford (16). Serial dilutions of bovine serum albumin (crystalline; Intergen Company, Purchase, NY) at 10.0, 5.0, 2.5, 1.0, and 0 µg/ml served as the standard. Absorbance was measured on a Gilford spectrophotometer at a wavelength of 595 nm. The sensitivity of the assay was 1 µg protein/ml. The inter- and intra-assay CV for a luteal homogenate pool as described for the DNA assay were 10.5% and 2.3%, respectively.
Progesterone Assay

An aliquot of the luteal tissue homogenate was extracted and assayed for progesterone as described previously (14). The sensitivity of the progesterone assay (0.05 ng) was defined as the amount of steroid that yielded 95% of the counts per minute. A luteal tissue homogenate pool was included in each assay to calculate interassay CV. Intraassay CV was determined by inclusion of 5 aliquots of the same luteal homogenate pool in one assay. The interassay and intraassay CV for this experiment were 17.0% and 2.6%, respectively.

Diacylglycerol Assay

Luteal tissue (=150 mg) was homogenized in a 1:2 solution of chloroform:MeOH (3ml) before addition of 1 M NaCl (.6ml) and additional homogenization. Mixtures were then transferred to a centrifugation tube and 1 ml of chloroform and 1 ml of a 1 M NaCl solution were added and vigorously vortexed. Samples were then centrifuged at 5000 x g for 5 min, the aqueous phase discarded and the chloroform phase transferred to another tube and dried under N₂ (no heat).

The radioenzymatic conversion of the extracted diacylglycerol to phosphatidic acid in the presence of diacylglycerol kinase and ³²P-[δ]-ATP (Amersham, Arlington Heights, IL) allowed for the subsequent separation of the ³²P-phosphatidic acid and the unused ³²P-[δ]-ATP on Si minicolumns (Amprep minicolumns, Amersham, Arlington Heights, IL). A standard curve was generated by adding 500 μl of chloroform to a vial of 50 nmol diacylglycerol followed by serial dilutions to yield a curve containing 31.25, 62.5, 125, 250, 500 and 1000 pmol standards and a chloroform blank. Serial dilutions of an extracted luteal tissue homogenate demonstrated parallelism to the standard curve. The intraassay CV was determined by inclusion of four aliquots of a CL extract and
was 15.4%.

Isolation and Purification of Luteal Tissue PKC

Luteal tissue was first pulverized to a fine powder while frozen in liquid nitrogen and then homogenized in 5 ml of ice-cold 25 mM Tris-HCl, pH 7.5, containing 0.25 M sucrose, 2.5 mM MgCl₂, 2.5 mM EGTA, 50 mM 2-mercaptoethanol, and 1.0 mM phenylmethyl-sulfonyl fluoride (homogenization buffer). The homogenate was then centrifuged at 1000 x g for 10 min at 4°C, the supernatant was collected and centrifuged at 100,000 x g for 60 min at 4°C. The resulting pellet and supernatant (cytosolic PKC fraction) were retained. To obtain the particulate PKC, the pellet was resuspended in 5 ml of homogenization buffer containing 0.3% Triton X-100 (Sigma, St Louis, MO). This suspension was then pulse sonicated for 20 sec and the supernatant (particulate PKC fraction) was retained.

Isolated cytosolic (8 mg protein) and particulate (1 mg protein) fractions were applied to individual DEAE Bio-Gel A agarose columns (1 x 3 cm) previously equilibrated with homogenization buffer. After each column was washed with 10 ml of homogenization buffer, the bound PKC was eluted with 7 ml of homogenization buffer containing 100 mM NaCl as previously described in our laboratory (17). These column elutrates were then assayed for PKC activity.

PKC Assay

Protein kinase activity was determined as previously described by McArdle and Conn (18) and validated in our laboratory (17). The PKC incubation mixture (0.25 ml) contained 20 mM Tris-HCl (pH 7.5), 5 mM MgNO₃, 200 µg/ml histone type III-S, 10 µM ³²P-[γ]ATP, and 50 µl of column eluate containing 0.1-20 µg protein. The assay solution was supplemented with either EGTA (1 mM) or CaCl₂ (1 mM) plus
phosphatidylserine (40 \mu g/ml) and 1, 2-diolein (4 \mu g/ml). After incubation for 6 min at 30°C, the reaction was stopped by transferring 100 \mu l onto filter paper and rapidly immersing in 10% trichloroacetic acid (TCA). After extensive washing in 10% TCA, \(^3\)P incorporation into precipitated protein was determined by liquid scintillation spectroscopy. Radioactivity nonspecifically trapped on filters ("filter blank") was determined in each experiment and was subtracted from radioactivity present on reaction filters.

PKC activity present in the 100 mM NaCl fraction was defined as that seen in the presence of Ca\(^{2+}\) plus lipids minus that seen in the presence of EGTA and no lipids.

Light Microscopy and Morphometry

Five micron thick paraffin sections of the vehicle- and the PGF\(_2\alpha\)-treated corpora lutea at the 6, 12 and 24 hr time points were stained with periodic acid Shiff’s reagent and hematoxylin in order to better visualize the luteal cell membrane boundaries (19). A total of nine fields (13000 \mu m\(^2\)/field) from three regions (inner, middle and outer) were evaluated for each CL. The inner region refers to those luteal cells nearest the centrally located implant, while the outer refers to the region nearest the capsule. Within each field, the number and diameter of the large luteal cells were determined by taking an average of two cross-sectional measures at 90 degrees to each other for each cell (20). Only luteal cells (large) meeting the following criteria were evaluated: the cell must be polygonal in shape (not spindle-shaped) with pale cytoplasmic staining, the nucleus must be present, and the cell must be greater than 20 \mu m in diameter.

