1988

Aspects of luteal function in pigs and sheep

Alan James Conley

Iowa State University

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Aspects of luteal function in pigs and sheep

Conley, Alan James, Ph.D.
Iowa State University, 1988
Aspects of luteal function in pigs and sheep

by

Alan James Conley

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
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For the Major Department

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For the Graduate College

Iowa State University
Ames, Iowa
1988
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GENERAL INTRODUCTION

Statement of the Problem

Luteal function in pigs is seen to differ from that in other domestic animal species in three major respects; 1) the porcine CL is less dependent on pituitary luteotropic support than the CL of other domestic animal species, 2) estrogen administration during the midluteal phase is luteotrophic not luteolytic in pigs and 3) PGF$_{2\alpha}$ is not an effective luteolysin until the second half of the porcine estrous cycle. These characteristics hamper the interpretation of data compiled from pigs, relative to other species such as the ewe. Therefore, the objectives of the following experiments were to gain further understanding into the latter two aspects of luteal function in pigs, the luteotrophic effects of estrogen and the apparent insensitivity of the CL to exogenous PGF$_{2\alpha}$.

With regard to the first of these objectives, previous studies have failed to eliminate the possible direct effects of estrogen on luteal function in pigs, as distinct from effects mediated through the hypothalamo-pituitary axis or those effects on the uterus. This can be attributed to the difficulty of simultaneously removing the influence of the latter two components in a single animal. The experiments to be described represent the development of such a model.

The second objective relates to the resilience of the porcine CL following exposure to PGF$_{2\alpha}$ in the first half of the estrous cycle. Although exogenous PGF$_{2\alpha}$ does not induce luteolysis in pigs prior to day 12 of the cycle, progesterone does decline transiently, and there is evidence of fatty degeneration of luteal tissue following PGF$_{2\alpha}$ administration early in the luteal phase. However, it has not been determined whether PGF$_{2\alpha}$ exposure during this period induces quantitatively recognizable changes in luteal composition or in subsequent luteal development. Therefore, various luteal measurements were taken prior to, and following, PGF$_{2\alpha}$ administration to gilts on day 9 of the estrous cycle.

The final component of the following studies involves the mechanism of action of PGF$_{2\alpha}$ at the cellular level. Evidence supports the notion that PGF$_{2\alpha}$ exerts its effect on the CL, at least in part, through a direct effect on the luteal cell, but the mechanism(s) involved remain unknown. Recent studies in the rat and the cow have established that phorbol esters, and presumably activation of
protein kinase C, have similar effects to those of PGF₂α on progesterone secretion by dispersed luteal cell preparations. PGF₂α has been shown to induce hydrolysis of luteal membrane phospholipids, and therefore appears capable of activating the C kinase pathway. Dispersed ovine luteal cell preparations have proven useful in many in vitro studies, but few reports have documented the effects of phorbol esters on progesterone secretion by ovine luteal cells, and none have investigated the additive effects of PGF₂α or changes in oxygen environments. Therefore additional experiments utilized dispersed ovine luteal cells to study the effects of a phorbol ester on progesterone secretion, how it relates to the effects of PGF₂α, and what factors may affect the response of either or each of these agents.

Explanation of Dissertation Format

This dissertation has been written in accordance with the specifications for the alternative dissertation format outlined in the I.S.U. Graduate College Manual (1985). It is comprised of four sections representing independent experiments completed in 1986, 1987 and 1988. Various parts have been presented at scientific meetings during this time. For clarity and continuity, each section appears in the style suitable for publication in the Journal of Animal Science although none have yet been submitted. The general bibliography includes all references appearing in the review of the literature and the general discussion but may not contain additional references included along with each manuscript.
REVIEW OF THE LITERATURE

Introduction

In a review of the progress of knowledge on ovarian physiology, Short (1977) gave credit to Aristotle for being the first to formally express an awareness of the ovaries and the effects of ovarian removal on the subsequent fertility and reproductive behavior of sows and camels. As early as 1897, Beard expressed an awareness that the presence of the corpus luteum (CL) suppressed ovulation during pregnancy. However, definitive recognition of the CL as a gland of internal secretion was made by Prenant in 1898, and its physiological significance suggested by Gustav Born, the so called 'father' of the CL. The subsequent studies of Fraenkel and Cohn (1901) and Magnus (1901; for review see Short, 1977) established definitively the importance of this structure for the maintenance of pregnancy in rabbits, an observation that has since become recognized as a general principle in mammalian reproduction.

The CL is an endocrine organ with many unique attributes. In mammals, on a tissue weight basis, it is one of the most metabolically active organs in the body and it commands an equally impressive blood supply (Ellinwood et al., 1978). It forms and is functional only transiently, for a shorter or longer time period, depending on species, and whether or not pregnancy is successfully established. The major secretory product of the CL is progesterone although other peptide and protein hormones such as oxytocin (Sheldrick and Flint, 1987) and relaxin (Anderson, 1987), synthesized and released from the CL of several species, may play important roles in the control of reproductive function. For instance, it has been suggested that luteal oxytocin release in the ewe may play a role in the control of estrous cycle length (Sheldrick and Flint, 1987). More consideration will be given for and against this hypothesis in later sections.

Attempts have been made to explain the physiology of the CL, its control and function, in terms of an evolutionary development. Perhaps the most comprehensive of these reviews is that by Rothchild (1981) who argues that the appearance of the CL preceded and was essential to the development of viviparity (Porter et al., 1982). In lower oviparous or ovoviviparous vertebrates CL may play a phagocytic role resorbing the egg and yolk of oocytes within atretic
follicles (Hisaw, 1959). It is hypothesized that the acquisition of steroidogenic function, along with development of a mechanism to enable maintenance of the organ, enabled it to assume an endocrine role (Rothchild, 1981). In monoestrous species such as the dog, felids and mustelids, luteal lifespan is extended whether or not pregnancy is established. Polyestrous species are said to have further developed a mechanism to prematurely terminate the lifespan of the CL in the absence of conception. In many of these species hysterectomy results in extended luteal function similar in many respects to the extension seen in monestrous species. Luteal function is also maintained in pregnant animals through an effect of the conceptus and its products on the uterus and ovaries. Therefore, the evolutionary development and control of luteal function involves luteal maintenance (luteotrophic factors), premature regression (luteolytic factors) and mechanisms to prevent regression (luteoprotective or antiluteolytic factors).

A scheme such as that outlined above would predict that in mammalian species there would be a good deal of similarity in the basic function of the CL and in its control. The limits of the present review do not allow the presentation of arguments and examples that contradict the notion that the CL truly evolved in both structure and function, or that CL of all mammalian species share a common basic physiology. Instead luteal physiology will be discussed in general terms except where major species differences exist. In these the sheep and the pig will be emphasized so that an understanding of the apparent peculiarities of luteal function of these two species can be achieved. More general information can be obtained from the numerous reviews that have appeared over the years including the following not specifically referred to in this summary (Harrison, 1948; Ginther, 1967; Pharriss, 1970; Pharriss, 1972; Channing et al., 1980; Inskeep and Murdoch, 1980; Hansel and Convey, 1983; Keyes et al., 1983; Ford, 1985; Thatcher, 1985; Richardson, 1986; Smith, 1986; Thatcher et al., 1986; Xavier, 1987; Auletta and Flint, 1988.

Morphology and Development

Despite the fact that recognition of the CL as an endocrine gland did not occur until the close of the 19th century, the CL of several species had been
studied morphologically by several investigators. Debate raged between those scientists who believed that luteal cells were derived from the theca interna and those who believed that they were derived from the follicular granulosa layer (for reviews see Marshall, 1904; Corner, 1920; Brambell, 1956). More recently, it is generally accepted that the granulosa contributes the major endocrine cellular component of the CL (Mossman and Duke, 1973; Harrison and Weir, 1977). Even so, two distinctly different types of secretory cells can be recognized both by their morphological characteristics and by their size. Attempts have been made to trace the origins of the two cells types to either granulosa or theca interna cells, but at the present time no definitive data exists to support either possibility (O'Shea et al., 1979).

In general, morphological development is similar among most mammalian species (Harrison and Weir, 1977). Following ovulation in the ewe, contraction and disruption (Hadek, 1958) of the basement membrane allows invasion of the granulosa cells of the postovulatory fossa by thecal cells, capillaries and the surrounding connective tissue (Marshall, 1904). In these early stages, the first two days following ovulation, thecal cells migrate deep into the developing organ (O'Shea et al., 1980) but their fate after this time is difficult to trace. Subsequent growth consists of expansion of capillary and lymphatic networks (Andersen, 1926), and marked hypertrophy of granulosa cells (Marshall, 1904; McClellan et al., 1975; O'Shea et al., 1980).

Changes in granulosa cell morphology during the period of luteinization have been well described. In the sheep, prior to ovulation, granulosa cells are joined by both gap junctions and desmosomes, nuclei rarely contain nucleoli and occasional mitoses are seen in these cells or those of the theca interna (McClellan et al., 1975). Within hours of the LH surge, gap junction numbers decline, nuclear size increases and nucleoli appear, features commensurate with activation of cellular function and protein synthesis. These changes are accompanied by further development of the cellular organelles associated with steroidogenic function (Christensen and Gillim, 1969) including increases in size and number of mitochondria and size of smooth endoplasmic reticulum. Luteal cells also acquire additional features including some rough endoplasmic reticulum and a distinct Golgi complex suggestive of the development of protein hormone secretion or at least packaged hormone release mechanisms (Enders,
A similar sequence of events occurs in the pig (Corner, 1915, 1920; Bjersing, 1967; Cavazos et al., 1969; Belt et al., 1970, 1971) with minor variation in subcellular morphology such as whorl-like rather than stacked arrangements of smooth endoplasmic reticulum (Bjersing, 1967), and more lipid droplets.

Several factors are thought to be involved in the control of luteinization. For instance, the degree of follicular maturity appears to be important because mid-luteal or immature follicles will not luteinize normally (Channing, 1970a). Although both FSH and LH appear to be important in the preparation of follicles the major stimulus for luteinization appears to be through LH-induced increases in cAMP (Channing, 1970b). In addition, the studies of El-Fouly et al. (1970) and Nekola and Nalbandov (1971) suggested that the ovum had an inhibitory influence on the initiation of luteinization. However Lindner et al. (1974) observed LH-stimulated luteal transformation in the presence or the absence of the ovum and suggested that the major influence on luteinization was LH alone. Within the granulosa cell itself many of the morphological changes are brought about by the action of microfilaments (Amsterdam and Rotmensch, 1987). Major biochemical alterations also occur including large increases in the transcription of genes coding for steroidogenic enzymes such as cytochrome P-450 side chain cleavage enzyme (ssc) (Richards et al., 1987) and peptide hormones (Ivell et al., 1985).

Subsequent to the first two days of development, growth of the CL proceeds in a less dramatic manner with continued vascular development and an increase in cell volume reflected by luteal weight. In the sheep, luteal weight increases continually until around day 14 of the estrous cycle (Grant, 1933; Smith and Robinson, 1969; Farin et al., 1986; Schwall et al., 1986a). In the pig however, it has been reported that luteal weight peaks around day 8 or 10 then slowly declines (Duncan et al., 1960). It is generally agreed that growth is brought about mainly by hypertrophy of lutein cells (Harrison and Weir, 1977) because a low mitotic rate is observed particularly in granulosa-lutein cells (O'Shea et al., 1980).

Recently studies have observed changes in the relative populations of large and small luteal cells of the ovine CL during the estrous cycle. Preparations of dispersed luteal cells made on days 4, 8, 12 and 16 of the estrous cycle indicated an increase in both small and large cell populations as defined by cell diameter.
alone (Schwall et al. 1986a). Large cells increased at a proportionally faster rate resulting in a significant increase in the large:small luteal cell ratio. Farin et al. (1986) used a morphometric approach to defining large and small luteal cell populations during the development of the ovine CL. Although an overall increase in cell number was observed, contrary to the findings of Schwall et al. (1986a) this was due mainly to an increase in small cell numbers and therefore it resulted in a decline in the large to small cell ratio. Large luteal cell size also increased through the cycle but small luteal cell size did not. It is difficult to resolve the discrepancies in the results of these two studies particularly in view of the data of Rodgers and O'Shea (1982) who demonstrated that large luteal cells were more readily disrupted during cell dispersion than were small luteal cells (Rodgers et al., 1984; O'Shea, 1987), an effect which would tend to decrease or minimize the increased ratio of large to small cells seen by Schwall et al. (1986a).

It has been suggested that large luteal cells develop from small luteal cells in cows (Donaldson and Hansel, 1965). Studies by Alila and Hansel (1984) using monoclonal antibodies prepared against each of the theca and granulosa cell membranes were said to suggest that large luteal cells were derived from small (thecal) cells because thecal antibody binding to large luteal cells increased through the estrous cycle. No evidence was presented to verify that the cell membrane antigenic components did not also change through the estrous cycle in either cell population however, and the identity of the proteins labelled by the antibodies was not recognized so that the expected specificity of each of the components could not be confirmed. In the pig, Kineman et al. (1987) also presented data on large and small dispersed cell populations from CL collected from gilts at various times throughout the estrous cycle. These data also suggested that large luteal cells were more numerous during the later stages of the luteal phase.

In the ewe, Farin et al. (1985) presented data to suggest that HCG treatment effectively increased large luteal cell number indicating that since only small luteal cells contain LH receptors (Fitz et al., 1982), the large luteal cells may indeed be derived from the small cell population. The increase in small luteal cell numbers toward the end of the estrous cycle (Farin et al., 1986) tends to contradict this hypothesis, but it has been suggested that perhaps a stem cell population exists from which lutein cells may be derived (Farin et al., 1986;
Schwall et al., 1986b). Hoyer et al. (1988) did not observe mitoses of luteal cells maintained for long periods in culture in media containing calf serum even though progesterone secretion was constant and other cell types proliferated. The apparent lack of intermediate cell types in the study by Aliila and Hansel (1984) and the low mitotic index previously noted histologically in CL, would seem not to support either the small to large cell differentiation theory or the possibility of a stem cell population respectively.

Pituitary Luteotrophic Support

**Ewes**

Pituitary support appears to be important in the development and function of the CL in most mammalian species (Stormshak et al., 1987). In the ewe both pituitary stalk-transection and complete hypophysectomy have been used to investigate the components of the luteotrophic complex. Stalk-transection appears not to dramatically affect luteal function in ewes (Denamur et al., 1966; Mallory et al., 1986) and therefore the interplay between the hypothalamus and the pituitary is unclear in this species. Hypophysectomy, on the other hand, does influence luteal function in sheep but authors differ in their interpretation of the data depending on the measurements used to assess it and the actual time of assessment relative to the stage of the estrous cycle. Additional controversy involves the relative importance of prolactin as a luteotrophin in this species, therefore some of the data relating to this issue will be briefly reviewed as an introduction to those factors which have the potential to prevent luteolysis.

With regard to the importance of pituitary factors in the formation of the CL in the ewe, Denamur et al. (1966) found no effect of hypophysectomy performed on days 2 to 5 on ovarian venous progesterone concentration or luteal histology on day 9. Kaltenbach et al. (1968a) hypophysectomised ewes on day 1 and examined luteal weight and progesterone content and concentration on day 8. Although luteal weight was significantly reduced, luteal tissue progesterone concentration in hypophysectomized ewes was similar to that in controls (Kaltenbach et al., 1968a). These data suggest that a limited degree of luteal development is possible in ewes in the absence of pituitary support and
that existing luteal tissue although reduced in mass, may maintain ovarian venous concentrations of progesterone within normal limits during the first half of the cycle.

Data relating to the components comprising the pituitary luteotrophic complex are more difficult to assess. Earlier experiments employed relatively impure hormone preparations. Even so, data supporting a role for prolactin in luteal maintenance in ewes includes those of Hixon and Clegg (1969) who demonstrated a greater effect of LH and prolactin combined, than either hormone alone, in maintaining luteal function in ewes hypophysectomized on day 8 or 9 of the estrous cycle. Additional support was provided by the study of Schroff et al. (1971) who demonstrated that LH maintained luteal function in ewes in which hypophysectomy was incomplete. In addition, Kaltenbach et al. (1968b) was able to maintain luteal function in ewes following hypophysectomy with a crude LH preparation but not with one more purified in its LH content. These data generally support a role for an additional pituitary factor such as prolactin, in the luteotrophic complex in ewes.

Some mention must also be made of those reports supposedly not supporting an important role for prolactin in luteal maintenance in ewes. The studies by Karsch et al. (1971a, b) were performed in ewes hypophysectomized on day 12 of the estrous cycle and infused with LH and prolactin preparations. In these ewes luteal function was maintained to day 20 by infusion of LH (Karsch et al., 1971b) but not by prolactin (Karsch et al., 1971a). Whether or not LH and prolactin interacted, as suggested by Hixon and Clegg (1969), was not investigated in ewes administered both hormones. It is important to note that these experiments and others (Kaltenbach et al., 1968b) were performed on uterine-intact ewes during the latter half of the estrous cycle and may relate not only to the requirements for luteal maintenance but also the prevention of luteolysis, as discussed more fully later. Therefore, these data appear not to preclude an effect of prolactin which may modify the LH response either by effects on LH receptor number (Denamur et al., 1973; Hansel et al., 1973) or mobilization of the cholesterol precursor pool (Bartosik and Romanof, 1969).