Statistical Analysis

Data were analyzed within each time period using the GLM procedures of SAS (21) with gilts and treatment (type of CL)
as the independent variables. Luteal cell size as determined by light microscopy was analyzed as a two x three x three factorial with treatment (vehicle and PGF₂α) and regions (inner, middle and outer) and time (6, 12, 24 hr) as factors. Least square means were used to determine differences between treatments.
RESULTS

Progesterone concentrations decreased (P < .05) in PGF$_2\alpha$-implanted CL by 12 hr post-treatment, and the reduction was sustained through 24 hr (Figures 1c,d; P < .08). Luteal weight also declined (P < .05) in those CL treated with PGF$_2\alpha$, but only for the 24 hr post-treatment group (Figure 2d). Intraluteal implantation of the silastic material alone had no effect on luteal tissue progesterone concentrations or weights, as vehicle-implanted and unimplanted CL were similar at all 4 time points (Figures 1a-d and 2a-d). Furthermore, the control (time 0) CL were similar to the vehicle-implanted and unimplanted CL of the treated ovary at 6, 12 and 24 hr post-implantation. In contrast, at 3 hr, luteal progesterone concentrations for all of the CL on the treated ovary (i.e., unimplanted, vehicle- and PGF$_2\alpha$-implanted) were reduced significantly in comparison to the control CL (Figure 1a); however, luteal weights were not different for the unimplanted, vehicle-implanted and PGF$_2\alpha$-implanted CL at 3 hr when compared to the control CL (Figure 2a).

The protein:DNA ratio which can be used as an indicator of cell size (22) was not different for the control, unimplanted, vehicle-implanted and PGF$_2\alpha$-implanted CL at 3 and 6 hr post-implantation (Figure 3a,b). However, by 12 hr a slight decline (P=.10) in the protein:DNA ratio was observed for the PGF$_2\alpha$-implanted CL and by 24-hr the protein:DNA ratio was reduced (P < .07) for the PGF$_2\alpha$-implanted CL Figure 3c,d). Morphometric analysis of the luteal cross-sections was consistent with the predicted changes in cell size as estimated by the protein:DNA ratio (Table 1). Comparison of the vehicle-implanted and PGF$_2\alpha$-implanted CL at 6 hr showed no difference in cell size for any of the three regions (inner, middle, outer) within the CL. In contrast, by 12 and 24 hr large luteal cell size (diameter) had declined (P < .05) in
Figure 1. Progesterone concentrations (ng/mg of luteal tissue) for the time 0 control (CONTROL), PGF$_2\alpha$-implanted (PGF), vehicle-implanted (VEH) and unimplanted (UNI) corpora lutea at the 3 (A), 6 (B), 12 (C) and 24 (D) hr time points. \textsuperscript{a,b}Means ± SEM within a panel with unlike superscripts differ: Panels A, B, C (P < .05); Panel D (P < .08).
Figure 2. Weights (mg) for the time 0 control (CONTROL), PGF$_2$α-implanted (PGF), vehicle-implanted (VEH) and unimplanted (UNI) corpora lutea at the 3 (A), 6 (B), 12 (C) and 24 (D) hr time points.

Mean ± SEM within a panel with unlike superscripts differ (P < .05).
Figure 3. Protein:DNA ratio (µg protein/mg luteal tissue:µg DNA/mg luteal tissue) for the time 0 control (CONTROL), PGF₂α-implanted (PGF), vehicle-implanted (VEH) and unimplanted (UNI) corpora lutea at the 3 (A), 6 (B), 12 (C) and 24 (D) hr time points.

Means ± SEM within a panel with unlike superscripts differ: Panels A, B, D (P < .07); Panel C (P = .10).
Table 1. Morphometric analysis of large luteal cell diameter (μm) for vehicle and PGF₂α-treated CL

<table>
<thead>
<tr>
<th>Time</th>
<th>Treatment</th>
<th>n</th>
<th>Region within luteal crosssection</th>
<th></th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inner</td>
<td>Middle</td>
<td>Outer</td>
</tr>
<tr>
<td>6</td>
<td>Vehicle</td>
<td>160</td>
<td>25.7 ± .6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.2 ± .8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.8 ± .7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PGF₂α</td>
<td>183</td>
<td>27.1 ± .6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.0 ± .6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.4 ± .5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>Vehicle</td>
<td>165</td>
<td>28.3 ± .7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.1 ± .5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.1 ± .6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PGF₂α</td>
<td>178</td>
<td>23.8 ± .5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.5 ± .6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.1 ± .5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>24</td>
<td>Vehicle</td>
<td>132</td>
<td>26.6 ± .7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.3 ± .7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.2 ± .6&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>PGF₂α</td>
<td>135</td>
<td>23.6 ± .8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.5 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.4 ± .7&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a,b</sup>Means ± SEM within a column with unlike superscripts differ (P < .05).

the PGF₂α-implanted CL, whereas no change was seen in the vehicle-implanted CL.

Cytosolic protein kinase C activity accounted for ~53% of the total protein kinase C activity in the vehicle-implanted and PGF₂α-implanted CL across all 4 time periods. No change in membrane-bound protein kinase C activity was observed at any of the four time periods (Table 2). Further, cytosolic protein kinase C activity was not different at 3, 6 or 12 hr for vehicle-implanted and PGF₂α-implanted CL. At 24 hr a trend (P = .11) for an increase in luteal cytosolic protein kinase C activity was seen in the PGF₂α-implanted CL in comparison to the vehicle-implanted CL (Table 2). Luteal tissue concentrations of diacylglycerol ranged between 195-583 pmol/mg wet weight and were not different for vehicle-implanted and PGF₂α-implanted CL at 3, 6, 12 and 24 hr (data not shown).
Table 2. Cytosolic and membrane-bound protein kinase C activity for the vehicle-implanted and PGF$_2$α-implanted corpora lutea

<table>
<thead>
<tr>
<th>Time</th>
<th>Treatment</th>
<th>Number of Gilts</th>
<th>PKC Activity pmoles/mg Crude Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cytosolic</td>
</tr>
<tr>
<td>3</td>
<td>Vehicle</td>
<td>4</td>
<td>118 ± 5$^a$</td>
</tr>
<tr>
<td></td>
<td>PGF$_2$α</td>
<td>4</td>
<td>109 ± 22$^a$</td>
</tr>
<tr>
<td>6</td>
<td>Vehicle</td>
<td>4</td>
<td>109 ± 26$^a$</td>
</tr>
<tr>
<td></td>
<td>PGF$_2$α</td>
<td>4</td>
<td>95 ± 9$^a$</td>
</tr>
<tr>
<td>12</td>
<td>Vehicle</td>
<td>4</td>
<td>136 ± 10$^a$</td>
</tr>
<tr>
<td></td>
<td>PGF$_2$α</td>
<td>4</td>
<td>118 ± 14$^a$</td>
</tr>
<tr>
<td>24</td>
<td>Vehicle</td>
<td>4</td>
<td>117 ± 19$^a$</td>
</tr>
<tr>
<td></td>
<td>PGF$_2$α</td>
<td>4</td>
<td>152 ± 15$^b$</td>
</tr>
</tbody>
</table>

$^a,b$Means ± SEM within a time period with unlike superscripts differ (P => .11).
A sustained reduction in luteal concentrations of progesterone was observed for the PGF$_2$α-implanted CL at 12 and 24 hr, while decreased luteal weight was evident only at 24 hr post-implantation for the PGF$_2$α-implanted CL. The transient decline in progesterone concentrations observed at 3 hr for all CL (i.e., unimplanted, vehicle-implanted and PGF$_2$α-implanted) on the treated ovary may have resulted due to the lack of adequate time for complete recovery from surgical trauma. Regardless, by 6 hr post-implantation CL progesterone concentrations for the unimplanted and vehicle-implanted CL as well as PGF$_2$α-implanted CL returned to levels observed in control (time 0) CL. The results of the present study are also consistent with previous long-term (8 day) implantation studies, where vehicle-implanted and unimplanted CL were not different and remained fully functional (i.e., similar progesterone concentrations as CL collected on day 12), while PGF$_2$α-treated CL did not (9,14).

Prostaglandin F$_2$α-treated CL exhibited a decreased protein:DNA ratio that was temporally associated with the decreases in luteal progesterone concentrations at 12 and 24 hr. Further, a decrease in large luteal cell size was also evident via morphological analysis of luteal cross-sections suggesting that the declining protein:DNA ratio was the result of a decreased large luteal cell size. Braden et al. (23), in a time course study of PGF$_2$α-induced luteolysis, observed that one of the first morphological differences following PGF$_2$α treatment was a decline in ovine large luteal cell diameter, followed closely by losses in small luteal cell number. While the results of our study demonstrate an effect of PGF$_2$α on large luteal cells, they do not preclude the possibility that PGF$_2$α also affected other cell populations within the CL which may have contributed to the observed decrease in the
Changes in protein kinase C activity were not reflected by changes in either PGF₂α-induced functional or structural luteolysis, exhibiting only a small increase in cytoplasmic activity at 24 hr post PGF₂α treatment, which might be associated with the breakdown of the cell membrane and an influx of extracellular Ca²⁺. The relative PKC activity levels observed for the cytosolic and membrane-bound portions are similar to those previously reported for luteal tissue (5). These data are consistent with that of Yuan and Connor (24) who demonstrated that PKC activity was not different for isolated porcine large luteal cells collected before the onset of luteolysis (day 10) and after its natural onset (day 15). Moreover, most studies linking PKC activity to PGF₂α-luteolysis do so indirectly by activating PKC pharmacologically with phorbol esters and then evaluating its effects on either basal luteal steroidogenesis and/or gonadotrophin responses (i.e., cAMP or progesterone production; 4,5,25). Interestingly, in rat luteal cells the antigonadotrophic effects of a phorbol ester (TPA) and PGF₂α were shown to be mediated by separate intracellular processes (26). In fact these authors demonstrated that the maximally effective dose of each compound when given together had an additive antigonadotrophic effect (26). Further, inclusion of the PKC inhibitor, staurosporine, in the medium completely blocked TPA-induced antigonadotrophic effects, while not affecting the antigonadotrophic effects of PGF₂α. Lastly, these authors demonstrated that TPA caused a rapid and large translocation of PKC activity from the cytosol to the membrane, whereas PGF₂α had only a slight effect (26).

Maximal activation of protein kinase C requires the presence of Ca²⁺, diacylglycerol and a membrane binding site, usually phosphatidylyserine (27,28). In addition to measuring PKC activity, we also evaluated diacylglycerol concentrations
in vehicle- and PGF$_2$α-treated CL. As an important regulator of protein kinase C activity, diacylglycerol induces a conformational change in protein kinase C which then allows it to phosphorylate proteins (27). After phospholipase-induced production, diacylglycerol remains associated with the cell membrane and therefore it is also an excellent estimate of the amount of membrane-bound protein kinase C. The lack of change in diacylglycerol concentrations in our study is therefore consistent with the absence of a change in membrane-bound protein kinase C activity.

Utilizing this unique intraluteal implant system, whereby PGF$_2$α-treated and vehicle-treated CL reside on the same ovary, enabled us to investigate for the first time both specific functional changes as well as structural changes within an individual CL following in vivo PGF$_2$α treatment. These data suggest that PGF$_2$α does not appear to mediate its antisteroidogenic activity through changes in PKC activity or by altering its translocation to the cell membrane. The results of our study also provide for the first time evidence of a change in porcine large luteal cell diameter which occurred coincidentally with the first evidence of decreased luteal tissue progesterone concentrations following PGF$_2$α treatment.
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REFERENCES


28. Wheeler MB, Veldhuis JD. Catalytic and receptor-binding properties of the calcium-sensitive phospholipid-
PAPER 3

LUTEAL MAINTENANCE DURING EARLY PREGNANCY IN THE PIG:
ROLE FOR PROSTAGLANDIN E₂
LUTEAL MAINTENANCE DURING EARLY PREGNANCY IN THE PIG:
ROLE FOR PROSTAGLANDIN E$_2^{1,2}$

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ABSTRACT

We previously demonstrated that prostaglandin E₂ (PGE) directly inhibits prostaglandin F₂α (PGF)-induced regression of individual pig corpora lutea (CL) in a dose dependent manner. The present experiments were conducted to 1) characterize and compare uterine secretion of PGE and PGF during the estrous cycle and early pregnancy and 2) evaluate the local effect of the conceptus on uterine prostaglandin secretion and associated CL function in unilaterally pregnant pigs. In Exp. 1, utero-ovarian venous blood samples were collected from two nonpregnant and two pregnant gilts at 3-h intervals from day 10 through 16 (first day of estrus or mating = day 0) for quantitation of uterine PGE and PGF secretion. In Exp. 2, gilts (n=4) were made unilaterally pregnant on day 2, and utero-ovarian venous catheters were placed bilaterally to determine if differences in PGE and/or PGF secretion might account for the known luteotrophic/antiluteolytic effect of the gravid uterine horn on the CL of the ipsilateral ovary. During the estrous cycle (Exp. 1), pulsatile secretion of PGF increased markedly on day 13 and continued to increase through day 16. PGE secretion also increased from day 13 to 16 of the estrous cycle; however, concentrations of PGE remained at least 3-fold lower than those of PGF. In contrast to changes in non-mated gilts, prostaglandin secretion in mated gilts peaked earlier (day 11-12), with PGE predominating. Thereafter, both PGE and PGF secretion declined to basal levels where they remained through day 16 of pregnancy. During unilateral pregnancy (Exp. 2), PGF concentration in nongravid and gravid horns was similar (P > 0.8). In contrast, PGE concentrations were greater (P < 0.06) in utero-ovarian venous blood draining the gravid uterine horn. This increase in PGE was associated with enhanced CL function on the ipsilateral ovary as evidenced by
an elevated progesterone content and concentration as well as increased CL weights. These data are consistent with a role for conceptus-associated increases in uterine PGE secretion in the local stimulation of luteal function during early pregnancy in the pig.
INTRODUCTION