More recently, Niswender et al. (1986) stalk-sectioned ewes on day 5 of the estrous cycle and investigated the effect of LH infused 6 times each day along with ergocryptine treatment which reduced prolactin levels by greater than
95%. Several measurements were taken including luteal weight and
progesterone concentration, unoccupied luteal LH receptors and systemic plasma
progesterone concentration. Without ergocryptine, LH maintained luteal
weight and progesterone concentration at levels that were lower but not
significantly so, from those of intact controls. In the presence of ergocryptine,
LH treated ewes had significantly lower luteal weight and progesterone
concentration than intact controls although no significant difference was found
between LH with ergocryptine treatment and LH treatment alone with regard to
these two measurements. However, LH markedly increased systemic
progesterone concentrations of stalk-sectioned ewes, an effect that was blocked
by ergocryptine treatment. These results suggest that in these experiments,
performed during the luteal phase prior to the time of onset of luteal regression,
LH may not have completely compensated for reduced pituitary support.
Contrary to the conclusions drawn by the authors themselves, the studies of
Niswender et al. (1986) also demonstrate that reduction of circulating prolactin
levels to less than 95% of normal values modified the ovarian response to LH
treatment of stalk-sectioned ewes. The mechanism appears not to involve the
maintenance of LH receptors unless this involves the prevention of down
regulation, since ergocryptine treatment did not alter unoccupied LH receptor
numbers. In any event, the failure of prolactin alone to extend luteal function
and the observations that prolactin does not stimulate luteal progesterone
secretion in vivo (Domanski et al., 1967; McCracken et al., 1971) or in vitro
(Kaltenbach et al., 1967) suggest a supportive rather than a stimulatory role of
this hormone in luteal function in the ewe.

As discussed above, the data of Karsch et al. (1971a,b) indicate that LH
administration may be able to prevent natural luteolysis and extend luteal
function. Others have investigated the ability of LH to prevent induced
luteolysis. Adams et al. (1976) administered LHRH in a pulsatile manner on days
12 to 15 of the estrous cycle of ewes and noted increased luteal weight and
progesterone content and systemic progesterone concentrations. LHRH
administration also reduced the luteolytic effect of estrogen. Denamur and
Kann (1973) had previously demonstrated that hysterectomy prevented the
luteolytic effect of estrogen administered in the latter part of the luteal phase in
ewes indicating that this effect may be mediated by endogenous release of a
uterine luteolysin. Bolt et al. (1971) also prevented estrogen induced luteolysis by the simultaneous administration of HCG but not LH on day 10 of the estrous cycle, however, luteal weights in both groups were less than untreated controls suggesting that luteal maintenance was not complete. The above mentioned studies suggest that LH or HCG can at least partially overcome the luteolytic effect of the uterus in the ewe.

In contrast to those studies relying on endogenous luteolytic factors, Sasser et al. (1977) administered prostaglandin F2α in two 6 mg doses 12 hours apart, a regime suggested by previous data to be the minimally effective luteolytic dose in ewes. Treatments consisting of LH, prolactin or LH plus prolactin were not able to prevent luteal regression whether or not they were administered as 12 hour constant infusions or whether they were administered beginning 2 or 6 hours before PGF2α injection. Similar data were reported by Cerini et al. (1973) following PGF2α infusion into the ovarian artery. However, the possibility remains that under more physiological conditions, during natural luteolysis, pituitary luteotrophins may prevent luteolysis.

**Pigs**

Studies concerned with hypophysial control of luteal function in pigs have yielded results which are essentially similar. Several early studies utilized progesterone administration to inhibit pituitary gonadotrophin release. Sammelwitz and Nalbandov (1958) showed that progesterone administration induced luteal regression by day 20 in pregnant pigs. Similarly, Spies et al. (1959) demonstrated that administration of progesterone from day 4 to day 18 or 25 post-mating decreased luteal weight and adversely affected embryo survival at day 25. The inhibitory effect of progesterone on luteal weight was subsequently shown to occur until day 13 whether progesterone treatment was begun after ovulation (Sammelwitz et al., 1961) or even before the LH surge as long as ovulation took place (Brinkley et al., 1964b). In agreement with these results, hypophysectomy at estrus (du Mesnil du Buisson and Léglise, 1963) or the day after (Anderson et al., 1967), did not affect the length of the luteal phase or luteal progesterone concentration. However, contrary to the results of
Sammelwitz et al. (1961) luteal weights of hypophysectomized pigs were significantly less than intact controls by day 12 (Anderson et al., 1967).

Several reports confirm the earlier suggestion that luteal maintenance beyond day 12 or 14 requires pituitary support in pigs. Crude pituitary extracts and HCG maintain luteal function under certain experimental conditions. For instance, during pregnancy, continuous HCG infusion maintained normal luteal weight but not luteal progesterone concentration at day 40 in pigs which were pituitary-suppressed by progesterone administration (Brinkley et al., 1964a). Anderson et al. (1965, 1967) demonstrated that pituitary extracts and HCG maintained and stimulated increased luteal weights in hysterectomized pigs following either stalk-transection or hypophysectomy early in the luteal phase. However, gonadotrophin administration was not able to maintain luteal function in these pigs if the uterus was left intact (Anderson et al., 1965, 1967), and it was concluded that the uterus interfered with the action of gonadotrophins on the CL (Anderson et al., 1965). A negative or inhibitory influence of the uterus was demonstrated by the results of Spies et al. (1959) which showed that hysterectomized pigs had heavier CL than intact controls at day 25 post-estrus. Although Guthrie and Rexroad (1981) claimed to have prevented luteal regression of intact, nonpregnant gilts by HCG administration towards the mid-luteal phase, these authors used systemic plasma progesterone concentration as the sole criterion for maintained luteal function and failed to confirm that the grossly elevated progesterone concentrations obtained following treatment were not due to the induction of accessory CL. Spies et al. (1967) used anti-ovine LH antiserum administered between days 25 to 29 to passively immunize pregnant gilts against LH. This treatment resulted in a significant reduction in luteal weight by day 30. These results and those above suggest that LH plays a major role in providing luteotrophic support for the CL of pregnancy and those maintained by hysterectomy in pigs after day 14.

The role of prolactin in luteal function in pigs is less clear. A general lack of effect of prolactin in maintaining luteal weight was found in both progesterone treated (Sammelwitz and Nalbandov, 1958) and hypophysectomized pigs (Anderson et al., 1965) and this is consistent with the fact that prolactin (and FSH) receptors have been difficult to demonstrate on luteal cells in the pig (Jammes et al., 1985; Bramley and Menzies, 1987; Ziecik et al., 1988). These
results indicate that LH may be the only important component of the pituitary luteotrophic complex in pigs during early pregnancy or early in the luteal phase following hysterectomy.

The results of experiments involving unilateral ovariectomy also provide additional insights into the maintenance of luteal function by pituitary gonadotrophins. Compensatory ovarian hypertrophy is a well recognized phenomenon following unilateral ovariectomy of pigs. However, several studies have failed to demonstrate hypertrophy of CL at day 14 of the estrous cycle of nonpregnant pigs unilaterally ovariectomized following ovulation (Brinkley et al., 1964c; Short et al., 1965; Brinkley and Young, 1969). Evidence to support the contention that the failure to demonstrate luteal hypertrophy by day 14 was probably not due to the failure of expression of the compensatory mechanism was presented by Staigmiller et al. (1972). It was shown in unilaterally ovariectomized pregnant and hysterectomized pigs that although no detectable increase in luteal weights was evident by day 15 that significant increases had occurred in follicular fluid weights and therefore that follicular compensation had at least begun by that stage. Luteal weight, however, was increased by day 27 (Staigmiller et al., 1972). These data are consistent with a minimal effect of pituitary factors on luteal growth in the nonpregnant pig.

Similar results had been reported previously by Rathmacher et al. (1967) who unilaterally ovariectomized pregnant and hysterectomized gilts early in the luteal phase and showed an increase in luteal weights in both groups by day 24 to 26 post-mating or estrus. However, luteal compensatory hypertrophy at days 24 to 27 was not accompanied by an increase in luteal tissue progesterone concentration (Rathmacher et al., 1967). These results were confirmed by Staigmiller et al. (1974) and extended to show that systemic progesterone concentrations were higher in intact control pregnant pigs than in unilaterally ovariectomized pregnant pigs. When corrected for CL number there was only a trend toward an increase in progesterone production per gram of luteal tissue (Staigmiller et al., 1974). Therefore, the luteal hypertrophic response seen by day 24 may be more structural than it is functional. However, it can be concluded, with more certainty, that luteal hypertrophy in the pig does not occur during the period when CL are believed to be less responsive to pituitary gonadotrophins.
The mechanism whereby luteal hypertrophy is brought about is not clear. Redmer et al. (1984) examined the effects of unilateral ovariectomy of pigs on day 1 of the estrous cycle, on pituitary gonadotrophin levels in systemic plasma and estrogen levels in ovarian venous plasma on days 2, 4 and 8 of the cycle. FSH levels increased within 24 hours of ovariectomy. Ovarian venous estrogen concentration was also elevated at day 2 and 4 but not day 8 of the cycle. No change was seen in systemic LH concentrations (Redmer et al., 1984). These results suggest that if luteal hypertrophy occurs in response to LH then since no increase in systemic LH levels occurs, there must be an increased responsiveness of the tissue to the existing levels as suggested by Anderson et al. (1966).

Rexroad and Casida (1976) unilaterally ovariectomized gilts at days 4 and 15 of pregnancy and examined follicular growth at day 27. Both an increase in follicular size and estrogen concentration in follicular fluid were noted (Rexroad and Casida, 1976). Garverick et al. (1982) reported an increased number of LH receptors on days 15 to 21 in the CL of both intact and hysterectomized gilts treated with estradiol on day 12 after estrus. These data, and the previous studies cited above, are consistent with the hypothesis that unilateral ovariectomy of pregnant or hysterectomized pigs early in the luteal phase results in compensatory luteal hypertrophy by day 27 and that this effect may be mediated by local increases in ovarian estrogen concentrations resulting from increased follicular growth in response to elevated FSH levels in systemic plasma.

In conclusion, the differences in the effects of pituitary gonadotrophins on luteal function in the sheep and pig can be summarized as follows. The luteotrophic complex in the sheep probably consists of both LH and prolactin, the latter influencing responsiveness to LH. The luteotrophic complex is important in the development and maintenance of the CL and LH can induce changes in luteal structure and morphology. In addition, luteotrophic support can overcome the luteolytic effect of the uterus and the general health of the CL may depend on a balance between luteotrophic and luteolytic influences in the ewe. In the pig LH appears to be the major luteotrophin but luteal responsiveness does not develop until after day 14. LH appears to be unable to prevent natural luteolysis but it may induce structural alterations in CL maintained by prevention of the luteolytic signal.
Prostaglandin F₂α as the Uterine Luteolysin

Uterine luteolytic effects

It has long been recognized that an interdependence of function exists between the uterus and the ovaries (Anderson, 1973; 1977). Conception is reliant on an active CL and in most species the CL is influenced by both lytic and trophic factors from the nongravid and gravid uterus, respectively. Among the earliest evidence that the uterus might exert negative influences on luteal lifespan was the demonstration by Loeb (1923) that hysterectomy extended the luteal phase of guinea pigs. Similarly, in several other species including the sheep, cow and pig, hysterectomy prolongs luteal function for a period equal to or exceeding that of pregnancy (Anderson and Melampy, 1964; Anderson, 1966; Kiracofe and Spies, 1966; Anderson et al. 1969). Additionally, placement of intrauterine devices early in the luteal phase causes premature luteal regression in many domestic and laboratory animal species (Anderson, 1973) including the sheep (Ginther, 1968a). However, in the pig, estrous cycle length and luteal tissue progesterone concentration are not affected by placement of Intrauterine device’s. Although placement of Intrauterine device’s results in only local elevations of uterine PGF₂α release in the pig (Magrini et al., 1978), luteal weight is decreased (Gerrits et al., 1968) suggesting a negative uterine influence at least on luteal development with perhaps less dramatic consequences on cycle length.

Uterine effects on luteal function have been shown to have locally mediated components in both sheep and pigs. Moor and Rowson (1966a) and Inskeep and Butcher (1966) demonstrated in ewes that unilateral hysterectomy of the uterine horn ipsilateral to a functional CL early in the luteal phase significantly extended estrous cycle length. A similar extension of luteal function occurred if either the ovary or the uterus was transplanted to the neck (Goding et al., 1967) but not if the uterus and its attached ovary were transplanted together (Harrison et al., 1968). In the pig, however, unilateral regression of CL can be achieved only if all but one quarter of one uterine horn remains in situ (du Mesnil du Buisson, 1961) and transplantation of the ovary to another site within the abdominal cavity or to the neck results in a continuation of normal
ovarian cyclicity (Binns et al., 1967; Hagen et al., 1981; Harrison, 1982) and maintenance of gestation (Martin et al., 1978; Harrison, 1982). These results indicate that in the ewe the uterus controls luteal function in a very locally restricted manner the operation of which is critical to continued reproductive function. By comparison, although there is evidence that a similar mechanism of uterine influence on luteal function may exist in the pig, in this species normal cyclical luteal regression occurs in the absence of the normal anatomical association between the uterus and the ovary and these mechanisms can operate normally on a purely systemic level.

Although the experimental results discussed above indicated that a substance of uterine origin - a uterine luteolysin - was inducing luteal regression, studies designed to demonstrate its presence in the uterus yielded mixed results (for early review see Nalbandov and Cook, 1968). For instance, Kiracofe et al. (1966) failed to induce luteolysis in mid luteal phase ewes by administration of uterine extracts prepared from early and late luteal phase ewes. Similarly, Stormshak and Kelley (1967) found no inhibitory effects of endometrium on progesterone secretion by ovine luteal tissue cultured in vitro. In contrast, Caldwell et al. (1968) showed that endometrial extracts prepared from ewes on day 14 or 15 of the estrous cycle, (but not on days 3 to 6), would reduce luteal lifespan in hysteredomized hamsters. Myometrial extracts were ineffective at any stage.

In the pig, Anderson et al. (1961) demonstrated that endometrial damage induced by intrauterine infusion with cauterizing agents extended luteal lifespan suggesting that endometrial integrity was important in the luteolytic process. Christenson and Day (1972) infused porcine endometrial extracts into the nongravid uterine horn of uni-laterally pregnant gilts on day 23 of pregnancy. Extracts prepared from pigs on days 13 to 17 of the estrous cycle or day 19 of pregnancy induced luteolysis and embryonic death. There was no significant effect of extracts prepared from pigs at earlier stages of the cycle or later (day 29) stages of pregnancy on either luteal function or pregnancy rate.

The above mentioned data, in the sheep and the pig, provided evidence that the luteolysin was of endometrial origin and that it was present in endometrium in increased quantities around the time of luteolysis in nonpregnant animals. Furthermore, the results of Caldwell et al. (1968)
suggested that it might not necessarily be species specific. Further support for these data were provided by Caldwell and Moor (1971) and Baird et al. (1973) who were able to induce luteolysis in mid-luteal phase ewes by ovarian arterial infusion of uterine venous plasma collected from donor ewes in the late luteal phase of their estrous cycles. In addition, Caldwell et al. (1969a) observed local areas of degeneration around endometrial tissue that had been implanted into CL of hysterectomized ewes. These animals had functional CL for only about 90 days compared with unimplanted hysterectomized ewes who experienced continued luteal function for up to 140 days. Local degeneration immediately surrounding the implant was observed at various stages up to day 90 (Caldwell et al., 1969a).

Soon after the isolation and identification of prostaglandins it was recognized that at least one member of the group, notably prostaglandin F$_{2\alpha}$, had potent luteolytic properties, firstly in rats (Pharriss and Wyngarten, 1968), and subsequently in other animal species (Horton and Poyser, 1976). Since this monumental discovery an enormous amount of research has been done to establish the role of PGF$_{2\alpha}$ as the physiological uterine luteolysin (McCracken et al., 1972a,b; Goding, 1974).

Properties of prostaglandin F$_{2\alpha}$

Several biochemical and physiological properties make PGF$_{2\alpha}$ a suitable candidate for the physiological luteolysin. Prostaglandins, in general, are small molecular weight fatty acids. The precursor molecule arachidonic acid, is stored mainly in the form of membrane phospholipids and is released by the action of phospholipases A$_2$ or C. In general, the former enzyme is the most important in prostanoid synthesis (Moore, 1985). Cyclooxygenase is involved in catalyzing the formation of prostaglandins from arachidonic acid and requires both heme and molecular oxygen for the process. However, the role of oxygen is complex because although complete anoxia prevents prostaglandin synthesis, reductions in oxygen concentrations may initially stimulate it (Lands, 1979). The enzymes mentioned, the membrane phospholipids and therefore the potential for prostaglandin synthesis, are ubiquitous and almost every tissue in the body including endometrium (Wlodawer et al., 1976) has this capacity (Moore, 1985).
Although most tissues also appear to possess the capacity for prostaglandin catabolism, prostaglandins that spill over into the systemic circulation are generally metabolized by the lung, liver, kidney and endometrium (Maule Walker et al., 1977; Guthrie and Lewis, 1986). The lung appears to be particularly important in this regard (Piper et al., 1970; Eling and Anderson, 1976) and in the ewe an estimated 99% of PGF$_{2\alpha}$ is inactivated in a single passage through the pulmonary circulation (Davis et al., 1980). Species differences in the efficiency of prostaglandin metabolism by the lung do exist (Davis et al., 1985) so that in the pig only 18% of PGF$_{2\alpha}$ is inactivated in a single pulmonary passage (Davis et al., 1980). Such differences may have important consequences within a species as to whether luteolysis is locally mediated as in the ewe or has a greater systemic component as in the pig. Pulmonary endothelium is the cellular component responsible for prostaglandin metabolism, and uptake into these cells appears to be via active transport (Bito and Spellane, 1974; Moore, 1985).