In the pig, as in other domestic animals, prostaglandin F\textsubscript{2\alpha} (PGF) of uterine origin causes regression of the corpora lutea (CL) during the estrous cycle (1, 2). However, the sequence of signals during early pregnancy resulting in luteal maintenance in the pig remains an enigma (3, 4). In the ewe and cow, unique proteins of conceptus origin (i.e., ovine/bovine trophoblastic protein-1, respectively) have been postulated to be the key luteotrophic/antiluteolytic factors (5). In addition to the presence of ovine trophoblastic protein-1, prostaglandin E\textsubscript{2} (PGE) concentrations are also elevated during early ovine pregnancy (6). Furthermore, PGE administered to nonpregnant ewes and cows during the days corresponding to maternal recognition of pregnancy in each species has been shown to extend luteal progesterone production (7, 8, 9, 10). In the pig, conceptus secretory proteins do not seem to have luteotrophic activity (11); however, evidence for a luteotrophic/antiluteolytic effect of PGE in the pig has been documented (12, 13).

During the period of maternal recognition of pregnancy in the pig, conceptus estrogen and PGE production increase markedly (14, 15). The role of embryonic estrogen production in luteal maintenance has been investigated thoroughly (see 14 for review). Estrogen is thought to protect the CL by decreasing uterine PGF secretion during days 11-13 of pregnancy (16, 17). Systemic injections of estrogen on days 11-13 of the estrous cycle in pigs can mimic many of the effects of the conceptus, including the delay of luteal regression (18). In addition to its ability to decrease uterine PGF secretion, exogenous estrogen has also been shown to increase uterine luminal PGE concentrations (18). A local and systemic effect of estradiol-17\beta on CL progesterone production has been observed in cyclic gilts receiving
estradiol-17ß unilaterally into an isolated uterine horn on
days 11-15 (19). It appeared that the local luteotrophic
effect of estrogen was independent of uterine PGF secretion,
as no difference in utero-ovarian venous PGF concentrations
was observed between the estrogen- and vehicle-treated horns
in that study. This indicates that estrogen either directly
affected the CL or stimulated the local secretion of another
luteotrophic or antiluteolytic substance, possibly PGE.

Recently, we demonstrated that PGE could protect
individual CL from the luteolytic effects of intraluteal
administered PGF (20). Utero-ovarian venous plasma PGE
concentrations have not been evaluated over the period of
maternal recognition of pregnancy in the pig. An objective of
the present investigations was to characterize and compare the
patterns of uterine secretion of PGE with those of PGF during
the estrous cycle and the corresponding days of early
pregnancy in the pig. The second objective was to determine
if increased secretion of PGE from the gravid horn could
explain the elevated function of CL on the ipsilateral ovary
in unilaterally pregnant pigs.
MATERIALS AND METHODS

Experiment 1

Yorkshire gilts with at least one previous estrous cycle of normal duration (19 to 21 days) were either mated (n = 2) to a boar at 0 and 24 h after the first expression of estrus (day 0) or were not mated (n = 2). On days 4 or 5 of the estrous cycle or pregnancy, gilts were anesthetized with thiamylal sodium\(^1\) and then maintained with a 3-5% halothane/oxygen mixture in a closed-circuit system (21). At surgery, the utero-ovarian vein draining a randomly selected uterine horn of each gilt was catheterized, and the catheter was exteriorized via a puncture wound in the flank and maintained in a pouch glued to the side as previously described (22). At the completion of surgery, gilts were placed in elevated farrowing crates for blood collection.

Utero-ovarian venous blood samples were collected at 3-h intervals beginning at 0600 h on day 10 and continuing until 0600 h on day 16. Blood samples (5 ml) were collected in chilled (4°C) tubes containing 10 IU of heparin and .2 mg of acetylsalicylic acid as a prostaglandin synthesis inhibitor. Immediately after collection, each sample was centrifuged and plasma collected and frozen in two tubes at -90°C until analyzed for PGE, PGF and progesterone. At the completion of the blood collection period, gilts were slaughtered at the Animal Science Dept. abattoir, and catheter placement (i.e., tip of the catheter located in the utero-ovarian vein) and pregnancy (i.e., presence of conceptus tissue in uterine lumen) were verified.

Experiment 2

Four Yorkshire gilts 6 to 8 months of age with two

\(^1\)Surital®, Parke-Davis, Morris Plains, NJ
previous estrous cycles of normal duration (19-21 days) were checked for estrous activity once daily with a boar. Gilts were bred to mature boars at the first expression of estrous activity (day 0) and then again 24 h later (day 1). On day 2, gilts were anesthetized and laparotomized to allow bilateral catheterization of the utero-ovarian veins as described for Experiment 1. Immediately after catheterization, the uterine tube ipsilateral to the ovary with the fewest ovulations was flushed with 20 ml of phosphate-buffered saline as previously described (22). The uterine tube flush was examined, and the number of embryos recovered was compared with the number of ovulations to verify that all embryos were removed. To prevent transuterine migration of embryos, each uterine horn was isolated with double silk ligatures at its junction with the uterine body (22). The gilts were returned to individual recovery pens where they remained for the duration of the experiment.

Beginning on day 3 of pregnancy and continuing through day 14, blood samples (5 ml) were collected twice daily (0600 h and 1800 h) and were processed as described in Experiment 1. Since no consistent differences were observed between the a.m. and p.m. PGE and PGF concentrations, daily averages are reported to indicate uterine secretion rates. On the morning of day 15, the gilts were anesthetized and the reproductive tracts were again exteriorized. Catheter placement (same as Exp. 1) was verified prior to its removal. To confirm unilateral pregnancy, the lumen of each uterine horn was flushed with 20 ml of phosphate-buffered saline and the presence or absence of conceptus tissue was noted. Both ovaries were removed, and the CL were dissected free of the ovarian stroma, individually weighed, and frozen at -90°C until tissue progesterone determinations were made.
Assays

Progesterone. Concentrations of progesterone in luteal tissue and blood plasma were measured via radioimmunoassay exactly as described by Magness and Ford (21). Assay sensitivity (50 pg/tube) was defined as the amount of steroid that yielded 95% of the counts in buffer control tubes. A plasma pool standard was included in each assay for calculation of intra- and inter-assay coefficients of variation (CV). The intra- and inter-assay CV were 7.2% and 10.0%, respectively. Luteal tissue progesterone determinations (Exp. 2) were completed in a single assay; a pooled luteal tissue homogenate was included in this assay and the intra-assay CV was 19.2%.

Prostaglandin E and F. One ml of each heparinized plasma sample was acidified with 1.5 ml of 0.01 M acetic acid and 0.1 ml of 1.5 M citric acid. Prostaglandins were extracted from acidified plasma using Bond Elut® solid phase extraction cartridges\(^2\). Cartridges were activated initially with acetonitrile and were rinsed with water immediately prior to sample application. Following rinses with water and petroleum ether, the prostaglandins were eluted with ethyl acetate. Extraction efficiency averaged 90% for both prostaglandins. The ethyl acetate extract was taken to dryness under air and reconstituted in 250 µl of HPLC column solvent [acetonitrile: benzene: acetic acid: water (225:2:1:772)]. A 200 µl aliquot was chromatographed on a Waters\(\text{TM}\) Free Fatty Acid column (3.9 x 150 mm, 4 micro bonded, spherical silica). Prostaglandins were eluted at a flow rate of 1 ml/min. The elution patterns for radiolabeled PGE and PGF were determined each day in order to accurately define the fractions containing the prostaglandins. HPLC recovery of tritiated prostaglandins was

\(^2\text{C}18\) Octadecyl; Varian and Assoc. Harbor City, CA
71.6 ± 1.1% and 66.7 ± 0.6% for PGE and PGF, respectively. Fractions containing each of the respective prostaglandin classes were taken to dryness and stored at -20°C until assayed at three dilutions using the previously validated radioimmunoassays for PGE (23) and PGF (24). The intra- and inter-assay coefficients of variation for each prostaglandin were 7.4% and 12.1% (PGE), and 8.2% and 17.3% (PGF), respectively.

Statistics/Data Analysis

Changes in utero-ovarian venous plasma progesterone and prostaglandin concentrations were analyzed by split-plot analysis of variance for repeated measures (25). Paired t-tests were used to evaluate differences in luteal weight and CL progesterone content and concentration between the ovaries ipsilateral to the gravid and nongravid uterine horns (Exp. 2).
Results

Experiment 1

Utero-ovarian venous progesterone concentrations (ng/ml; \( \bar{x} \pm \text{SEM} \)) for the pregnant gilts were similar \((P > 0.1)\) from day 10 to 16 (350 ± 80). During days 10 to 13 of the estrous cycle, utero-ovarian venous progesterone concentrations (272.9 ± 58.5) were similar \((P > 0.1)\) to those measured during pregnancy. However, on day 14 of the estrous cycle, utero-ovarian venous progesterone concentrations (88 ± 23) declined sharply, marking the onset of luteolysis and continued to decline to day 16 (15 ± 8).

The decline in luteal progesterone production after day 14 in the nonpregnant gilts was associated with the initial large pulses of PGF emanating from the uterus (Figures 1A and 1B). Pulsatile secretion of PGF continued to increase in nonpregnant gilts through day 16 of the estrous cycle. Although pulsatile secretion of PGE also increased from day 14 to 16 of the estrous cycle, its concentration remained ≈3-fold lower than those of PGF. In contrast to the pattern of prostaglandin secretion exhibited by the nonpregnant gilts, pregnant gilts had an earlier (days 11-13) transient rise in PGE and PGF, during which PGE was the predominant uterine prostaglandin secreted (Figures 2A and 2B). Thereafter, uterine secretion of both PGE and PGF declined to basal concentrations where they remained throughout the sampling period.

Experiment 2

The concentrations of progesterone in the utero-ovarian veins draining the gravid and nongravid horns were similar \((P > 0.1)\) throughout the sampling period. Progesterone concentrations (ng/ml; \( \bar{x} \pm \text{SEM} \)) increased progressively from day 3 (72 ± 31) to day 10 (298 ± 75), remained high from day
Figures 1A,B. Utero-ovarian venous plasma prostaglandin E (\(-\Delta--\)) and F (\(--\theta--\)) profiles during the estrous cycle. Panels A and B each represent an individual gilt.
Figures 2A, B. Utero-ovarian venous plasma prostaglandin E (Δ) and F (θ) profiles during pregnancy. Panels A and B each represent an individual gilt.
11 through day 13 (294 ± 78), and declined slightly on day 14 (162 ± 32). Individual CL weights, as well as progesterone content and concentration, were significantly (P < 0.01) greater for CL on the ovary ipsilateral to the gravid uterine horn when compared with CL on the ovary adjacent to the nongravid side (Table 1).

Table 1. Luteal Characteristics in Unilaterally Pregnant Gilts

<table>
<thead>
<tr>
<th></th>
<th>Number of CL/ovary</th>
<th>CL weight (mg)</th>
<th>Progesterone content (ng/CL)</th>
<th>Progesterone concentration (ng/mg CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravid uterine horn</td>
<td>11 (4)*</td>
<td>354.4</td>
<td>7467</td>
<td>19.9</td>
</tr>
<tr>
<td>Nongravid uterine horn</td>
<td>8 (4)</td>
<td>330.4</td>
<td>5087</td>
<td>14.2</td>
</tr>
<tr>
<td>Average difference (gravid-nongravid)</td>
<td>24.0 ± 2.7a</td>
<td>2379 ± 290a</td>
<td>5.8 ± .6a</td>
<td></td>
</tr>
</tbody>
</table>

*Number of ovaries.
aDifferences were significant (P < 0.01) as determined by paired t-test.