Prostaglandins are known to have several physiological actions including control of hormone and neurotransmitter release, smooth muscle contractility, renal tubular absorption, gastric acid secretion, platelet aggregation and involvement in several other aspects of the inflammatory response (Lauderdale, 1974; Moore, 1985). Despite the vast literature on prostaglandin action, the cellular basis of activity is still largely unknown (Harris et al., 1979). As stated by Moore (1985), an action through activation of membrane bound cellular receptors is consistent with a surprisingly limited ability of these lipids to traverse plasma membranes.

Cellular membrane receptors for PGF$_{2\alpha}$ have been characterized in CL of sheep (Powell et al., 1974) and other species (Robertson, 1986). Although Mattioli et al. (1985) failed to detect PGF$_{2\alpha}$ receptors in luteal cell membrane preparations collected from gilts slaughtered on days 3 to 15 of the estrous cycle, a preliminary report by Gadsby et al. (1988) described high affinity PGF$_{2\alpha}$ binding sites which increased markedly on day 14 above levels found both at earlier and later stages of the estrous cycle. The physiological significance of changes in PGF$_{2\alpha}$ receptor numbers is obscured by the lack of knowledge as to the importance of receptors in eliciting a response to PGF$_{2\alpha}$.
Cyclic uterine prostaglandin release

As previously discussed, several reports provided evidence suggesting that the uterus was the source of a luteolytic substance. With the discovery of the potent luteolytic ability of PGF$_{2\alpha}$, subsequent investigations were conducted to identify PGF$_{2\alpha}$ in the uterine compartment (Harrison et al., 1972), and to correlate its occurrence with the onset of luteolysis. In the ewe it was established that both the content and concentration of PGF$_{2\alpha}$ increased in the endometrium on day 14 when compared to day 3, 5 or 11 of the estrous cycle (Wilson et al., 1972a,b). Alwachi et al. (1979) and Findlay et al. (1981, 1983) also showed increased endometrial PGF$_{2\alpha}$ synthesis two or three days prior to expected estrus and Huslig et al. (1979) demonstrated increased cyclooxygenase activity in ovine endometrium late in the luteal phase. Levels of PGF$_{2\alpha}$ increase in the uterine vein late in the luteal phase of the ewe (Bland et al., 1971; Thorburn et al., 1972). Also in this species, blockade of uterine prostaglandin synthesis by inhibitors such as indomethacin extends luteal function (Lewis and Warren, 1977a) and prevents the luteolytic effect of estrogen (Lewis and Warren, 1977b) and Intrauterine devices (Spilman and Duby, 1972). Immunization of ewes against PGF$_{2\alpha}$ also increases cycle length (Scaramuzzi and Baird, 1976). Together, these data suggest that PGF$_{2\alpha}$ plays an important role in luteolysis in the ewe.

It is accepted that uterine release of PGF$_{2\alpha}$ in ewes is pulsatile (Thorburn et al., 1972) and that the pulsatility is important in determining the luteolytic potency (McCracken et al., 1984a). During PGF$_{2\alpha}$-induced luteolysis in sheep, the CL accumulate PGF$_{2\alpha}$ (Stacy and Gemmell, 1976b) but ovine CL are also capable of PGF$_{2\alpha}$ synthesis (Rexroad and Guthrie, 1979). It has been suggested that intraluteal PGF$_{2\alpha}$ production may participate in the luteolytic chain of events, however, blockade of luteal PGF$_{2\alpha}$ production by indomethacin administration to ewes in vivo appears not to interfere with luteolysis induced by administration of prostaglandin analogues (Rexroad and Guthrie, 1979).

In the pig, it has been demonstrated that both the endometrium and CL synthesize and release greater quantities of PGF$_{2\alpha}$ late in the luteal phase (Patek and Watson, 1976; Guthrie et al., 1978, 1979). Uterine venous plasma
concentrations of PGF$_{2\alpha}$ increase in the late luteal phase of the estrous cycle in gilts (Gleeson and Thorburn, 1973) and are negatively correlated with systemic progesterone concentrations (Killian et al., 1976). Indomethacin treatment of gilts from day 10 to 17 of the estrous cycle prevents the normal decline in systemic progesterone and return to estrus (Kraeling et al., 1981). As in the ewe inhibition of PGF$_{2\alpha}$ synthesis in the CL of pigs by concomitant indomethacin treatment does not prevent luteolysis induced with synthetic prostaglandin analogues (Guthrie et al., 1979; Guthrie and Rexroad, 1980).

**Counter current transfer and utero-ovarian lymphatics**

Demonstration of the luteolytic properties of PGF$_{2\alpha}$ and accumulating evidence that this compound might be the uterine luteolysin (Goding, 1974) led to studies designed to investigate the mechanisms that might operate and explain the previously described local luteolytic effect (Goding et al., 1967). Restall et al. (1973) reported that 3 of 4 ewes given PGF$_{2\alpha}$ along with a ganglionic blocking agent, guanethidine, did not experience luteolysis while 3 of 4 control ewes, receiving PGF$_{2\alpha}$ only, did. It was therefore suggested that sympathetic innervation may mediate the luteolytic effects of PGF$_{2\alpha}$ in ewes. However, several other investigators (Barrett et al., 1971; Thorburn and Nicol, 1971; McCracken et al., 1972b; Mapleloft et al., 1976) demonstrated that PGF$_{2\alpha}$ was luteolytic when infused directly into the ovarian artery. These data, and the fact that PGF$_{2\alpha}$ induces regression in autotransplanted ovaries suggests, therefore, that innervation of the ovary itself may not be necessary for luteolysis to occur. These data do not exclude a possible interaction with adrenergic receptors, but further evidence supporting such a concept has not yet been obtained.

Nevertheless, the requirement for anatomical integrity of the ovarian and uterine vasculature in natural regression suggested the possibility that the luteolysin might be transferred from the uterine vein to the ovarian artery this being the final pathway in the process (Baird and Land, 1973; Lamond and Drost, 1973). It has been demonstrated in several different laboratories that tritiated-PGF$_{2\alpha}$ infused into the uterine vein appeared in the ovarian artery as PGF$_{2\alpha}$ in higher concentrations than appeared in the aorta (McCracken et al., 1972a,b; Land et al., 1976; Einer-Jensen and McCracken, 1981). These elevated levels in
PGF$_{2\alpha}$ in the ovarian artery appeared after a 30 to 60 minute delay and disappeared slowly following cessation of infusion suggesting the saturation and depletion of a large tissue reservoir (McCracken et al., 1972a; Land et al., 1976). Transfer was observed on days 8, 10 or 11 and day 15 without any suggestion of an alteration in transfer rate with stage of the estrous cycle (Land et al., 1976), despite the suggestion by McCracken et al. (1984b) that the rate of transfer from the uterine vein to the ovarian artery might be concentration dependent.

While the above experimental results demonstrate transfer of PGF$_{2\alpha}$ from the uterine vein to the ovarian artery, the mechanism by which this occurs is less clear. The degree of association between the uterine veins and the ovarian artery is variable among species (Ginther, 1976). In the ewe the ovarian artery is very convoluted and follows a tortuous path across the surface of the uterine vein resulting in extensive areas of contact and apposition between these two vessels (Del Campo and Ginther, 1973). In addition, histological evidence suggested a thinning of the walls of these two vessels including the sharing of a single adventitia (Del Campo and Ginther, 1974). In swine the ovarian artery in the region of contact with the uterine vein is straighter and therefore the area of contact and possible exchange is less extensive than in the ewe (Del Campo and Ginther, 1973). These findings agree with the previously discussed greater systemic component of the luteolytic signal in the pig compared with that in the sheep.

The above anatomical studies lend support to the theory of transfer of PGF$_{2\alpha}$ from the uterine vein to the ovarian artery in the ewe but few reports deal with the counter-current exchange of PGF$_{2\alpha}$ in the pig. However, using in vitro perfused porcine uteri (day 14 of the estrous cycle) with ovaries and broad ligament intact, Kotwica (1980) demonstrated that following injection of $^3$H PGF$_{2\alpha}$ under the serosa of the uterus, labelled PGF$_{2\alpha}$ appeared in the ovarian artery. In a similar, subsequent experiment in which the mesosalpinx of the PGF$_{2\alpha}$-injected horn was draped over the remaining ovary which had also been excised with its vasculature, and perfused with autologous blood, $^3$H PGF$_{2\alpha}$ was identified in the ovarian venous effluent of both ovaries (Kotwica et al., 1982). These authors interpreted these results to suggest that PGF$_{2\alpha}$ may travel either via the interstitial fluid or lymph. In view of the less intimate association
between the ovarian artery and uterine vein in the pig and the more obvious systemic luteolytic effects previously discussed, the physiological relevance to luteolysis of either of these reports is not clear (Kotwica et al., 1982; Krzymowski et al., 1987).

However, the notion that the lymphatics may play a role in the pathway of unilateral luteal regression (Inskeep and Butcher, 1966) has gained increasing support in ewes and has formed the basis of a number of recent studies. The complex nature of the lymphatic drainage surrounding the ovary of the ewe was first described in detail by Morris and Sass (1966) and that of the uterus by Hoggan and Hoggan (1881). These and several other studies have failed to find any direct lymphatic connections between the uterus and the ovary (Lindner et al., 1964; Cicmanec, 1972; Meckley and Ginther, 1969; Fabian, 1981; Staples et al., 1982). In view of the frequent occurrence of lymphatic valves (Meckley and Ginther, 1969; Staples et al., 1982; Abdel Rahim and Bland, 1985) it is unlikely that any back flow occurs to the ovary and therefore mixing of lymph is restricted to the basal portions of the ovarian pedicle only (Staples et al., 1982). However, several investigations have described close associations between uterine lymphatics and the ovarian artery (Cicmanec, 1972; Staples et al., 1982) and more detailed examination of this association confirmed it to be quite intimate (Abdel Rahim and Bland, 1985). Since lymph has previously been shown to contain and drain considerable quantities of steroids from the ovaries in the ewe (Lindner et al., 1964; Staples et al., 1982), it has been suggested that lymphatic vessels might also serve to transport high concentrations of PGF$_2\alpha$ (Staples et al., 1982). Indeed, Abdel Rahim et al. (1983, 1984b) demonstrated that ovine lymph contained PGF$_2\alpha$ in concentrations comparable to those in the uterine vein and that PGF$_2\alpha$ concentrations in lymph increased towards the later stages of the luteal phase.

Additional evidence exists to support a functional role for uterine lymphatics in the events leading to luteolysis. Stacy and Gemmell (1976a,b) demonstrated that luteolysis, as indicated by decreased systemic progesterone concentrations and luteal histology, could be induced by local lavage of the ovarian vasculature with PGF$_2\alpha$ at a rate of 1 to 40µg/hr for 6 hours. Infusion of PGF$_2\alpha$ into the uterine lymphatics also induces luteolysis (Whylie et al., 1984; Heap et al., 1985) as does infusion into the uterine vein or uterine lumen (Land et
al., 1976; Heap et al., 1985). In the study by Heap et al. (1985), tritiated PGF$_{2\alpha}$ was infused into both a uterine lymphatic and a uterine vein and $^3$H PGF$_{2\alpha}$ was quantified in the ovary and CL one hour later. Higher ovarian and luteal $^3$H PGF$_{2\alpha}$ accumulation occurred following lymphatic infusion as compared to uterine venous infusion which suggested a higher rate of transfer of $^3$H PGF$_{2\alpha}$ from the lymphatics.

Further evidence supporting an essential role of the uterine lymphatics in the events leading to luteolysis was provided by the experiments of Abdel Rahim et al. (1984a). These researchers suggested that the extremely close anatomical relationship between the uterine lymphatics and the uterine venous drainage previously described precluded completely obliterating lymphatic flow without complete severance of the veins themselves together with the mesometrium. Therefore, a glass cannula was placed into the lumen of the uterine vein and secured at each end. Between these ligatures, over the surface of the glass cannula, a segment of the uterine vein was completely removed and the attached broad ligament was completely severed. In this way, interaction between the uterine vein and the ovarian artery at their usual points of association was supposedly undisturbed but participation of the lymphatics was prevented. Surgery was performed in these ewes in the first week of their estrous cycle and animals were subsequently sacrificed at 18 to 24 days following that estrus. Systemic plasma progesterone concentration and visual appraisal of CL indicated that CL were maintained in all cannulated ewes but regressed in all sham-operated controls (Abdel Rahim et al., 1984a). These and the other previously discussed data indicate that in the ewe, lymphatic drainage associated closely with the ovarian vasculature may provide an essential link between uterine luteolytic (and possibly luteotrophic) influences and the ovary which has been described as counter-current transfer.
Factors Involved in the Stimulation of Uterine Prostaglandin F₂α Release

Effects of ovarian steroids

As numerous as are studies investigating counter-current transfer of PGF₂α, until recently much less attention has been given to the mechanisms controlling its release from the uterus. For instance, several different kinds of stimuli can induce uterine PGF₂α release (Roberts et al., 1975) in the ewe but the stimulus for the release which induces normal luteal regression in domestic animals is unknown. Among the hormonal factors which have been implicated in modifying PGF₂α release are steroid hormones (Blackwell and Flower, 1975) such as estrogen and progesterone and also the peptide hormone oxytocin (McCracken, 1981). These agents have different effects depending on species and the reproductive stage of the animal at the time of exposure. The following discussion will summarize what is known of the effects of each of these hormones in the ewe and the pig where data are available.

Woody et al. (1967a,b) and Woody and Ginther (1967) investigated the effect of progesterone administered early in the luteal phase of ewes, cows, guinea pigs and gilts on subsequent estrous cycle length of these species. Progesterone was administered during the first 6 (ewes and guinea pigs) or 10 (cows and gilts) days of the estrous cycle at twice the levels previously shown by other authors to inhibition ovulation in these species. A significant shortening of the estrous cycle was observed in ewes (3.8 days), cows (4.0 days) and guinea pigs (1.3 days) but not in gilts. Ginther (1968b, 1969) confirmed and extended these observations in both sheep and guinea pigs. In particular, it was shown that the most important treatment period included the first 3 days of the cycle because estrous cycles of ewes treated with progesterone on days 4 to 7 were not greatly reduced in comparison with controls. The lack of effect of progesterone on estrous cycle length of gilts is in accord with previously discussed data also concerned with the effects of this hormone on the hypothalamo-pituitary axis. It appears that neither a reduction of pituitary luteotrophic support or progesterone priming of the uterus affects cycle length of pigs.
More recent studies in ewes demonstrated that a single injection of 40 mg of progesterone on day 2 significantly shortened the estrous cycle as a result of the appearance of earlier pulse of PGF$_{2\alpha}$ in the utero-ovarian vein (Ottobre et al., 1980). Studies by Louis et al. (1977) demonstrated that progesterone administered for 9 days to ovariectomized ewes increased the caruncular PGF$_{2\alpha}$ concentration and release in vitro from tissues collected on day 10. Increased jugular venous concentrations of the prostaglandin metabolite, 13,14-dihydro, 15-keto prostaglandin F, (PGFM), were also observed in progesterone primed ewes on day 10 (Louis et al., 1977). Together, these data suggest that progesterone enhances the synthesis and subsequent release of uterine PGF$_{2\alpha}$ in the ewe which brings about early regression of the CL.

In contrast to the shortening of the luteal phase induced by progesterone administration to ewes during the first few days of the estrous cycle, estrogen has a more rapid effect on cycle length. Stormshak et al. (1969) demonstrated that estrogen administered to mid-luteal phase ewes caused luteal regression as indicated by luteal weight and luteal progesterone concentration measured two days following injection. However, estrogen administered once or twice prior to day 7 did not induce luteolysis (Hawk and Bolt, 1970). Since the luteolytic effect is prevented by hysterectomy (Stormshak et al., 1969), it is presumed to be mediated by the uterus. A local effect of the uterus on the CL of estrogen-treated sheep was demonstrated by Akbar et al. (1971) who administered estrogen on days 11 and 12 to unilaterally hysterectomized ewes. On day 14 of the estrous cycle, those ewes which had ovulated bilaterally were found to have heavier CL on the side contralateral to the remaining horn suggesting a local luteolytic influence of the remaining uterine horn. Overall, CL in control ewes were heavier than CL on either ovary of treated ewes. The results of these studies are consistent with the release of PGF$_{2\alpha}$ from the uterus in response to estrogen and several authors have demonstrated this to be the case under a variety of experimental conditions (Barcikowski et al., 1974; Ford et al., 1975; Louis et al., 1977; Scaramuzzi et al., 1977). Consistent with these data also is the observation that indomethacin blocks estrogen-induced luteolysis in ewes (Barcikowski et al., 1974; Lewis and Warren, 1977b).

The results of several studies provide both direct and indirect evidence to suggest that a period of progesterone priming is necessary to demonstrate the
luteolytic effect of estrogen. Barcikowski et al. (1974) showed that very low levels of estrogen, 1 ng/min, infused into the ovarian artery of ewes bearing ovarian autotransplants failed to induce prostaglandin release on day 6 or 10 of the estrous cycle whereas prostaglandin release was stimulated by estrogen infusion on day 14. Similarly, Warren et al. (1973) demonstrated that luteal regression could be induced by estrogen as early as day 5 or 6 of the cycle following progesterone priming on days 1 to 4. Ford et al. (1975) found an increase in uterine venous and endometrial PGF$_{2\alpha}$ concentrations one day after exogenous estrogen administration to ewes on days 9 or 10 but not after administration on days 4 or 5. Louis et al. (1977) reported similar findings with regard to PGF$_{2\alpha}$ release from endometrial cultures collected from ovariectomized ewes treated with lower more physiological levels of estrogen and progesterone. It was demonstrated that estrogen dramatically increased PGF$_{2\alpha}$ release from caruncular sites in progesterone primed ewes (Louis et al., 1977).

A further complexity of interaction between estrogen and progesterone was suggested by the studies of Scaramuzzi et al. (1977). These researchers also used ovariectomized ewes, in which the uteri had been transplanted to the neck, to demonstrate the important priming effect of progesterone on eliciting a maximal PGF$_{2\alpha}$ release in uterine venous blood following estrogen treatment. While estrogen treatment increased PGF$_{2\alpha}$ release following progesterone priming, continuous progesterone administration, encompassing the time of estrogen challenge, prevented PGF$_{2\alpha}$ release. It was suggested that even though a period of progesterone priming was necessary to induce a maximal PGF$_{2\alpha}$ response, withdrawal of progesterone for one or two days prior to estrogen administration of exposure was essential for PGF$_{2\alpha}$ release (Scaramuzzi et al., 1977). Similar results were also reported more recently by Vincent and Inskeep (1986) who studied the effects of estrogen and progesterone administration and withdrawal on uterine PGF$_{2\alpha}$ release in ovariectomized ewes.

Because these above mentioned events somewhat resemble the endocrine events occurring around the time of luteal regression and the onset of estrus it has been suggested that increasing follicular estrogen initiates uterine PGF$_{2\alpha}$ release and luteolysis during the normal estrous cycle of ewes (Cox et al., 1972, 1974; Horton and Poyser, 1976). In support of this concept Barcikowski et al.
(1974) noted that although the smaller peaks of PGF$_{2\alpha}$ observed prior to day 13 did not coincide with elevations in estrogen concentrations, after day 13 peaks in estrogen in ovarian venous plasma were temporally associated with peaks of PGF$_{2\alpha}$.

It has been further suggested that since destruction of ovarian follicles by x-irradiation (Karsch et al., 1970) or cauterization (Ginther, 1971) slowed the rate of luteal regression in ewes and reduced the LH requirement for luteal maintenance (Karsch et al., 1970), that estrogen may be important in the responsiveness of the CL to PGF$_{2\alpha}$. Additional evidence in support of this hypothesis was presented by Hixon et al. (1975) using intact ewes and Gengenbach et al. (1977) using hysterectomized ewes. These studies demonstrated that otherwise subluteolytic doses of PGF$_{2\alpha}$ administered to ewes whose ovarian follicular populations had been destroyed by prior X-irradiation, were luteolytic when given in conjunction with exogenous estradiol benzoate. These data are consistent with the possibility of an interaction between PGF$_{2\alpha}$ and estrogen in the final demise of the CL. However, there has been a general failure in finding any similar association between the initial peaks of PGF$_{2\alpha}$ and increased estrogen concentrations at earlier stages of luteal demise and therefore the role of increased follicular estrogen secretion in causing the onset of luteolysis has been disputed (Baird et al., 1976a; Ottobre et al., 1980, 1984).

In the ewe an alternative hypothesis to that discussed above concerns the previously mentioned ability of progesterone to prime the uterus for prostaglandin production and release (Louis et al., 1977; Ottobre et al., 1980; Alwachi et al., 1981). In a series of experiments conducted on ewes between days 9 to 14 and 14 to 17 in nonpregnant ewes and between days 12 to 17 in pregnant and nonpregnant ewes, Ottobre et al. (1984) examined the effect of progesterone, estrogen or both on PGF$_{2\alpha}$ concentrations in utero-ovarian venous plasma. Rises in PGF$_{2\alpha}$ were noted on days 13 and 14 in ewes which had been ovariectomized on day 9 of the estrous cycle whether or not supplemental progesterone or estrogen was administered at surgery and on subsequent days. Estrogen did not elevate PGF$_{2\alpha}$ concentrations in ewes ovariectomized on day 14, however, pulses of PGF$_{2\alpha}$ may have been already occurring at this time (Ottobre et al., 1984). These authors concluded that progesterone priming in the 9 days prior to the beginning of the study "programmed" uterine PGF$_{2\alpha}$ release
which occurred regardless of the presence or absence of steroid hormones (Ottobre et al., 1984). However, the apparent lack of a requirement for estrogen in the events leading to uterine PGF$_{2\alpha}$ release does not preclude a role for estrogens of follicular origin in luteolysis at the level of the ovary as previously discussed (Ottobre et al., 1984; Vincent and Inskeep, 1986).

The role of progesterone priming in the release of uterine PGF$_{2\alpha}$ in pigs is less clear. As previously stated, progesterone administration has no effect on the estrous cycle length of gilts (Woody et al., 1967b), however, whether or not progesterone priming has a similar effect on uterine release of PGF$_{2\alpha}$ is not known. Because administration of PGF$_{2\alpha}$ itself does not cause significant shortening of the cycle in gilts if administered prior to days 10 to 12 (Diehl and Day, 1974), it is possible that progesterone priming might induce early PGF$_{2\alpha}$ release without affecting cycle length. Therefore, the apparent difference in the response of the ewe and the gilt to exogenous progesterone administered early in the luteal phase may not be due to differences in the response of the uterus but to differences in luteal response to PGF$_{2\alpha}$.

**Role of oxytocin**

More recently, attention has turned to the possible role of oxytocin in the control of uterine prostaglandin release. The earliest observations on the possible effects of oxytocin on luteal function were that oxytocin administration on days 3 to 6 of the estrous cycle shortened luteal lifespan of cows (Armstrong and Hansel, 1959). Oxytocin has not been shown to affect the cycle length of ewes (Wathes, 1984) although Milne (1963) observed histologically recognizable signs of degeneration following oxytocin administration to ewes during the first 6 days of the estrous cycle. Oxytocin stimulates PGF$_{2\alpha}$ release from the uterus of estrogen-primed (Sharma and Fitzpatrick, 1974), early or late luteal phase ewes (Roberts et al., 1975, 1976; Roberts and McCracken, 1976). In anestrous ewes, Sharma and Fitzpatrick (1974) demonstrated that PGF$_{2\alpha}$ concentrations in the anterior vena cava were increased 3-fold by estrogen treatment and 10-fold when oxytocin was given to estrogen-primed ewes. Oxytocin has no effect of PGF$_{2\alpha}$ concentrations in the anterior vena cava without estrogen priming (Sharma and Fitzpatrick, 1974). In vitro, oxytocin stimulated PGF$_{2\alpha}$ release from
ovine endometrium in a dose dependent manner and the amount released increased the later in the cycle the tissue was collected with peak release observed from tissue taken from estrous ewes (Roberts et al., 1976). Both ovine myometrium and endometrium possess high (Roberts et al., 1976) and low affinity binding sites for oxytocin (Pliska et al., 1986) which are present in highest concentrations at estrus (Sheldrick and Flint, 1985). McCracken et al. (1984a) summarized data on the influence of steroid hormones on oxytocin receptor levels in ovine endometrium. Estrogen was shown to increase oxytocin receptor concentrations but not in the presence of high concentrations of progesterone. Upon progesterone withdrawal oxytocin receptor concentrations in caruncular endometrium increased markedly in a 6 to 12 hour period (McCracken et al., 1984a; Hixon and Flint, 1987). It was proposed, based on the work of others, that progesterone suppressed nuclear estrogen receptors and that progesterone withdrawal was first followed by increases in nuclear estrogen receptors, then oxytocin receptors (McCracken et al., 1984a).

That oxytocin may play a role in the events of luteolysis in ewes under physiological conditions is suggested by the demonstration that immunization both actively (Sheldrick et al., 1980) and passively (Schams et al., 1983) extends the luteal phase of ewes. Measurements of PGFM in the systemic plasma of ewes, and of the oxytocin peptide cleavage unit neurophysin simultaneously released with oxytocin itself, suggest that oxytocin and PGF₂α secretion occur coincidentally (Fairclough et al., 1980; Flint and Sheldrick, 1983). It was subsequently suggested, on the basis of bio-, immuno- and chemical assays that oxytocin may arise from the CL itself (Wathes and Swann, 1982; Flint and Sheldrick, 1983). Oxytocin concentrations in systemic plasma of ewes closely follow those of progesterone, declining shortly before progesterone at the end of the luteal phase (Webb et al., 1981; Flint and Sheldrick, 1983; Sheldrick and Flint, 1983b). Evidence that the source of circulating oxytocin throughout the estrous cycle of ewes is primarily of ovarian origin includes positive arteriovenous oxytocin concentrations differences across the ovary (Flint and Sheldrick, 1982), the increase in ovarian venous but not jugular venous oxytocin concentrations following prostaglandin administration (Flint and Sheldrick, 1982, 1983) and the observation that oxytocin concentrations decline markedly in systemic plasma following ovariectomy (Schams et al., 1982). Large luteal cells
of the ovine CL appears to contain oxytocin (Rodgers et al., 1983a; Fields and Fields, 1986; Fehr et al., 1987) stored inside secretory granules (Rice and Thorburn, 1984) in higher concentrations than other luteal cell types. Highest rates of oxytocin secretion by dispersed ovine luteal cells was found from cells collected from day 6 CL (Harrison et al., 1987). Synthesis of oxytocin in the bovine CL is initiated at estrus as measured by levels of mRNA which reach a peak within the first few days then decline to low or undetectable levels at the time of luteolysis and throughout pregnancy (Ivell et al., 1985; Jones and Flint, 1988). The low levels of mRNA for oxytocin in bovine CL suggest that once secreted, levels may not be able to be regenerated (Ivell et al., 1985; Jones and Flint, 1988). Oxytocin concentrations in jugular venous plasma and luteal tissue from pregnant ewes decline to day 15, recover over days 15 to 17 then decline again progressively thereafter to a nondetectable level (Sheldrick and Flint, 1983b). Similarly, in ewes following hysterectomy on day 6, nondetectable luteal oxytocin concentrations were found on days 28, 53 and 56 in 3 ewes and concentrations of oxytocin in jugular venous plasma were nondetectable by day 53 and nonstimulatable with cloprostenol by day 48 in two others (Sheldrick and Flint, 1983a). These changes in luteal oxytocin synthesis and secretion by the CL in pregnant and hysterectomized ewes are not due to a loss of large luteal cells (Sheldrick and Flint, 1984). These data confirm that the ability of the ovine CL to synthesize and secrete oxytocin is restricted to the early to mid-phases of the estrous cycle only.

The temporal relationship between PGFM and neurophysins in jugular venous blood of ewes found at the time of luteolysis (Fairclough et al., 1980) and the observation that each can stimulate release of the other (Sharma and Fitzpatrick, 1974; Flint and Sheldrick, 1982) suggested that pulses of oxytocin and PGF\(_{2\alpha}\) may be caused by one another in a positive feedback loop (McCracken et al., 1984a; Lamsa et al., 1988). The increased potency of ultra-low pulses of PGF\(_{2\alpha}\) infused into the ovarian artery during a 24 hour period emphasizes the possible physiological importance of pulsatile release of uterine prostaglandins (Schramm et al., 1983). Sheldrick and Flint (1986) demonstrated that in ovariectomized, steroid primed ewes, oxytocin induced increased PGFM levels in systemic plasma initially but that second and subsequent oxytocin challenges at 1, 2, 4 or 6 hours later resulted in increases in PGFM of only 23, 23, 54 and 62%
respectively. Whether a more prolonged period would have restored the PGFM response completely, and how long this may have taken was not investigated. Hooper et al. (1986a) measured oxytocin, PGF$_{2\alpha}$ and progesterone in utero-ovarian venous plasma of ewes between days 13 to 16 of the estrous cycle. Pulses of PGF$_{2\alpha}$ were associated with oxytocin pulses 96.5% of the time but oxytocin pulses were associated with PGF$_{2\alpha}$ pulses on only 55.6% of the occasions when oxytocin pulses were observed (Hooper et al., 1986a). Lamsa et al. (1988) reported that continuous PGF$_{2\alpha}$ infusion (25 pg/min) for 2 hours into the ovarian artery of an autotransplanted ovary in ewes resulted in only a transient rise in luteal oxytocin secretion which was not due to depletion of luteal oxytocin stores. A refractory period of 6 hours followed in which oxytocin secretion was not able to be stimulated by PGF$_{2\alpha}$ administration. These data may explain the relatively lower correspondence between oxytocin pulses following PGF$_{2\alpha}$ pulses in the uteroovarian venous plasma of ewes late in the luteal phase.

In addition to the observations made in cyclic ewes, no difference was found in the frequency of oxytocin pulses in pregnant versus nonpregnant ewes and, in pregnant ewes, 86.4% of oxytocin pulses occurred in the absence of a PGF$_{2\alpha}$ pulse (Hooper et al., 1986a). PGF$_{2\alpha}$ pulses were fewer and of lower amplitude in pregnant than in nonpregnant ewes but were almost always coincident with an oxytocin pulse. Therefore it was concluded that reduced PGF$_{2\alpha}$ output in pregnant versus nonpregnant ewes was not a result of lack of oxytocin stimulation (Hooper et al., 1986a).

The cellular mechanisms of action by which oxytocin induces endometrial PGF$_{2\alpha}$ release and PGF$_{2\alpha}$ induces luteal oxytocin release have been investigated. Flint et al. (1986) demonstrated that oxytocin stimulated ovine endometrial phosphoinositide hydrolysis in a dose and time dependent manner and it was suggested that oxytocin stimulated endometrial prostaglandin synthesis by increasing release of arachidonic acid from diacylglycerol. In ovine luteal slices, phospholipase A$_2$, phospholipase C and arachidonic acid stimulated oxytocin secretion (Hirst et al., 1988). Oxytocin secretion was not stimulated by PGF$_{2\alpha}$ or PGE$_2$ nor was arachidonic acid-induced oxytocin release affected by several prostaglandin synthesis inhibitors (Hirst et al., 1988). It was reported by Luck (1988) that progesterone and oxytocin secretion from bovine granulosa cells
were independent of one another and that progesterone was not able to influence oxytocin secretion. Therefore, the data of Hirst et al. (1988) suggest that the effect of PGF$_{2\alpha}$ in inducing the release of oxytocin is indirect but it probably does not result from secondary effects on progesterone secretion. An inhibitor of 5-lipoxygenase prevented arachidonic acid-induced, and reduced phospholipase A$_2$-stimulated, oxytocin secretion suggesting that the lipoxygenase pathway may be involved in these events (Hirst et al., 1988). Other studies have confirmed that in vitro, oxytocin induces increases in phosphoinositide turnover in ovine caruncular endometrium and that exogenous estrogen administration in vivo increases this in vitro effect (Hixon and Flint, 1987).

In summary, the above mentioned studies demonstrate an association between luteal oxytocin secretion and uterine PGF$_{2\alpha}$ release, particularly evident after day 13 of the ovine estrous cycle. It seems plausible that ovarian oxytocin release may therefore contribute to the pulsatile release of uterine PGF$_{2\alpha}$ which culminates in the final demise of the CL. Perhaps the most impressive evidence in favor of this hypothesis is the demonstrated effectiveness of immunization against oxytocin to extend the luteal phase. However, no confirmation has yet been provided that these immunization procedures do in fact block the effects, or the biological activity, of oxytocin. Sheldrick and Flint (1987) presented evidence to suggest that overstimulation with oxytocin lead to down-regulation, lack of response to exogenous oxytocin and increased estrous cycle length. An increase in hormonal half-life following immunization against gonadotrophins was demonstrated by Cole (1973). Although the possibility of a similar effect of immunization against the much smaller peptide oxytocin seems remote, it remains to be proven definitively whether oxytocin plays an important physiological role in normal luteal regression. Finally, the evidence seems to suggest that if involved in luteolysis at all in the ewe, oxytocin probably is not involved in the initiation of luteolysis which occurs as early as day 12.

Oxytocin has also been identified in porcine CL both immunologically (Pitzel et al., 1984) as well as by detection of oxytocin mRNAs by hybridization techniques (Einspanier et al., 1986). A physiological role for oxytocin in luteal function in pigs has not yet been defined however, although in vitro studies with dispersed porcine luteal cells suggest that oxytocin inhibits both basal and LH-
stimulated progesterone secretion (Przala et al., 1986; Pitzel et al., 1988). No studies have reported in vivo levels of oxytocin in luteal tissue or plasma or in vivo effects of oxytocin administration on luteal function in this species.

Factors Involved in Luteal Maintenance

**Luteotrophic effects of estrogen in pigs**

While the uterine response to exogenous progesterone may or may not differ between the sheep and the pig the uterine response to estrogen in terms of PGF$_{2\alpha}$ release appears to differ radically. In the pig estrogen has been recognized as a luteotrophin since the first report of Kidder et al. (1955) that estrogen administration extended luteal function. Gardner et al. (1963) defined the period of sensitivity to estrogen to be between day 11 and 14 of the estrous cycle. Geisert et al. (1987) confirmed and extended these findings to suggest that treatment with 5 mg intramuscularly on day 11 and again between day 14 to 16 resulted in luteal maintenance for over 60 days. A single injection of estrogen on any day between 9.5 and 15.5 extended luteal function for only 30 days. It is generally believed that at least part of this luteotrophic effect is attributable to a reduction in the uterine release of PGF$_{2\alpha}$ (Bazer et al., 1986).

In vivo and in vitro results support an antiluteolytic role for estrogen in the pig. Frank et al. (1977) demonstrated a decrease in UOV PGF$_{2\alpha}$ concentrations following estrogen-induced luteal maintenance an observation later confirmed by Ford et al. (1982b). In a subsequent study by Frank et al. (1978) estrogen-treated gilts were shown to have elevated concentrations of PGF$_{2\alpha}$ in luminal flushings. Other studies have also demonstrated lower levels of PGFM in peripheral plasma of estrogen-treated versus control gilts during days 11 to 17 of the estrous cycle (Guthrie and Rexroad, 1981; Flowers et al., 1987). Additional in vitro studies suggest that endometrial tissues collected from pigs following estrogen treatment produce less PGF$_{2\alpha}$ than controls (Guthrie and Rexroad, 1981; Patek and Watson, 1983). This latter result seems somewhat to contradict the results obtained by Frank et al. (1978) concerning elevated luminal PGF$_{2\alpha}$ concentrations. However, Bazer and Thatcher (1977) suggested that estrogen may induce luteal maintenance by redirecting the secretion of PGF$_{2\alpha}$ from
secretion into the uterine vein to secretion directed toward the uterine lumen. Marengo et al. (1986) reported reduced transport of PGF$_{2\alpha}$ from the uterine lumen to the venous drainage in estrogen-treated gilts. Therefore, reduced endometrial synthetic rates might still allow an increase in luminal PGF$_{2\alpha}$ concentrations by virtue of a decrease in rate of uterine luminal transport.