Concentrations of PGF in utero-ovarian venous blood draining from both the gravid and nongravid uterine horns were similar (P > 0.8), increasing progressively from day 11 through day 14 post-mating (Figure 3). Similarly, progressive increases in PGE were detected in utero-ovarian venous blood draining both horns from day 11 through day 14 (Figure 4). However, in contrast to what was observed with PGF, concentrations of PGE emanating from the gravid uterine horn were elevated (P = 0.06) on days 11-14 when compared with those from the nongravid horn.
Figure 3. Utero-ovarian venous plasma prostaglandin F concentrations (pg/ml) from the gravid (---Δ--) and nongravid horns (---θ--) of 4 gilts from day 7 to 14 of unilateral pregnancy (SE = 20.2).

Figure 4. Utero-ovarian venous plasma prostaglandin E concentrations (pg/ml) from the gravid (---Δ--) and nongravid horns (---θ--) of 4 gilts from day 7 to 14 of unilateral pregnancy (SE = 17.1).
DISCUSSION

During days 12-16 of the porcine estrous cycle, utero-ovarian venous PGF concentrations increased progressively to peak levels of 10-12 ng/ml (Exp. 1); PGF was secreted in episodic pulses at approximately 9-hr intervals. Similar maximal concentrations of utero-ovarian venous PGF as well as similar pulse frequency have been observed in prior studies during the late luteal phase of the nonpregnant pig (26, 27) and ewe (28). Measurement of the stable metabolite of PGF (PGFM; 15-keto-13,14-dihydro-PGF\(_2\alpha\)) in peripheral blood samples of the nonpregnant pig has also been used to estimate cyclic changes in PGF emanating from the uterus (29). As observed in utero-ovarian venous blood, Shille et al. (29) observed a progressive increase in the pulsatile secretion of PGFM into the peripheral circulation from day 12-17 of the porcine estrous cycle.

The appearance of uterine PGF pulses prior to any decline in utero-ovarian venous progesterone concentrations in nonpregnant gilts in Experiment 1 confirmed the original findings of Gleeson and Thorburn (26). These researchers observed that the initial decline in progesterone concentrations on day 14 in the nonpregnant sow occurred after the first few large pulses of PGF. Recently, Gadsby et al. (30, 31) observed that luteal cell PGF receptors were transiently increased on day 13 or 14 of the estrous cycle in nonpregnant gilts, whereas luteal cell PGF receptor numbers remained unchanged in pregnant and estrogen-treated gilts. These investigators suggested that endogenous uterine PGF might cause the induction of its own receptor on the porcine CL (30). In support of this argument, Estill et al. (32) demonstrated that repeated injections of PGF during the early luteal phase (days 5-10) of the porcine estrous cycle advanced the time at which the CL became susceptible to the luteolytic
effects of PGF.

In contrast to observations during the estrous cycle, there was an increase in utero-ovarian venous PGF concentrations on days 11-12 of pregnancy followed by a rapid and sustained decrease through day 16. Shille et al. (29) also observed an initial transient increase in peripheral blood PGFM concentrations during days 11-12 of early pregnancy followed by a pronounced decrease on day 13. In contrast to what was observed during the estrous cycle, we observed a dramatic increase in the ratio of PGE to PGF during pregnancy. This increase in PGE occurred simultaneously with the increase in PGF on days 11-12, possibly to protect the CL from a luteolytic effect of PGF during this critical period. The increased secretion of PGE by the gravid versus nongravid horn of pigs in Experiment 2 was associated with an elevated progesterone concentration of CL on the adjacent ovary. This may explain the previously reported local luteotrophic effects of the porcine conceptus (22, 33, 34, 35).

Conceptus estrogens may change the ratio of PGE to PGF in uterine venous effluent by altering the contribution of uterine prostaglandins emanating from the stromal or glandular endometrial tissue (36). Porcine endometrial stromal cells isolated on day 13 of pregnancy had an increased ability to secrete PGE in vitro compared with those collected on days 11 or 15 of pregnancy (37). Interestingly, the stromal secretion of PGE observed by these investigators was ≈3-fold greater than that of PGF, consistent with the increase in PGE concentrations in pregnant versus nonpregnant gilts in the present study. Comparison of prostaglandin secretion from stromal and glandular cells isolated from day 13 nonpregnant and pregnant gilts also demonstrated that the PGE:PGF ratio was markedly increased only in stromal cells isolated from pregnant gilts (36). Due to the recent finding that uterine synthesis of epidermal growth factor (EGF) in mice is
modulated by estrogen (38), Zhang et al. (39) evaluated the effects of EGF on in vitro production of PGE and PGF by isolated porcine endometrial stromal and glandular cells. Upon exposure of stromal cells to EGF, PGE production increased 133%, whereas PGF production rose only 66%. The glandular cells also responded to EGF with a 30% increase in PGE secretion, however, no change in PGF was observed (39). This alteration in endometrial PGE and PGF production, therefore, may be modulated by estrogen via EGF. Providing further evidence for an estrogen-induced increase in PGE during early pregnancy, systemic estrogen administration on days 11-13 of the estrous cycle increased uterine luminal concentrations of PGE (18). In addition to uterine prostaglandin secretion, conceptus-derived prostaglandins may also contribute to the shift in the ratio of PGE:PGF emanating from the uterus on days 11-12 of pregnancy. Indeed, beginning on day 11 of pregnancy, the porcine conceptus increases prostaglandin synthesis markedly and, moreover, produces greater concentrations of PGE than PGF (15).

The observed shift in the ratio of luteotropic to luteolytic prostaglandins observed in utero-ovarian venous blood during early pregnancy in this study may have in part been due to a reduced uterine conversion of PGE to PGF. During early pregnancy (days 13-16) in the ewe, endometrial PGE_2-9-ketoreductase activity is markedly suppressed when compared to corresponding days of the estrous cycle (40). It is possible that a secretory product of the conceptus (estrogen?) and/or ovary (progesterone?) may depress conversion of PGE to PGF, thereby sustaining luteal function. Although the existence of endogenous luteal PGE_2-9-ketoreductase was confirmed by these researchers (40), it was shown to be unaffected by pregnancy status or day of collection and thus would not have contributed to the pregnancy-specific rise in PGE in utero-ovarian venous blood.
In contrast, in the pig, metabolism of PGF and PGE by both endometrial and luteal tissue is less than that in the ewe and moreover appears to be unaffected by pregnancy status (41).

Porcine conceptus homogenates have been shown to prevent PGF-induced luteolysis when administered simultaneously via gelatin implants into CL on day 30 of gestation (42). Further, the nature of this conceptus-derived luteoprotective factor(s) was shown to be charcoal extractable indicating that the factor(s) was of a low molecular weight, possibly a steroid or prostaglandin (42). The luteoprotective substance in the porcine conceptus homogenate does not appear to be estradiol-17β, as simultaneous administration of estradiol and PGF into individual CL via silastic implants at a ratio of 100:1 failed to prevent luteolysis (20). In contrast to estrogen's inability to prevent luteolysis directly at the level of the CL, PGE simultaneously administered with PGF at a 4:1 ratio into individual pig CL was capable of counteracting the luteolytic effects of PGF (20).

Additional evidence that PGE may be luteotrophic in the pig was provided by Akinlosotu et al. (12, 13). These investigators demonstrated that 4 times a day or continuous intra-uterine infusions of PGE (2400 ug/day) beginning on day 7 of the porcine estrous cycle delayed luteolysis until infusions were stopped on day 23. In contrast, other investigators using less frequent administration and lower concentrations of PGE were unable to show an effect of intrauterine infusions (43, 44) or i.m. injections (45) of PGE on porcine luteal function. Considering that the effectiveness of PGE in maintaining luteal function during pregnancy appears to be dependent on the availability of sufficient quantities of PGE to overcome the luteolytic effects of PGF, very high levels of exogenous PGE would be necessary to overcome the pulses of PGF observed during the estrous cycle. That insufficient doses of PGE were utilized
in the latter experimental protocols (43, 44, 45) is well illustrated by the fact that a single intra-uterine infusion of PGE (300 ug) on days 12 or 15 of the estrous cycle resulted in PGE concentrations that were 50% less than PGF levels over a 6-h blood sampling period after PGE administration (43).

Systemic administration of estrogen to nonpregnant pigs on days 11-12 of the estrous cycle does not extend luteal function for more than a few days (46) suggesting the necessity for additional luteotrophic stimuli. Geisert et al. (46) demonstrated that prolonged maintenance of CL function through day 60 could be achieved if injections of estrogen were administered on days 11 and 14-16 of the cycle. The injections of estrogen on days 14-16 are known to result in a prolonged inhibition of uterine PGF secretion (27, 46), resulting from a redirection of endometrial PGF from an endocrine (uterine vein) to an exocrine (uterine luminal) route. The rapid and sustained decline in utero-ovarian venous PGF and PGE concentrations on days 13 through 16 of pregnancy in the present study are consistent with an effect of continued conceptus estrogen secretion in prevention of uterine prostaglandin release. Additionally, this decline in utero-ovarian venous prostaglandin concentrations after day 13 may be, in part, a result of increased circulating progesterone levels. In support of this view, elevated progesterone concentrations in systemic blood have been observed on days 13-16 of pregnancy in the pig (47). Studies in the ewe support an inhibitory role for progesterone on the pulsatile secretion of uterine prostaglandins (48, 49).

Further, an inhibitory effect of progesterone on uterine endometrial cell PGE and PGF secretion has been observed for isolated porcine endometrial stromal and glandular cells (37).

Since estrogen is known to affect uterine PGE production, we speculate that conceptus estrogen may have two consecutive uterine effects: 1) an early effect to preferentially
increase the secretion of PGE over PGF (maternal recognition of pregnancy) and 2) a later effect to prevent both PGE and PGF synthesis/secrection (continued luteal function). The results obtained in these two experiments are consistent with a luteoprotective role for PGE during the period of maternal recognition of pregnancy in the pig.
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REFERENCES


49. Vincent, D.L., Meredith, S., and Inskeep, E.K. Advancement of uterine secretion of prostaglandin E₂ by
To date, the process of luteal regression in the pig has received very little investigative research. This is due primarily to the complicating fact that porcine corpora lutea do not become susceptible to the luteolytic effects of prostaglandin \( F_2 \alpha \) until day 12-13 of the estrous cycle, a time coincident with uterine prostaglandin \( F_2 \alpha \) secretion. This problem was overcome by use of estrogen-treated "pseudopregnant" gilts which have extended luteal function through day 19. The present studies examined both the biochemical and structural changes occurring in porcine luteal tissue during prostaglandin \( F_2 \alpha \)-induced luteolysis. An intraluteal implant system was utilized which allowed individual corpora lutea to be exposed to prostaglandin \( F_2 \alpha \), and compared to corpora lutea residing on the same ovary that were implanted with silastic material alone.

The results reported in Paper 1 (Experiments 1, 2 and 3) established the minimally effective luteolytic dose of prostaglandin \( F_2 \alpha \) and confirmed the presence of a conceptus-associated antiluteolysin. Experiment 1 illustrated that the inhibition of progesterone secretion by luteal cells was more sensitive to prostaglandin \( F_2 \alpha \) than structural changes in the corpora lutea (i.e., weight). Further, this experiment established that the minimally effective luteolytic dose of prostaglandin \( F_2 \alpha \) was 100 \( \mu \text{g} \)/corpus luteum. This dose was then used in all subsequent experiments. The rationale for this dose was that corpora lutea implanted with 100 \( \mu \text{g} \) underwent complete functional and structural luteolysis, while the remaining corpora lutea on the ovary remained fully functional through day 19.

The results of Experiment 2, demonstrated that the corpora lutea of pregnant gilts were equally susceptible to the luteolytic actions of 100 \( \mu \text{g} \) of prostaglandin \( F_2 \alpha \).
Interestingly, however, the corpora lutea of pregnancy were heavier than those obtained from estrogen-treated "pseudopregnant" gilts. This would suggest that the influence of the early day 11-19 porcine conceptus, cannot be mimicked by estrogen treatment alone. Previous studies in the pig have suggested that the gravid uterus is associated with a local luteotrophic and/or antiluteolytic effect on the corpus luteum as evidenced by the maintenance of unilateral pregnancy (Anderson, 1966; Christenson and Day, 1971). Additionally, elevated progesterone concentration and weight of corpora lutea adjacent to the gravid uterine horn of unilaterally pregnant gilts pointed to a local effect of the conceptus/gravid uterus on luteal function (Niswender et al., 1970; Ford and Christenson, 1979).