Although Geisert et al. (1987) attempted to better define the estrogen sensitive period and the minimum requirement for administration of 5 mg levels of estradiol by the intramuscular route with regard to the expected period of luteal maintenance, no studies have yet addressed the minimally effective dose of estrogen administered by any route which is required to extend luteal function in pigs. Such considerations may be important in view of the possible involvement of the hypothalamo-pituitary axis, particularly since, as discussed previously, gonadotrophic support may overcome and prevent induced luteolysis in the ewe (Bolt, 1979). It was demonstrated in hypophysectomized pigs that estrogen only maintained luteal function in the continued presence of gonadotrophic hormones (Anderson et al., 1967). Indeed, Rothchild (1966) suggested that estrogen-induced prolactin release may be involved in luteal maintenance in gilts following estrogen administration. Flowers et al. (1987) demonstrated lowered LH concentration from 1.5 ng/ml to 0.5 ng/ml in estrogen treated gilts, but prolactin levels were not measured. To date, no studies have been able to eliminate possible effects on the hypothalamo-pituitary axis which may in part mediate the luteotrophic effects of estrogen in swine.

Another example of the importance of using minimally effective doses of estrogen when interpreting data related to luteal maintenance in swine is provided by the study by Saunders et al. (1983). These authors infused 100 µg of estradiol into the uterus or administered the same dose subcutaneously to cyclic gilts. A similar response, an extension of luteal function for 4 to 5 days by either route, was interpreted to indicate a lack of uterine sensitivity thereby suggesting that the uterus was not the primary target organ (Saunders et al., 1983). However Ford et al. (1982b) were able to maintain luteal function bilaterally in gilts with the unilateral infusion of 1.5µg/day into a surgically isolated uterine horn from day 11 to 16 probably through the observed bilateral reduction in UOV PGF$_{2\alpha}$ concentrations. Therefore, it is likely that the levels of estrogen used by Saunders et al. (1983) were well above those minimally required to induce
maintenance and may have lead to erroneous conclusions. The evidence remains strong that estrogen exerts its effect at least partly through an effect on the uterus.

Unambiguous evidence for a direct effect of estrogen on the ovary in preventing the luteolytic effect of PGF$_{2\alpha}$ on the CL is lacking. It was reported by Kraeling et al. (1975b) that estrogen administration to hysterectomized gilts prevented regression of CL in response to a luteolytic dose of PGF$_{2\alpha}$. Unfortunately no details were provided on how luteal maintenance was actually assessed. Flowers et al. (1987) showed that PGFM levels which were associated with luteolysis in cyclic gilts were present also in gilts whose CL were maintained by zearalenone, suggesting that the CL were made resistant to PGF$_{2\alpha}$ action. However PGFM concentrations were lower in estrogen treated gilts (Flowers et al., 1987). In the previously mentioned study by Ford et al. (1982b) UOV plasma progesterone concentrations were higher on the side ipsilateral to the estrogen infusion. Unfortunately these studies do not rule out an interaction between estrogen and the uterus, perhaps the production of another luteotrophin. Therefore, studies so far have not been able to eliminate an effect of estrogen at either the uterus or the pituitary in the same experimental paradigm, and the possible direct luteotrophic effects of estrogen on the CL remain unproven.

Influence of pregnancy on luteal maintenance

Circumstantial evidence indicating a possible direct luteal effect of an embryonic luteotrophin which may include estrogen, is provided by the observed high incidence of unilateral luteal maintenance (Anderson et al., 1966; Niswender et al., 1970; Christenson and Day, 1971) and elevated progesterone concentrations (Ford and Christenson, 1979) of CL adjacent to the gravid uterine horn in unilaterally pregnant pigs. Ball and Day (1982a) demonstrated that uterine infusions of embryonic homogenates maintained CL bilaterally and Ford et al. (1982a,c) observed increased luteal blood flow in gilts on day 13 after mating suggesting a direct effect of the establishment pregnancy on the ovarian vasculature. More direct evidence in the pig of embryonic factors preventing the luteolytic effect of PGF$_{2\alpha}$ at the level of the CL is provided by Ball and Day (1982b). In that study luteal implants containing both embryonic extracts and
PGF$_{2\alpha}$ were prepared and placed into individual CL on day 30 to 35 of pregnancy. Implants with both embryonic extract and PGF$_{2\alpha}$ were heavier and contained more progesterone that those containing PGF$_{2\alpha}$ alone suggesting that embryonic factors prevented the luteolytic response directly (Ball and Day, 1982b). Furthermore, charcoal extraction of embryonic homogenates removed the luteotrophic principle(s) indicating that it was of low molecular weight, possibly a steroid. However the nature of the luteotrophic component(s) remains unknown.

The obvious physiological significance of the ability of estrogen to decrease the luteolytic signal lies in the observation that the porcine embryo produces estrogen (Perry et al., 1973; 1976) in proportion to its developmental state (Ford et al., 1982a; Geisert et al., 1982a). Therefore it has been suggested that estrogen is the embryonic signal responsible for the maintenance of luteal function enabling the establishment of pregnancy in pigs (Perry et al., 1976; Bazer and Thatcher, 1977). In view of the data discussed previously concerning the ability of estrogen to decrease PGF$_{2\alpha}$ release from the uterus in pigs, comparisons of uterine venous PGF$_{2\alpha}$ and systemic PGFM levels have been made between pregnant and nonpregnant gilts. Relatively few studies have been performed however and despite conclusions drawn by the authors, the data are not conclusive. Hunter and Poyser (1982) collected samples on various days postestrus from the uterine vein of both pregnant and nonpregnant pigs, while animals were under anesthesia. Pregnant pigs, one each day, were anesthetized on days 7 through 19. Nonpregnant pigs, a total of 32 animals, were anesthetised on days 7 through 19. On each day, several consecutive samples were taken at 15 minute intervals from pregnant pigs by indwelling uterine venous catheters. These data did not demonstrate marked differences in uterine venous PGF$_{2\alpha}$ concentrations between pregnant and nonpregnant gilts until days 17 to 19 when a clear downward trend in luteal progesterone secretion in the nonpregnant group was evident (Hunter and Poyser, 1982). Similarly Killian et al. (1976) reported higher PGF$_{2\alpha}$ concentrations in the utero-ovarian vein of nonpregnant versus pregnant gilts on day 16 but similar levels on day 10. Data between these times were not reported except to comment that there was considerable variation observed. Interesting, however, was the observation that PGF$_{2\alpha}$ and progesterone concentrations were negatively correlated in non-
pregnant animals \( r = -0.96 \) but not in pregnant gilts \( r = 0.02 \); Killian et al., 1976) suggesting that during pregnancy any elevations in PGF\(_{2\alpha}\) did not result in reduced luteal function or vica versa. Moeljono et al. (1977) reported PGF\(_{2\alpha}\) concentrations in UOV plasma of mated \( n = 5 \) and nonmated gilts \( n = 6 \) from day 12 of the cycle to the next estrus or day 25 of pregnancy. Samples were taken every 15 minutes during two 3 hour intervals each day. Again, data were highly variable (more so in nonpregnant group) but an overall increase in PGF\(_{2\alpha}\) concentrations was reported in nonpregnant compared to pregnant gilts. Data presented indicated that PGF\(_{2\alpha}\) concentrations in the utero-ovarian vein of pregnant and nonpregnant gilts were similar on day 12 but were increased in nonpregnant gilts on day 13 and thereafter.

Other studies have reported peripheral levels of the more stable (Martin and Liptrap, 1981) PGF\(_{2\alpha}\) metabolite (PGFM) in pregnant vs nonpregnant pigs. Guthrie and Rexroad (1981) found PGFM levels to be similar between pregnant and nonpregnant gilts on day 12 but significant increases in nonpregnant over pregnant animals on days 13 to 17 of the cycle or pregnancy. Data was collected from day 1 throughout the estrous cycle of 3 nonmated gilts and from day 1 to 30 of pregnancy in 3 mated gilts by Shille et al. (1979). Spikes of PGFM were observed from day 11 in nonpregnant gilts and continued for 6 days. In pregnant gilts an increase in peripheral PGFM was also noted on days 11 to 13 in the form of pulses which appeared to be as high (250 to 700 pg/ml) and even more sustained (24 to 36 hours) than were pulses in nonpregnant gilts but then levels declined and remained low throughout the rest of the sampling period. In vitro studies of PGF\(_{2\alpha}\) release from porcine endometrium in pregnant versus nonpregnant pigs have investigated later stages (day 16) of the estrous cycle and provide no data relevant to earlier events occurring at the initiation of luteolysis as discussed (Guthrie and Rexroad, 1981; Patek and Watson, 1983; Guthrie and Lewis, 1986).

In summarizing these data it is difficult to be definitive about the time at which reported events actually occur in relation to the variability inherent in the porcine estrous cycle. For instance Gleeson (1974) measured PGF\(_{2\alpha}\) in UOV plasma of 3 sows fitted with chronic UOV catheters which were sampled from days 8 to 11, 12 to 15 and 13 to 18, respectively. The corresponding days on which elevations of PGF\(_{2\alpha}\) were detected were days 10 or 11, 13 or 14 and 14 to
Although these data are commonly accepted as good indications of the initial events involved in luteolysis little attention is given to the between animal variability of these data or the fact that none of these animals returned to estrus for an additional 14 days (Gleeson 1974). In fact few authors who have investigated the onset of PGF\(_2\alpha\) release from the uterus in nonpregnant pigs have related the data from each animal to the length of the estrous cycle in which the data was collected, making it virtually impossible to relate the results of one study to that of any other but for the relative changes within individual animals. Regardless of these limitations to interpretation, none of the above mentioned data are inconsistent with the possibility indicated most clearly by the data of Shille et al. (1979) that although uterine PGF\(_2\alpha\) release is reduced in pregnant pigs over most of the period when natural luteolysis would otherwise occur, an initial increase in release which normally signals the onset of luteolysis in nonpregnant pigs also occurs in pregnant animals. The reason that no sustained elevation occurs subsequent to this may be related to a suppression of uterine PGF\(_2\alpha\) release as suggested by Bazer et al. (1986) in later stages of the cycle.

Added emphasis is given to the importance of the distinction made here between uterine PGF\(_2\alpha\) release at the onset of luteolysis and at the time of final luteal demise by the observation that luteal maintenance induced by the presence of embryos in the uterus is achieved in the pig at about day 12 or 13 (Dhindsa and Dziuk, 1968; Ford et al., 1982a) and that luteolysis cannot be prevented if the embryonic influence is introduced after this time.

More extensive studies on the levels of systemic PGFM or PGF\(_2\alpha\) in uterine and UOV plasma have been performed in pregnant and nonpregnant ewes. Thorburn et al. (1973) concluded that UOV PGF\(_2\alpha\) concentrations were lower in pregnant versus nonpregnant ewes but only one third of the ewes mated were confirmed pregnant and of those many demonstrated peaks not unlike those in the nonmated controls. Barcikowski et al. (1974) reported small peaks of PGF\(_2\alpha\) on day 13 and 14 in a single pregnant ewe, but found the larger peaks of nonpregnant ewes on day 14 and 15 were absent. PGFM was used by Peterson et al. (1976) and Webb et al. (1981) to detect uterine PGF\(_2\alpha\) release in pregnant and nonpregnant ewes through the normal period of luteal regression. Measuring PGFM in samples taken from pregnant ewes late on day 12 or day 13, Peterson et
al. (1976) found an absence of large peaks normally seen 3 days prior to estrus. Webb et al. (1981) demonstrated more stable PGFM levels in 3 pregnant versus 3 nonpregnant ewes between days 6 and 17 of mating or from day 6 until next recorded estrus. These authors generally conclude from their data that PGF$_{2\alpha}$ output from the uterus is less in pregnant than in nonpregnant ewes. Their data generally support this notion with particular regard to the large peaks of PGF$_{2\alpha}$ release associated with the final stages of luteal demise but they generally do not allow an assessment of or at least are not at odds with the idea that earlier increases signaling the initiation of luteolysis occur in both pregnant and nonpregnant ewes.

McCracken et al. (1984a) also reported that the PGF$_{2\alpha}$ spikes associated with final luteal demise were absent in pregnant ewes but large spikes of PGF$_{2\alpha}$ in uterine venous plasma of pregnant ewes were seen on day 13 postmating as they were in nonpregnant ewes. More recently still, Hooper et al. (1986a) failed to observe spikes of PGF$_{2\alpha}$ in UOV plasma obtained between day 13 and 16 by chronic catheterization of mated ewes. No mention was made of PGF$_{2\alpha}$ concentration in the utero-ovarian vein of pregnant animals on days 10 to 12 when spikes of PGF$_{2\alpha}$ were first recognized in the utero-ovarian vein of nonpregnant controls (Hooper et al., 1986a).

Many more studies than those cited above have concluded that uterine PGF$_{2\alpha}$ release is similar between pregnant and nonpregnant ewes. These studies include reports on concentrations of PGF$_{2\alpha}$ in uterine or UOV plasma taken at various times ranging from day 11 to day 16 of the estrous cycle or postmating either at surgery (Pexton et al., 1975a,b, day 15; Lewis et al., 1977, days 11 to 16; Ellinwood et al. 1979a, days 13, 15 and 17) or by collection from a cannula the day following surgery (Lewis et al., 1978, day 15 collection) and continuing daily thereafter (Nett et al. 1976a, days 11 to 14 and days 14 to 17; Silvia et al., 1984a,b; days 10 to 14; Vincent and Inskeep, 1986, days 13 to 16).

Elevations of PGF$_{2\alpha}$ in uterine or UOV plasma of both pregnant and nonpregnant ewes correlates well with increased PGF$_{2\alpha}$ content in uterine flushings and increased endometrial content and synthesis of PGF$_{2\alpha}$ at comparable stages of the cycle (Lewis et al., 1977, 1978; Ellinwood et al., 1979a; Findlay et al., 1981, 1983). However, Nett et al. (1976a) suggested that the frequency of PGF$_{2\alpha}$ spikes differed with pregnancy status being much more
frequent in nonpregnant ewes. The report of an extensive study by Zarco et al. (1988a,b) in which PGFM concentrations were measured in systemic plasma every 2 hours from days 10 to 18 in 12 nonpregnant and 10 pregnant ewes, failed to detect any pulsatile release of uterine prostaglandin release in pregnant animals. Silvia et al. (1984a) collected UOV blood at 15 minute intervals for 2 hours twice daily from day 10 to 14 of the estrous cycle (n = 8) or pregnancy (n = 6) ewes. No differences were found between pregnant and nonpregnant ewes in PGF$_{2\alpha}$ concentrations or logarithmic variances during this period suggesting that pulsatility was not different between groups. These data are not necessarily inconsistent with those of Zarco et al. (1988b) who found the major episodes of pulsatile release occurring on days 14 and 15 probably indicating final demise of the CL in nonpregnant ewes. In summarizing the above data, whether or not complete luteolysis is associated with increased pulsatility of release of uterine PGF$_{2\alpha}$ in ewes which does not occur at the same time in pregnant ewes (Thatcher et al., 1986), the process which may involve initial rises in prostaglandin output, may occur regardless of the presence or absence of conceptus tissue. Interpretation of the data in this way, provides rationale for the existence of embryonic luteotrophins in sheep and pigs which would be required to negate or otherwise counteract the possibly damaging effects of PGF$_2$ at the level of the ovary in both species.

In the ewe studies have provided evidence supporting a direct effect of the embryo at the level of the ovary antagonizing the luteolytic effects of PGF$_{2\alpha}$. Mapleton et al. (1975) surgically isolated the gravid and nongravid uterine horns of ewes and then established vascular anastomoses between the uterine veins from each side. Uterine venous blood from the nongravid horn induced ipsilateral luteal regression which was prevented by anastomosis and mixing with uterine venous blood from the gravid horn. This suggests both a protective effect at the ovary and a factor inducing it which is present in uterine venous blood draining the gravid horn reaching the ovary by a vascular route (Mapleton et al. 1975, 1976). These data are consistent with the observation made by Moor and Rowson (1966b) that luteal maintenance in bilaterally ovulating ewes occurs only on the side ipsilateral to the gravid horn. Various other approaches demonstrating the local effect of the embryo on luteal function in ewes have been reviewed (Ginther, 1974, 1981).
Additionally, it has been shown by several authors that pregnant ewes are more resistant to the luteolytic effects of PGF$_{2\alpha}$ (Inskeep et al., 1975; Mapletoft et al., 1976; Pratt et al., 1977; Silvia and Niswender, 1984; Silvia et al., 1984b; Lacroix and Kann, 1986), and estrogen (Lacroix and Kann, 1986). Furthermore the data presented by Silvia and Niswender (1984) suggested that greater doses of PGF$_{2\alpha}$ were required to induce luteolysis in ewes with two embryos than were required in ewes with singleton pregnancies.