The intraluteal implant system provided the unique opportunity to determine if the early-porcine conceptus (day 13) contained any compounds which had direct luteotrophic and/or antiluteolytic activity (Experiment 3). The day 13 conceptus tissue partially prevented prostaglandin F$_{2\alpha}$-induced regression in the estrogen-treated gilts. The substance in the conceptus, however, did not appear to be directly luteotrophic, as corpora lutea implanted with the conceptus tissue alone exhibited no increases in progesterone concentrations or weight over those levels seen in the control corpora lutea on the same ovary. The results of these studies would, however, suggest that the early porcine conceptus produces factors (antiluteolytic) which can inhibit prostaglandin F$_{2\alpha}$-induced luteolysis. Because the conceptus was only partially able to prevent luteal regression, it would appear that conceptus-stimulated uterine secretory products may also be vital for early maintenance of pregnancy in the pig.

The focus of Paper 2 was to investigate the time course of both functional (i.e., progesterone, protein kinase C...
activity, diacylglycerol levels) and structural (cell size, weight) regression in response to prostaglandin F₂α. The results of this study indicated a close association between declining steroidogenesis (i.e., progesterone production) and a decreased size of large luteal cells, which occurred by 12 h post-prostaglandin F₂α treatment. Further, the protein:DNA ratio, an estimator of cell size, indicated that by 12 h prostaglandin F₂α-treated corpora lutea had smaller cells (P < .05) than the adjacent vehicle (silastic alone) and unimplanted corpora lutea on the same ovary. These data were confirmed by morphologic analysis which indicated that large luteal cells were smaller in diameter at 12 h and 24 h in prostaglandin F₂α-treated corpora lutea. Previous studies in the pig have shown that the large luteal cells express the high affinity prostaglandin F₂α receptors and therefore are the likely primary target of this luteolytic compound (Gadsby et al., 1990, 1993). Further, the large luteal cells in the pig, as in other species are responsible for most of the progesterone production by the luteal tissue.

Many studies have evaluated the role that the protein kinase C second messenger system plays in prostaglandin F₂α-induced luteal regression. In the ewe, there is a preponderance of indirect evidence which suggests that the antisteroidogenic activity of prostaglandin F₂α is mediated by protein kinase C (Wiltbank et al., 1989, 1990). These studies however have been conducted entirely in an in vitro culture system and have incorporated the use of the non-physiologic protein kinase C activators called phorbol esters. The authors demonstrate that protein kinase C activation leads to a decline in progesterone accumulation in the cell culture media, however, they did not attempt to directly link prostaglandin F₂α treatment with changes in protein kinase C activity. The results of the present study indicated no association of protein kinase C activity with changes in
luteal progesterone concentrations or changes in large luteal cell size. In support of these data, Yuan and Connor (1992) reported that porcine large luteal cell protein kinase C activity does not change between day 10 (before uterine prostaglandin F₂α secretion) and day 15 (after uterine prostaglandin F₂α secretion) of the estrous cycle. In addition to protein kinase C, concentrations of diacylglycerol, the endogenous membrane associated activator of protein kinase C, did not change in this study. The present results bring into question the relevance of results obtained from in vitro systems to natural luteolysis in vivo.

Previous studies in the pig have shown that prostaglandin E₂ can prevent prostaglandin F₂α-induced regression both directly at the level of the corpus luteum (Ford and Christenson, 1991) and when administered into the uterine lumen (Akinlosotu et al., 1986). Paper 3 (Experiments 1 and 2) in this dissertation was designed to determine the role if any of prostaglandin E₂ in maternal recognition of pregnancy. The results of Experiment 1 demonstrated that utero-ovarian venous prostaglandin E₂ concentrations were transiently elevated over those of prostaglandin F₂α on days 11-12 of pregnancy followed by a sharp decline of both prostaglandins to basal levels on day 13 through day 16. In contrast during the estrous cycle, pulsatile secretion of prostaglandin F₂α predominated. In Experiment 2, elevated prostaglandin E₂ emanating from the gravid uterus versus the nongravid uterus of unilaterally pregnant pigs was associated with the increased luteal function on the ipsilateral ovary.

Conceptus estrogens may play a key role in changing the ratio of prostaglandin E₂ to prostaglandin F₂α during early pregnancy by altering the local contributions of uterine prostaglandins from stromal and glandular endometrial tissue. Porcine endometrial stromal cell isolated on day 13 of pregnancy produced greater concentrations of prostaglandin E₂
than cells collected on days 11 or 15 of pregnancy (Zhang and Davis, 1991). Interestingly, stromal secretion of prostaglandin E₂ was approximately three-fold greater than that of prostaglandin F₂α consistent with the observed increase seen in the pregnant gilts (Experiment 1). In addition to estrogen, the early porcine conceptus also exhibits a pronounced increase in prostaglandin production beginning on day 11 of pregnancy and produces greater levels of prostaglandin E₂ than prostaglandin F₂α.

Systemic estrogen administration to nonpregnant pigs on days 11-12 of the estrous cycle extends luteal function only a few days (Geisert et al., 1987). The results of Experiments 1 and 2 are consistent with a brief luteoprotective role for prostaglandin E₂ during the period of maternal recognition of pregnancy in the pig. Subsequent estrogen treatment of these same gilts with additional estrogen on days 14-16 of the estrous cycle prolonged luteal function in excess of 60 days due to a prolonged inhibition of uterine prostaglandin F₂α secretion. The marked and sustained decline in prostaglandin concentrations (prostaglandin E₂ and prostaglandin F₂α) we observed in pregnant pigs on days 13-16 are consistent with an effect of continued conceptus estrogen secretion in preventing any further uterine prostaglandin release. This would remove the need for continued prostaglandin E₂ secretion to maintain corpora luteal viability.
REFERENCES CITED


supplementation with hydroxycholesterol analogues and serum lipoproteins on ovine luteal cell progesterone secretion in vitro: demonstration of prostaglandin F$_2\alpha$ luteolytic actions in a defined model system. J. Reprod. Fertil. 97:57-63.


rat corpora lutea of various ages. Endocrinology 123:1044-1052.


Patek, C. E., and J. Watson. 1983. Factors affecting steroid and prostaglandin secretion by reproductive tissues of


Sawada, M., and J. C. Carlson. 1991. Rapid plasma membrane changes in superoxide radical formation, fluidity, and phospholipase A_2 activity in the corpus luteum of the rat


Swann, R. T., and N. W. Bruce. 1987. Oxygen consumption, carbon dioxide production and progestagen secretion in
the intact ovary of the Day-16 pregnant rat. J. Reprod. Fertil. 80:599-605.


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