**Luteotrophic effects of prostaglandin E$_2$**

Due to the local manner in which the embryo can affect luteal function in both the sheep and the pig, it has been suggested that the luteotrophin would be expected to have similar properties to PGF$_{2\alpha}$ (Vincent and Inskeep, 1986). In the ewe PGE$_2$ has been shown to antagonize the luteolytic properties of PGF$_{2\alpha}$ both in vivo (Pratt et al., 1977; Henderson et al., 1977; Pratt et al., 1979; Ginther, 1981; Magness et al., 1981; Reynolds et al., 1981; Weems et al., 1985) and in vitro (Fitz et al., 1984a,b). In vivo, the luteoprotective effects of PGE$_2$ were demonstrated by intermittent (Pratt et al., 1977) or continuous intrauterine infusion (Pratt et al., 1979; Magness et al., 1981), simultaneous infusion with PGF$_{2\alpha}$ into the ovarian artery (Henderson et al., 1977) or into the ovarian pedicle (Reynolds et al., 1981). Although PGE$_2$ was generally found to be luteotrophic, luteal extension results were inconsistent in some studies (Pratt et al., 1979), of short duration (Pratt et al., 1977, 2 days) or even more transient lasting only hours (Henderson et al., 1977). Lacroix and Kann (1986) reported that PGF$_{2\alpha}$ was luteolytic when administered to indomethacin-treated ewes on days 10 or 11 of the estrous cycle but not when given to similarly indomethacin-treated ewes on days 19 or 20 of pregnancy. It was concluded that because both uterine PGE$_2$ and PGF$_{2\alpha}$ synthesis would be expected to be inhibited by indomethacin in pregnant ewes, the maintained resistance of the CL of pregnancy must be due to factors other than PGE$_2$ (Lacroix and Kann, 1986). Ginther (1981) reported the administration of various levels of PGE$_2$ along with 80 µg of PGF$_{2\alpha}$ into the perivascular space of the ovarian pedicle of ewes in the mid-luteal phase of the estrous cycle and luteal weights were examined 48 hours later. A biphasic response to PGE$_2$ in terms of maintained luteal weights was indicated such that
the most effective level of PGE_2 appeared to be 300 to 500 µg but higher levels tended to reduce luteal weights (Ginther, 1981). Collectively, the above mentioned data suggest that PGE_2 does not consistently extend luteal function although this may be dependent on the level of PGE_2 administered. More consistent inhibition of the luteolytic effects of concomitantly administered PGF_2 does not consistently extend luteal function although this may be dependent on the level of PGE_2 administered. More consistent inhibition of the luteolytic effects of concomitantly administered PGF_2α is obtained with PGE_2α. In support of a physiological role for PGE_2 in pregnancy maintenance, several studies have demonstrated increased PGE_2 concentrations in response to progesterone administration (Vincent et al., 1986) and in pregnant versus nonpregnant ewes in uterine or UOV plasma (days 10 to 14, Silvia et al., 1984a; days 13 to 16, Vincent and Inskeep, 1986) and in endometrium (Ellinwood et al., 1979a; Marcus, 1981; Lacroix and Kann, 1982).

Several studies have investigated the effect of PGE_2 on luteal function in swine. Sander et al. (1982) injected PGE_2 (0.008 mg/kg IM) into gilts either on day 10, 14 or 16 of the estrous cycle without effects on plasma progesterone or cycle length. In fact, a 10 fold higher dose administered on day 10 caused a transient decline in plasma progesterone which returned to pretreatment levels by 24 hours post-treatment. Schneider et al. (1983) administered various amounts of PGE_2 to gilts every 6 hours from day 12 to 22 of the cycle via intrauterine catheters. No consistent effect on cycle length was observed with even the highest level (400 µg every 4 hours). However, some animals appeared to experience some delay in the decline in systemic progesterone as indicated by higher progesterone concentrations on days 14 and 15 in the 200 and 300 µg groups (Schneider et al., 1983).

In contrast to the negative results obtained by others, Akinlosotu et al. (1986) maintained luteal function in gilts by administering 2400 µg of PGE_2/day by constant infusion from day 7 until day 23 or by pulse infusion of 400 µg of PGE_2 every 4 hours over the same time period. PGE_2 was infused in 10% ethanol-PBS solution and constant vehicle infusions had no effect on luteal function. Systemic progesterone concentrations measured twice daily remained at normal midluteal phase levels (25 to 30 ng/ml) after increasing in an apparently normal manner from day 7 to 12 of the cycle. However, progesterone declined precipitously immediately following cessation of infusion on days 24 and 25 in both groups. No information was provided on subsequent estrous cycles (Akinlosotu et al., 1986). The failure of previous attempts with PGE_2 to maintain
luteal function may be due to the later time of the start of the infusion period or the less rigorous infusion regime over those days.

Okrasa et al. (1985) infused 150 µg of PGE₂ into each uterine horn of gilts on days 12, 15 or 18 of the estrous cycle. Concentrations of both PGE₂ and PGF₂α were monitored in utero-ovarian plasma from 15 minutes before infusion then every 15 minutes for 2 hours and again at 3, 4, 5 and 6 hours post-infusion. Significant increases in both PGE₂ and PGF₂α were observed on all days but were higher and more persistent on day 15 than at either day 12 or 18. Furthermore, PGF₂α concentrations following PGE₂ infusions on day 15 remained elevated throughout the 6 hour sampling period at higher levels than PGE₂. These data might suggest that the transient decline in systemic progesterone noted by Sander et al. (1982) following PGE₂ administration on day 10, and the lack of effect of PGE₂ administered by Schneider et al. (1983) on days 12 to 22, may have been due to a stimulation of uterine PGF₂α release which was great enough to overcome any luteotrophic effects of PGE₂.

Few reports have measured PGE₂ concentrations in the reproductive tissues of pregnant versus nonpregnant pigs. Patek and Watson (1983) demonstrated that arachidonic acid stimulated more PGE₂ than PGF₂α secretion from day 20 pregnant porcine endometrium. Synthesis of PGF₂α and PGE₂ were equally inhibited by progesterone and estrogen. Geisert et al. (1982a) reported increasing PGF₂α and PGE₂ in uterine luminal flushing from pregnant gilts on days 11 to 14 post-mating. Although levels of these prostaglandins were comparable in medium collected from uterine flushings (PGF₂α = 4 ng; PGE₂ = 3 ng), apparently higher levels of PGE₂ (197 ± 12 ng) than PGF₂α (108 ± 23 ng) were present in the conceptus tissue itself on day 14 (Geisert et al., 1982a). Treatment of gilts with estrogen on day 11 increased both PGF₂α and PGE₂ concentrations in uterine luminal flushings in a similar manner to the presence of embryos (Geisert et al., 1982b). These data give some support to a physiological role for PGE₂ in the control of luteal function during the establishment of pregnancy in pigs, but it is clear that a substantial amount of work is still required to clarify this point.
Effects of Prostaglandin F$_2$α on the Corpus Luteum

**Effects on cycle length**

Prostaglandin F$_2$α or its analogues cause luteolysis in sheep and pigs depending on the route and mode of administration and the day of the estrous cycle on which it is given. Luteolysis has been induced in the ewe by various routes of PGF$_2$α administration including infusion into the ovarian artery (McCracken et al., 1971; Thorburn and Nicol, 1971; Chamley et al., 1972; McCracken, 1981), uterine vein (Thorburn and Nicol, 1971; Hearnshaw et al., 1973), utero-ovarian vein (Restall et al., 1973), perivascular space of ovarian pedicle (Reynolds et al., 1981), uterine lumen (Goding et al., 1972a; Douglas and Ginther, 1973; Nett et al., 1976b), ovarian follicles (Inskeep et al., 1975; Fogwell et al., 1977) or directly into the CL itself (Chamley and O'Shea, 1976). Minimum effective dosages were determined for ovarian intra-arterial (Chamley et al., 1972; Goding et al., 1972a) or intra-venous infusion (Restall et al., 1973) and parenteral administration (Douglas and Ginther, 1973). These and similar data were used to determine effective luteolytic levels in studies investigating the time of onset of susceptibility which is reported in general to be day 5 (day 0 = first day of estrus, Thimonier, 1981). Hearnshaw et al. (1973) infused 45 and 67 µg PGF$_2$α/hr into the uterine vein and observed inconsistent return to estrus in ewes during days 4 to 6 but consistent regression on day 8. Mellin and Busch (1976) also reported that 10 mg of PGF$_2$α administered intramuscularly on day 8 was effective in reducing peripheral plasma progesterone concentrations and cycle length in ewes but if given on days 3 or 4 only a transient decline in progesterone was observed with no effect on cycle length. Prostaglandin analogues are also reported to be inefficient if administered on day 3 but efficient in inducing luteolysis and a rapid return to estrus in ewes between days 5 to 14 (Acritopoulou and Haresign, 1980). Also, while a single 5 mg dose was generally ineffective at day 8, a second injection of 5 mg on day 9 consistently induced luteal regression suggesting that more prolonged exposure increases the effectiveness of lower amounts of PGF$_2$α (Mellin and Busch, 1976). Deaver et al. (1986) also found that two 5 mg injections 3 hours apart were effective in
inducing a return to estrus in ewes on days 5, 8 or 11. Ewes injected earlier in the luteal phase returned to estrus sooner after injection than those receiving PGF$_{2\alpha}$ later in the estrous cycle, but it was not determined whether this reflected more rapid luteolysis or folliculogenesis (Deaver et al., 1986).

In pigs PGF$_{2\alpha}$ is very much less effective at inducing premature luteal regression and thereby shortening the estrous cycle (Bosc et al., 1981). Early studies by Neill and Day (1964) and more extensive confirmatory experiments conducted by Caldwell et al. (1969b) suggested that several days were required before porcine CL became susceptible to luteolysis. These experiments involved the induction of accessory CL on the ovaries of normally cycling gilts at various stages of the cycle from day 6 to 16. Accessory CL induced on day 6 of the estrous cycle regressed at the normal time along with existing spontaneous CL on the ovaries but CL induced after this time were maintained while the pre-existing CL around them regressed (Neill and Day, 1964; Caldwell et al., 1969b). Numerous other studies since have demonstrated that PGF$_{2\alpha}$ or its analogues are not effective at inducing complete luteolysis and shortening of the estrous cycle of pigs prior to day 12 whether administered by an intraluteal (Lindloff et al., 1976), intrauterine (Diehl and Day, 1974; Lindloff et al., 1976), uterine venous (Gleeson, 1974) or parenteral route (Douglas and Ginther, 1974; Halford et al., 1975; Connor et al., 1976; Guthrie and Polge, 1976; Moeljono et al., 1976). Similar doses of PGF$_{2\alpha}$ are luteolytic, however, when given to pigs whose CL have been maintained by estrogen administration (Guthrie, 1975; Kraeling et al., 1975a), hysterectomy (Moeljono et al., 1976) or pregnancy (Kraeling and Rampacek, 1977; Guthrie and Polge, 1978; Ball and Day, 1982b). Prior to the time at which it will cause a shortening of the estrous cycle, transient decreases in plasma progesterone occur following PGF$_{2\alpha}$ administered by intrauterine (Lindloff et al., 1976) uterine venous (Gleeson, 1974) or parenteral (Connor et al., 1976) routes but levels return to pretreatment values within two days and luteal lifespan remains unaffected. These data and those previously discussed suggest that in both sheep and pigs an interval exists preceding the acquisition of complete susceptibility to prostaglandin F$_{2\alpha}$ during which exposure to luteolytic agents causes a transient depression of luteal function. Whether or not this depressed luteal function is accompanied by significant structural events or loss of luteal elements has not been addressed.
Effects on morphology

Morphological studies have described changes occurring in the ovine and porcine CL during natural cyclical luteolysis and luteolysis induced by administration of prostaglandin F₂α. Most descriptions refer to changes in the appearance of large luteal cells at least in the initial phases of regression. Deane et al. (1966) reported that the first alterations in intracellular morphology of ovine CL which may have heralded regression were noted after day 10. These included slow accumulation of lipid droplets, swelling of mitochondria and cytoplasmic vacuoles, a decrease in the number but increase in the size of dense bodies and the development of irregular nuclear outline recognizable in some areas by days 12, 13 and in many areas by day 15. Also, although lipid accumulation was evident between days 13 and 14, enzymatic activity measured by histochemistry, remained uniform and unchanged even up to day 15. However, some specimens did appear to contain cells that had completely lost the staining reaction. The authors concluded by remarking that no single histological, histochemical or electron microscopic feature appeared with enough consistency to be used as a marker of the onset of luteolysis (Deane et al., 1966).

Similar findings in regressing CL of ewes were reported by Bjersing et al. (1970a,b) who also described some karyolysis (not further defined) between days 10 and 14 with overt karyorrhexis obvious in most specimens by day 15. Also described as particularly evident by day 15 was swelling of mitochondria and the appearance of what were apparently autophagic vacuoles. Glycogen accumulation began from day 12 onwards and although it was suggested that this might represent a cessation of metabolic activity, it could not positively be attributed to definite signs of the initiation of luteolysis. It was also noted that while in some areas of the CL these changes were particularly obvious, in others apparently healthy luteal tissue persisted suggesting that vascular occlusion, as seen in several micrographs may have lead to the focal degeneration (Bjersing et al., 1970a). Gemmell et al. (1974) described changes in ovine luteal cells throughout the cycle with an emphasis on the disposition of cytoplasmic granules but noted also the appearance from day 12 onwards of large densely
staining cytoplasmic bodies which became numerous by day 15 when cells also began to shrink.

The particular changes occurring in the vascular channels of the ovine CL were closely investigated and reported by O'Shea et al. (1977). Similar morphological changes in luteal cells associated with luteal demise were also noted to be most prominent by day 14. In addition, by days 14 and 15, cellular debris was often observed in the lumens of blood capillaries. Endothelial gap junctions, protruding endothelium and ultimate disintegration of vessels was also correlated with the appearance of autophagic vacuoles in endothelial cells and other luteal elements. Areas of severe degeneration which were flanked by areas appearing relatively normal morphologically again suggested active vascular involvement in the luteolytic process. However, the earlier appearance of recognizable degeneration of luteal cells prior to changes in the luteal vasculature did not exclude the possibility of direct luteolytic effects initiated at the level of luteal cells (O'Shea et al., 1977). Further morphological evidence implicating changes in the luteal vasculature in the process of luteolysis was provided by Niswender et al. (1976) who noted no change in the relative volumes within the ovine CL occupied by granulosa-lutein, theca-lutein or connective tissue components but a progressive decline in the volume occupied by capillaries was demonstrated from day 10 through day 17.

Several authors have fixed ovine CL at defined times following injection of PGF$_{2\alpha}$. However, no two experiments used the same dose of PGF$_{2\alpha}$ which was administered by various routes including the uterine vein, dorsal aorta or intramuscularly and included both single and double injection regimens. Therefore differences among the reported findings may relate to differences in the way in which luteolysis was induced regardless of other methodological differences such as fixation techniques. Similar reservations can be expressed when considering comparisons between induced and natural luteal regression. Accepting these interpretive limitations, McClellan et al. (1977) reported findings emphasizing the accumulation of dense bodies which were thought to represent the formation of autosomes first recognizable 6 hours after PGF$_{2\alpha}$ administration and which appeared to be associated in the final stages with cellular fragmentation and macrophage phagocytosis. These changes were similar between CL from ewes undergoing natural or induced luteolysis and
supported the earlier suggestion by Dingle et al. (1968) that lysosomes and possibly PGF\(_{2\alpha}\) induced changes in lysosomal activity may be involved in luteolysis. Stacy et al. (1975) also reported autophagic bodies and decreased appearance of granules as among the first signs of definitive luteolysis following PGF\(_{2\alpha}\) administration. Rounding and a decrease in the size of mitochondria as well as a decrease in the number of membrane bound granules and lipid accumulation were observed by 12 hours post-PGF\(_{2\alpha}\) by Umo (1975).

More emphasis was given to morphological changes in the vascular compartment of the CL of superovulated ewes by Nett et al. (1976b). These researchers observed accumulating cellular debris in vascular channels by 12 hours following the second of two 5 mg doses of PGF\(_{2\alpha}\) injected intramuscularly. A progressive decline in the volume occupied by capillary lumen was also noted from 6 to 48 hours post-treatment. Earlier changes such as lipid accumulation were also observed as early as 6 hours after injection of PGF\(_{2\alpha}\).

The study reported by Van der Walt (1978) merits particular mention. Ewes which were used in the study were naturally high ovulators and those utilized had four or more CL on one or both ovaries. One CL was removed under anaesthesia on day 10 and used as a control while a second, third and fourth CL were removed 1, 2 and 3 hours after PGF\(_{2\alpha}\) administration. By 2 hours post-injection increased glycogen granules, ADP and substrate content of mitochondria were associated with mitochondrial rounding and were interpreted to suggest increased respiratory rate. A reduction in cytoplasmic volume and extracellular debris accumulation together with lipid formation was obvious by 3 hours post-injection. These features developed to dramatic proportions very rapidly compared to the results of other workers but since PGF\(_{2\alpha}\) was injected directly into the dorsal aorta, CL exposure to the luteolysin may also have been more dramatic. Corteel (1975) observed several morphological changes beginning as early as 30 minutes following infusion of PGF\(_{2\alpha}\) into the uterine vein but found these changes to differ somewhat from those observed in ewes undergoing natural luteal regression. These differences included more rapid disappearance of dense bodies following PGF\(_{2\alpha}\), maintenance of electron density of Golgi elements which did not occur in natural luteolysis in addition to larger lipid droplets accumulating in greater numbers, no development of the rough endoplasmic reticulum, no migration of
endoplasmic reticulum to peripheral areas and no modification of mitochondria all features typical of prostaglandin-induced but not natural luteolysis. It was concluded that the mosaic like distribution of these changes following prostaglandin administration together with irregular or ineffective fixation of smooth endoplasmic reticulum and the features noted above suggested strongly that induced luteolysis may result from acute pharmacological vasoconstriction (Corteel, 1975). In addition, Niswender et al. (1976) infused radiolabelled HCG into the ovarian artery and found that radioactivity was distributed in a very patchy manner, again suggesting differential rates of blood flow to various parts of the CL.

In the pig, only the study by Cavazos et al. (1969) appears to have detailed the morphological events during natural regression of CL. It was emphasized that features initiated during the secretory phase up to day 14 were accelerated in the regressive phase after this time and also that the events were not synchronous in all luteal cells. Regression was typified by lipid accumulation and an increasing abundance of dense bodies sometimes containing cytoplasmic organelles. Smooth endoplasmic reticulum became dilated and presenting as swirling arrays early in the cycle became vesiculated along with general cytoplasmic disruption. These final changes were accompanied by increases in intercellular collagen which appeared more general by day 18 and were considered irreversible once lysosomal activity was underway (Cavazos et al., 1969). As with natural luteolysis, it appears that few studies have examined morphological changes in porcine CL following luteolysis induced by PGF$_{2a}$ administration. In the study by Krzymowski et al. (1978) pigs received PGF$_{2a}$ infused into the uterine vein on days 6 and 12 and tissue was collected and fixed on day 15. Little descriptive histology was reported but to note that fatty degeneration was seen in tissues from both groups of pigs and in both cases degeneration was greatest on the side ipsilateral to the site of PGF$_{2a}$ infusion.

**Effects on blood flow**

These studies in the sheep and the pig leave open the possibility that luteolysis might be induced by vasoconstrictive or direct cellular effects of prostaglandins on the CL. In particular, as suggested by Corteel (1975) those
studies utilizing induced regression may give bias toward a mechanism not involved in natural regression. Little has been advanced in this area particularly as regards to the pig but evidence supporting a primarily blood-flow mediated event initiating luteolysis in these species will be reviewed briefly.

Several reports document changes in blood flow to the ovary of the ewe throughout the estrous cycle. Using various techniques flow rates have been shown to be highest during the luteal phase of the cycle and range from 5 to 10 ml/min as estimated by timed blood collection from the ovarian vein (Mattner and Thorburn, 1969; Cook et al., 1967), doppler ultrasound of the ovarian artery (Niswender et al., 1975; Nett and Niswender, 1981) and microsphere techniques (Niswender et al., 1975; Bruce and Moore, 1976; Niswender et al., 1976; Nett et al., 1976b; Ford et al., 1979; Nett and Niswender, 1981). Blood to the ovary of the ewe decreases around day 14 or 15 of the estrous cycle to less than 1 ml/min in most of these studies and systemic progesterone concentrations are highly correlated with ovarian and luteal blood flow (Niswender et al., 1975, 1976).

Much less data have been collected concerning ovarian or luteal blood flow in pigs throughout the estrous cycle. However, ovarian arterial blood flow has been observed to increase from estrus to day 6, then remain relatively constant until day 14 to 16 (Ford et al., 1982c). After this time, blood flow to the ovary (Magness et al., 1983) and the luteal vascular compartment (Rathmacher and Anderson, 1968, Ford et al., 1982c) declines markedly to reach minimal levels 2 days prior to the next exhibited estrus. During the establishment of pregnancy in pigs, a transient increase in ovarian and luteal blood flow occurs on day 13 (Ford et al., 1982c). As for the ewe, it is not yet clear whether or not the decline in blood flow at the end of the cycle is the cause or is an effect of luteolysis. Nor is it known if the increase in blood flow coincident with the maternal recognition of pregnancy in pigs is associated directly with luteal maintenance.

The observed decline in ovarian blood flow at the time of luteolysis in the ewe lead to more precise investigations of the temporal relationships between these two events (Thorburn and Hales, 1972). In order to know exactly when luteolysis was initiated it was necessary to perform these experiments in animals following the mid-cycle administration of PGF\textsubscript{2a} or its analogues. Nett et al. (1976b) found a significant decrease in ovarian blood flow within 4 hours of infusion of 5 mg of PGF\textsubscript{2} into the uterine lumen of ewes on day 9 of the cycle.
Systemic progesterone concentrations did not decline significantly until 6 hours but were highly correlated with ovarian blood flow \((r = 0.89, P < 0.01, \text{Nett et al., 1976b})\). In addition changes in ovarian blood flow also preceded measurable changes in LH receptors in luteal tissue following PGF\(_{2\alpha}\) administration to ewes \((\text{Nett and Niswender, 1981})\). These data suggest that a reduction in ovarian blood flow may be one of the earliest recognizable and possibly initiating events in degeneration of luteal tissue in ewes following administration of a luteolytic dose of PGF\(_{2\alpha}\).

It is presumed that luteal blood flow is controlled at the level of resistance vessels \((\text{Johnson, 1986})\), the arterioles, in both the sheep and the pig. Few anatomical studies have described the microvasculature of the ovary in either species, however. \(\text{Andersen (1926)}\) studied the ovarian vasculature of pig during the estrous cycle and early pregnancy. Arterioles were seen to enter the mature CL along with tongues of connective tissue of the theca interna, and gave off capillaries at right angles as they looped into and out of the substance of the gland. Similar studies in ewes appear not to have been performed with this emphasis.

Even though almost 90% of ovarian blood flow in the ewe goes to the CL \((\text{Bruce and Moor, 1976; Niswender et al., 1975, 1976})\), it has been suggested that during the period of luteolysis ovarian arterio-venous anastomoses divert blood away from luteal tissue. These conclusions have been drawn from the observation that significantly fewer 15 \(\mu\)m than 50 \(\mu\)m microspheres were trapped by luteal tissues during luteal regression \((\text{Mattner et al., 1981; Ellinwood et al., 1978})\). Ovarian arterio-venous shunts may provide a mechanism whereby blood flow to the nonluteal compartment may increase at the expense of luteal flow and enable internal redistribution of flow as has been demonstrated in the rabbit \((\text{Novy and Cook, 1973})\). In contrast to the above data, others have presented data to suggest that luteal capillary flow does not decline during induced regression. \(\text{Einer-Jensen and McCracken (1977, 1981)}\) utilized miniature Geiger-Muller probes to impale CL in ewes with ovarian autotransplants or CL in situ in ewes under anesthesia. The \(\gamma\)-emission following infusion of \(^{85}\)Krypton into a branch of the ovarian artery was detected by the probe and varied with capillary flow. Prostaglandin \(F_{2\alpha}\) was also infused into the ovarian artery at a rate of 10 \(\mu g/hr\) for 2 hours. Ovarian venous plasma
progesterone concentration decreased to half of the preinfusion value by 2 hours in the autotransplanted preparations and 1.3 hours in the in situ preparations but no change was seen in luteal capillary blood flow.

Additional evidence on the involvement of vascular occlusion in the mechanism whereby PGF$_{2\alpha}$ induces luteolysis has involved the use of prostaglandin analogues with differing smooth muscle contractile activities. Nett et al. (1976b) monitored ovarian blood flow and systemic progesterone concentrations in ewes during luteolysis induced with PGF$_{2\alpha}$ and two analogues with potencies 10 fold higher and 50 fold less than PGF$_{2\alpha}$ for inducing smooth muscle contraction. No dramatic differences were noted between the responses to the 3 prostaglandins in terms of reduced ovarian blood flow and systemic progesterone concentration but there was a higher correlation between flow and progesterone concentration in the group receiving the more potent than the less potent vasoconstrictive prostaglandin analogue (Nett et al., 1976b). In contrast, McCracken et al. (1979) found no correlation between the potency of a prostaglandin analogue with respect to luteolysis compared to its smooth muscle contractile potency. However, smooth muscle contractile potency was estimated using myometrium and may not have reflected vasoconstrictor activity. No mention was made by Nett et al. (1976b) as to how contractile potency of the two prostaglandin analogues used in their experiments was determined so it is difficult to draw any conclusions about the apparent discrepant results of these two studies.

Few studies have examined the effects of other vasoactive agents on ovarian arterial blood flow in ewes. However, the data of Phernetton and Rankin (1978) suggested that ovarian blood flow in late pregnant ewes was reduced by norepinephrine and angiotensin II and that PGE$_2$ was able to antagonise the vasoconstriction induced by these agents. In vitro perfusion studies of the ovarian and utero-ovarian arteries of sheep, have demonstrated greater contractility of these vessels during the luteal phase (Kuhl et al., 1974; Ford et al., 1976). Similar results were obtained in vessels collected from ovariectomised sheep treated with steroid hormones to mimic the luteal and follicular phases (Ford et al., 1975). These data suggest that the local exposure of blood vessels associated with the reproductive tract to ovarian steroids affects their vasoactivity and therefore play an important role in the control of flow.
Similar studies to those discussed for the ewe suggest that the contractility of vessels of the reproductive tract change with concentrations of secreted ovarian steroids in pigs also (Ford and Stice, 1985). Indeed, Reynolds and Ford (1984) perfused whole ovaries collected from luteal and follicular phase pigs. Contractility, as evidenced by an increase in ovarian vascular resistance in response to norepinephrine, PGF$_{2\alpha}$ and nerve stimulation, was enhanced in ovaries collected from pigs during the luteal phase over those from the follicular phase of the estrous cycle. These data and those discussed for the sheep, provide compelling evidence that the control of blood flow to the ovary, and therefore ovarian function, are influenced by steroid hormones and reproductive state. However, whether or not luteolysis involves the activation of similar mechanisms is still conjectural.

These data collectively suggest that ovarian and luteal blood flow is decreasing markedly at the time of terminal luteal demise. The relative lack of earlier changes in systemic progesterone concentrations with the initial increases in uterine prostaglandin release occurring prior to this time may indicate that early prostaglandin spikes do not induce functional or structural changes of any great magnitude in CL. Finally as previously discussed, these data provide further evidence that luteal regression as evidenced by uterine prostaglandin release, morphological signs of regression and concomitant declines in blood flow occur at least two days after maternal recognition of pregnancy in the sheep and the pig when logically it might be assumed that the initiation of luteolysis is blocked.

In summary, most morphological studies which have recognized patchy degeneration of luteal tissue have concluded that focal ischemia best explains this regressive pattern. None, however, attempt to explain how a vasoconstrictive effect of PGF$_{2\alpha}$ might operate on selected parts of an open capillary bed (Keyes and Wiltbank, 1988) which contains no vascular smooth muscle. In the ewe, data were presented on capillary, luteal and total ovarian blood flow during PGF$_{2\alpha}$ induced luteolysis some of which indicated a decline in flow while other data did not. Goding et al. (1972b) presented data on luteal function of 4 ewes following clamping of the dorsal aorta above the origin of the ovarian artery for periods ranging from 46 to 130 minutes. Two ewes exhibited luteal regression but two ewes experiencing ischemia for 46 and 130 minutes respectively recovered luteal function, and progesterone secretion
returned to preoperative levels 24h later (Cain et al., 1972). Similar experiments performed in the guinea pig have also demonstrated no significant effect of total ischemia lasting up to 2 hours on the histological appearance of luteal cells (Azmi and O'Shea, 1984). In support of these results, Kiracofe et al. (1966) hysterectomized 37 ewes between days 4 and 7 of the estrous cycle. Existing CL were charcoal marked and all major ovarian arteries and veins supplying both ovaries were ligated. At slaughter 22 to 28 days post-estrus, 7 ewes had marked CL averaging 544 mg in weight. Together, these data strongly suggest that even if revascularization was complete within a couple of days, severe interruption of the arterial supply to the CL need not necessarily lead to structural regression. It might be argued that peaks of PGF$_2$α in the uterine vein during natural luteolysis occur over a 24 to 48 hour period. However, luteolysis can be induced with a single brief exposure to high levels of PGF$_2$α which would be expected to maintain its effects for only minutes at best, well within the time limits of anoxia described above that are still compatible with continued luteal function. These data suggest that luteal tissue is not acutely dependent on blood supply for maintained viability despite its high metabolic rate although changes in blood flow would still be expected to bring about changes in metabolic and functional activity.

Additional evidence suggests that luteal steroidogenesis itself is not acutely dependent on oxygen supply and that functional changes in the CL can occur without structural changes taking place. In the rat, progesterone secretion or steroidogenesis accounted for less than 2% of the oxygen consumed in luteal metabolism (Swann and Bruce, 1987). Therefore, although not precluding a need for oxygen for other cellular functions essential for the maintenance of cellular viability, tissue anoxia itself might not be expected to lead to rapid reductions in progesterone secretion. Under these circumstances, a dramatic reduction in luteal blood supply would be expected to result in a decrease in peripheral progesterone levels due to reduced transport from the ovary, but an increase in luteal tissue progesterone concentration. This does not appear to be consistent with the data at hand. It is pertinent also that in ewes UOV oxygen concentrations are higher than jugular venous concentrations at the peak of luteal activity and declining oxygen concentration at the time of luteolysis seems not to precede declines in progesterone concentration (Hooper et al., 1986b).
Finally, Jenkin et al. (1984) inhibited conversion of pregnenolone to progesterone by the administration of a competitive enzyme blocker to ewes during the mid-luteal phase of the estrous cycle. Although peripheral progesterone concentration decreased to less than 40% of pretreatment levels and remained so for several hours, progesterone concentrations returned to normal within 24 hours and cycle length was normal. These data suggest that inhibition of progesterone synthesis in itself is probably not a stimulus for luteolysis in ewes.

In the pig, a different approach was taken to evaluate the effect of blood supply on luteal function. Pope et al. (1987) attempted to increase ovarian blood flow by ligating branches of the uterine artery distal to the point where anastomoses occur with the ovarian arterial supply thereby forcing or shunting uterine arterial blood into the ovarian vascular bed. Hysterectomy was performed concomitantly leaving various lengths of the ipsilateral uterine horn intact. Resistance to luteolysis was observed in the ovary receiving additional uterine blood supply and with 20 to 40 cm of ipsilateral uterine horn intact while the contralateral ovary with no uterine horn regressed. It was concluded that ovarian blood flow was involved in the regulation of luteal function (Pope et al., 1987).

The conclusion drawn by Pope et al. (1987) appears contradictory to the notion that the control of blood flow resides in the terminal arterioles. It also appears contradictory to the results of the studies by Neill and Day (1964) and Caldwell et al. (1969b) previously discussed. These studies examined the susceptibility of two populations of CL of different ages and on the same ovary to natural luteolysis in pigs. Accessory CL were induced to form during mid-cycle and CL were subsequently examined on day 20. Those naturally formed CL had regressed while all accessory CL were still functional as suggested by the lack of exhibited estrous activity of gilts by the time of slaughter. These data demonstrate that ovarian blood flow was adequate to maintain some CL in a functional state while those adjacent regressed. In order for uterine PGF$_{2\alpha}$ to have induced regression selectively under these circumstances arterioles immediately surround those older CL would have had to have been severely constricted while adjacent arterioles remained unaffected. If a vasconstrictive effect of PGF$_{2\alpha}$ brings about luteolysis in the pig and by inference the ewe, then
these data emphasize the importance of intraovarian events such as vascular shunting presumably brought about by constriction of terminal arterioles. In conclusion, focal patchy degenerative lesions recognized in CL of the pig and the sheep would seem not to be adequately explained by an alteration in blood flow or ischemia alone. Instead, this pattern may represent areas of susceptibility, containing populations of luteal cells sensitive to the effects of PGF2α. In support of this possibility, separate populations of large porcine luteal cells sensitive or not sensitive to the stimulatory effects of PGF2α on relaxin secretion have been reported by Taylor and Clark (1987). Alternatively, PGF2α may be preferentially transported to particular areas of the CL by differential flow patterns. Data in support of this notion are provided by Niswender et al. (1976) who noted that 125I-HCG injected into the ovarian arterial supply of the ewe was later found accumulated in focal hot spots. In either case degeneration would be expected to result from direct cellular effects of PGF2α on the luteal cells themselves because the open capillary bed of the CL would not provide a means whereby blood flow to particular areas could be compromised. Whether or not this cellular effect might also be influenced by level of tissue oxygen is not known. The following section will deal with what is known of the direct effects of PGF2α on ovine and porcine luteal cells.

In Vitro Studies of Luteal Function in Sheep and Pigs

Studies in ewes

In preceding sections, the anatomical developmental changes relative to populations of small and large ovine luteal cells was discussed. Detailed biochemical and physiological studies in the sheep have firmly established differences between large and small luteal cell populations purified by separation on the basis of size. Although large luteal cells were initially reported to possess few LH receptors (Fitz et al., 1982) more recent experiments have observed similar numbers of LH receptors on large and small ovine luteal cells (Harrison et al., 1987). However, while small luteal cells respond to stimulation with LH by substantial increases in progesterone production, large luteal cells are unresponsive to LH or activation of adenylate cyclase in vitro in this regard (Fitz
et al., 1982; Rodgers et al., 1983b; Hoyer et al., 1984; Harrison et al., 1987).

Large luteal cells were also observed to possess greater numbers of PGF$_{2\alpha}$ receptors than did small cells (Fitz et al., 1982) and a selective loss of large luteal cells during luteolysis (Braden and Niswender, 1985) suggests that this cell population may be the target for the luteolytic action of PGF$_{2\alpha}$.

After adjusting for estimated numbers of small and large cells, Rodgers et al. (1983b) considered that small luteal cells produced as much progesterone as large luteal cells and under conditions of normal LH stimulation probably produced more. However, basal production and content of progesterone is much higher in large than small ovine luteal cells (Fitz et al., 1982; Rodgers and O'Shea, 1982; Rodgers et al., 1983b) in the order of 20 times greater according to Fitz et al. (1982). These observations have lead to speculation as to the relative contribution of each cell type to systemic progesterone concentrations in the ewe. Niswender et al. (1985, 1986) suggested that large luteal cells contribute up to 80% of the progesterone secreted by the ovine CL (Niswender et al., 1986).

The lack of correlation between peaks of progesterone and LH in jugular venous plasma of luteal phase ewes (Baird et al., 1976b) is suggested by Niswender et al. (1986) to further support the notion, that the CL is not under tonic LH stimulation. However, these data might equally indicate near maximal LH stimulation of luteal progesterone production under the conditions of that experiment. Baird et al. (1976b) suggested that infusion of LH into the ovarian artery in physiological concentrations in vivo, did not result in an increase in progesterone secretion (Baird and Collett, 1973). These data lend support to the suggestion that under normal physiological conditions the CL of the ewe is under maximal LH stimulation. Nonetheless it is apparent that the relative large and small luteal cell contributions to progesterone secretion by the ovine CL are unlikely to be determined by calculations of this type.

It has been suggested that the mechanism by which PGF$_{2\alpha}$ induces luteolysis may involve a reduction in luteal LH receptors. Studies in the ewe have demonstrated a significant decline in luteal LH receptors by 22 hours after ewes were administered a luteolytic dose of PGF$_{2\alpha}$ on day 9 of the estrous cycle (Diekman et al., 1978a). The binding of labeled HCG also declined by 18 hours post-injection (Diekman et al., 1978a) but systemic progesterone had declined by 7 hours post-treatment and therefore it was concluded that receptor numbers
alone did not account for the reduction in luteal steroidogenesis following PGF$_{2\alpha}$ administration. In the hysterectomized pig, a more synchronous decline in luteal LH receptors and plasma progesterone concentrations were observed in gilts given PGF$_{2\alpha}$ on day 20 post-estrus (Barb et al., 1984). In that study, a small but significant decrease in luteal LH receptors and systemic progesterone concentrations was noted by 4 hours after PGF$_{2\alpha}$ treatment. In this context it is perhaps significant that the time at which PGF$_{2\alpha}$ becomes able to induce luteal regression (day 12), is about the time that continued luteotrophic support is required for luteal maintenance. It would be interesting to know whether or not PGF$_{2\alpha}$ induced any decline in luteal LH receptors in the pig following PGF$_{2\alpha}$ administration prior to day 12 when it would not be expected to induce luteolysis. At present it is not possible to determine from the available data whether or not reduced receptor numbers are important in the process of luteolysis in the pig (Bramley and Menzies, 1981) or the sheep particularly since less than 2% of receptors are required to be bound to elicit a full steroidogenic response (Diekman et al., 1978b).

Regardless of possible changes in luteal LH receptor numbers in response to PGF$_{2\alpha}$, several in vitro reports have suggested that PGF$_{2\alpha}$ prevents the normal response of ovine luteal tissue to LH (Henderson and McNatty, 1975). LH stimulates progesterone secretion by luteal cells from most species by activation of adenylate cyclase, through binding of the catalytic subunit to a guanine nucleotide regulatory subunit (Spiegel et al., 1981), and generation of cyclic AMP from ATP (reviews by Marsh, 1976; Niswender et al., 1980; Hoyer and Niswender, 1985). Ewes given a luteolytic dose of PGF$_{2\alpha}$ on day 10 of the estrous cycle experience a decrease in basal and LH stimulatable adenylate cyclase activity by 2 hours following injection concomitant with an increase in phosphodiesterase activity (Agudo et al., 1984). Both the change in adenylate cyclase and phosphodiesterase activities preceded a significant decline in systemic progesterone concentrations noted by 4 hours post-injection (Agudo et al., 1984). These data suggest that PGF$_{2\alpha}$ induced luteolysis is characterized by a rapidly developing block to the adenylate cyclase second messenger system of ovine luteal tissue independent of effects directly on the level of LH receptors.

In vitro studies with ovine luteal tissues have generally tended to support the conclusions concerning adenylate cyclase activity drawn from the studies
described above. Using both minced ovine luteal tissue and whole CL from ewes on day 10 of the estrous cycle, Evrard et al. (1978) demonstrated that PGF$_{2\alpha}$ inhibited LH-stimulated progesterone secretion. Fletcher and Niswender (1982) confirmed these data and extended their observations by showing that PGF$_{2\alpha}$ inhibited adenylate cyclase activity after 3 hours of culture of day 13 ovine luteal slices. Basal progesterone secretion was not affected after 2 hours exposure to PGF$_{2\alpha}$ which also seemed to affect only LH sensitive but not fluoride or PGE$_{2\alpha}$ stimulatable adenylate cyclase activity (Fletcher and Niswender, 1982). Progesterone secretion from ovine luteal slices can also be stimulated by changes in membrane potential (Higuchi et al., 1976) suggesting steroidogenic controls independent of cAMP mediated events (Hoyer et al., 1984). Further consideration to adenylate cyclase independent control of luteal steroidogenesis will be given in more detail in later sections.

Studies in pigs

Several studies have been conducted investigating in vitro progesterone production by porcine luteal tissue. Duncan et al. (1960) reported that day 4 luteal slices produced more progesterone basally than did slices from tissue taken at later stages of the estrous cycle or pregnancy. Pregnenolone increased progesterone synthesis but tissues were unresponsive to gonadotrophins (Duncan et al., 1960). A subsequent study, similarly found no effect of gonadotrophin preparations, lactogenic hormones or oxytocin (Duncan et al., 1961). Experiments performed by Cook et al. (1967) found a small (15%) and variable but significant effect of gonadotrophic stimulation on progesterone secretion by porcine luteal slices but no systematic effort was made to define steroidogenic capacity throughout the cycle.

More recent studies using minced (Hunter, 1981) and dispersed porcine luteal cells (Hunter, 1981; Mattioli et al., 1985) confirms that early luteal phase tissues synthesize more progesterone than tissues from the mid- to late-luteal phase. In addition, both minces and dispersed cell preparations of porcine CL are responsive to LH only during the mid-luteal phase (day 10 to 12) or after (Hunter, 1981; Mattioli et al., 1985). Although LH and cAMP increase progesterone accumulation by dispersed luteal cells collected on days 11 to 13 of
the estrous cycle in a dose dependent fashion, other studies have indicated a consistently greater response is obtained in the presence of LH (Hunter, 1981; Kineman et al., 1987). Grazul et al. (1986) examined LH stimulated progesterone secretion by dispersed porcine luteal cells collected from early pregnant, estrogen-treated (pseudopregnant) and HCG-treated gilts on day 18 of the cycle. Cells from HCG-treated gilts produced more progesterone basally but were unresponsive to LH. In both cases cells from pseudopregnant or pregnant gilts responded to LH by increased progesterone production but cells from estrogen-treated gilts were more responsive at lower LH concentrations (Grazul et al., 1986).

Lemon and Loir (1977) first isolated partially purified small and large luteal cell populations from CL of day 60 pregnant pigs. It was reported that large luteal cells synthesized more progesterone than small luteal cells but that only small cells responded to LH when cells were maintained in a superfusion system. Later studies (Lemon and Mauleon, 1982) indicated that when small cell superfusate subsequently perfused large luteal cell populations more progesterone was synthesized than if the reverse perfusion were performed or if either cell type were perfused alone. It was concluded that small cells, responsive to LH might provide substrate for large luteal cells (Lemon and Mauleon, 1982). No interaction in the production of progesterone by large and small luteal cells of sheep has been demonstrated (Rodgers et al., 1985).

Buhr (1987) examined progesterone secretion by small and large porcine luteal cells collected on days 10, 15 and 18 of the estrous cycle. These data confirmed that basal progesterone secretion by large luteal cells was significantly greater than that by small cells. Contrary to the data obtained in the ewe only day 10 large cells responded to LH by increased progesterone synthesis, no effect of LH was observed on small luteal cells (Buhr, 1987). However, Hunter (1981) found both cell types to be responsive to LH if collected from pigs on day 12 of the estrous cycle. The reasons for these contradictory results are not apparent.

Superfusion has also been utilized in conjunction with luteal slices to examine responsiveness of porcine CL in vitro (Watson and Wrigglesworth, 1975). These studies have demonstrated an increase in progesterone secretion following superfusion with media containing pregnenolone (Watson and
Wrigglesworth, 1975) and gonadotrophin (Watson and Leask, 1975). Similar experiments were also performed to examine the effects of PGF$_{2\alpha}$ and uterine extracts or flushings administered in vitro to slices from mid-luteal phase CL. Prostaglandin F$_{2\alpha}$ induced a 55% decrease in progesterone secretion which was transient only. Uterine extracts or flushings induced a 65% reduction in progesterone secretion and inhibition persisted for a 10h perfusion if extracts or flushings were collected from late rather than mid-luteal phase sows (Watson and Maule Walker, 1977). Neither LH nor estrogen prevented the decline in response to PGF$_{2\alpha}$ (Watson and Maule Walker, 1978). In contrast to these results, Mattioli et al. (1985) reported that PGF$_{2\alpha}$ treatment of dispersed cell preparations from mid-luteal phase pig CL stimulated progesterone secretion without increasing cAMP accumulation in media. Prostaglandin F$_{2\alpha}$ actually increased the stimulatory effect of LH (Mattioli et al., 1985). Vast differences in methodology make it difficult to reconcile these data.

Adenylate cyclase activity of porcine CL has been examined by Andersen et al. (1974) during the mid-to-late luteal phase (days 12, 14, 16 or 17) and throughout the estrous cycle (days 3, 8, 13 and 18) by Ritzhaupt et al. (1986). Basal enzyme activity was found to be higher on days 12 and 14 of the cycle than on days 16 or 17 by Anderson et al. (1974) but Ritzhaupt et al. (1986) reported elevated activity only on day 8 and no difference at the other time measured. Also Anderson et al. (1974) demonstrated LH stimulation of adenylate cyclase activity on days 12 and 14 but neither Ritzhaupt et al. (1986) or Birnbaumer et al. (1976) were able to duplicate these results using similar or identical concentrations of LH. No explanation can be offered for the discrepancies among these studies.

**Luteal substrate supply**

Because luteolysis induced by PGF$_{2\alpha}$ may influence steroidogenesis at any of several points along the steroidogenic pathway, the following discussion will briefly summarize what is known about regulation of basal steroid secretion in luteal cells by substrate supply followed by some discussion of observations made during luteolysis. The much smaller contribution of stimulatable progesterone secretion compared to basal secretion of total progesterone
production by porcine luteal tissue was interpreted by Buhr (1987) to suggest that, in this species, steroidogenesis may be limited by substrate availability. Lipoprotein and cholesterol metabolism by endocrine tissues has been recently reviewed by Gwynne and Strauss (1982). Low density lipoproteins (LDL) stimulate luteal progesterone production in the cow (Pate and Condon, 1982) over that stimulated by high density lipoproteins (HDL; O'Shaughnessy and Wathes, 1985). Porcine granulosa cells also preferentially utilize homologous LDL for steroidogenesis (Veldhuis et al., 1984) and estradiol and LH synergize in enhancing the utilization of LDL and cholesterol itself for pregnenolone synthesis (Veldhuis et al., 1982a,b, 1985).

Pregnenolone has been shown to stimulate progesterone synthesis by porcine luteal slices and minces (Duncan et al., 1960; Weiss et al., 1976) but little has been studied concerning lipoprotein effects on luteal steroidogenesis. Grinwich et al. (1983) used a predominantly HDL preparation of canine lipoprotein to demonstrate a stimulation of progesterone secretion by luteal tissue from day 70 to 85 pregnant pigs. A positive interaction between prolactin and LH was reported which increased HDL utilization in these experiments. In contrast to these results, Buhr (1987) found homologous HDL to be inhibitory to progesterone secretion by day 10 luteal cells. LDL in addition to culture media caused a marked increase in progesterone synthesis by both small and large luteal cells on all days examined.

Few studies of the kind described above have been performed using luteal tissue from sheep, however, Hoyer and Niswender (1985) cultured small and large luteal cells collected from superovulated ewes on day 10 of the estrous cycle in the presence or absence of 25-hydroxycholesterol. On a cell for cell basis, addition of 25-hydroxycholesterol increased progesterone secretion by small luteal cells to a level equal to that of large luteal cells whose progesterone synthetic rate was less dramatically effected (Hoyer and Niswender, 1985). These data, although not greatly elucidating the mechanisms of control of steroidogenesis by lipoprotein substrates, do suggest that progesterone production by large luteal cells is not as substrate limited as is that of small luteal cells from CL of ewes on day 10 of the estrous cycle.

Progesterone secretion may also be regulated by the level of steroidogenic enzyme activity within luteal cells. The rate of pregnenolone synthesis from
cholesterol within mitochondria is catalyzed by cytochrome P450-side chain cleavage enzyme, and is generally accepted to be the rate limiting step in the production of progesterone and other steroid hormones (Hall, 1985). There are three major sources of cholesterol available for luteal steroidogenesis namely de novo synthesis from acetate, uptake of cholesterol (in the form of lipoprotein) from the blood as described above and release of cholesterol from esterified stores by the action of cholesterol esterase (Behrman and Armstrong, 1969). The latter source may support pregnenolone synthesis in the absence of exogenous substrate for short periods (Baranao and Hammond, 1986).

The anatomical studies previously described established that lipids accumulated in the CL with the onset of luteolysis in both the sheep McClellan et al. (1977) and pig (Cavazos et al., 1969). Biochemical analysis confirmed the lipid accumulation in regressing CL in the pig (Bloor et al., 1930; Waterman, 1980a,b) and the sheep (Waterman, 1988) and it is believed that there exists a negative relationship between cholesterol content in CL and their ability to synthesize progesterone particularly in the latter stages of the cycle (Dorrington, 1977).

The amount of cytochrome P450-side chain cleavage enzyme increases gradually throughout the cycle and decreases around the time of luteolysis in both the sheep (Simpson et al., 1973; Rodgers et al., 1987) and the pig (Torday et al., 1980). However, although LH has been shown to increase cholesterol esterase activity in rat CL (Behrman and Armstrong, 1969), the relatively slow rate of change of enzyme levels either within the mitochondria or the cytosol are unlikely to acutely regulate progesterone synthesis particularly when synthesis is declining rapidly at the time of luteolysis. This is also suggested by the nature of mitochondrial P450 which is an integral protein of the inner mitochondrial membrane (Hall, 1985) and whose levels therefore are unlikely to undergo dramatic changes. Also, this contention is supported directly by the observations of Caffrey et al. (1979) who reported that although luteal cholesterol esterase activity correlates well with luteal progesterone secretion from days 2 to 14 of the estrous cycle of sheep, at the time of luteolysis esterase activity actually increased.

The role of protein synthesis in luteal steroidogenesis and luteolysis appears complex and difficult to study. French and Casida (1973) prevented luteolysis by the intrauterine administration of actinomycin D on day 11 of the
cycle of ewes. However, cycloheximide infused into the ovarian artery on day 11 of the estrous cycle caused a pronounced reduction in ovarian progesterone secretion and luteal cell granule content (Gemmell and Stacy, 1980). These data suggest that at the uterine level transcriptional events are essential in the uterine release of PGF$_{2\alpha}$ while at the level of the ovary continued translation is required to maintain steroidogenesis. Kumar et al. (1987) demonstrated that HCG induced an increase in progesterone secretion by bovine luteal cells but that several inhibitors of de novo protein synthesis were capable of blocking that response.

Other studies have suggested an alternative regulatory point. In pigs Torday et al. (1980) measured the binding of cholesterol to P450 throughout the cycle and during PGF$_{2\alpha}$-induced luteolysis on day 13 in hysterectomized pigs. During the time of natural luteolysis (days 14 to 16) and following induced luteolysis (days 14 to 16) a rapid reduction in cholesterol binding to P450 was seen. The use of protein synthesis inhibitors to pseudopregnant rats was shown to reduce the synthesis of progesterone during culture of mitochondria collected subsequent to sacrifice (Hermier et al., 1971). Furthermore, Toaff et al. (1979) demonstrated that inhibition was observed only when rat mitochondrial membranes were intact suggesting that inhibition was due to a decrease in the transport of substrate into intact mitochondria by an as yet unidentified protein. Inhibition of substrate transport into mitochondria would be expected to inhibit steroidogenesis regardless of the cholesterol mobilized. Although Simpson et al. (1973) failed to demonstrate a difference in P450-bound cholesterol in ovine CL during the late luteal phase a decrease in mRNA for cytochrome P$_{450}$scc was demonstrated by immunoblot analysis (Rodgers et al., 1987). Further investigations are required to elucidate the effects of luteolysis on luteal steroidogenesis.

Membrane associated effects

More recently, attention has been drawn to changes in luteal cell membranes as a target for PGF$_{2\alpha}$ during luteolysis. Buhr et al. (1979, 1983) described changes in the gel phase of the lipid bilayer of luteal membranes from pseudopregnant rats. Specifically, a decrease in membrane fluidity was
indicated by a significant increase in the gel; liquid phase 24 hours following PGF<sub>2α</sub> administration on day 9 or on day 14 of pseudopregnancy in rats (Buhr et al., 1979; Carlson et al., 1984) which correlated negatively to the decline in plasma progesterone concentrations. Subsequent studies also using luteal membranes isolated from luteal cells from pseudopregnancy rats following in vitro treatment with PGF<sub>2α</sub> suggested that these changes were calcium and calmodulin dependent and could be prevented also by heating (Riley and Carlson, 1985). These data suggest that calcium/calmodulin dependent protein synthesis or activation may be involved in rat luteal phase-transitions during natural and PGF<sub>2α</sub>-induced regression (Riley and Carlson, 1985).

Similar data to those described above in rats were also reported in studies of the bovine CL. Membrane rigidification as suggested by liquid to gel phase transitions during natural and PGF<sub>2α</sub>-induced luteal regression was reported in CL collected from late cycling or mid-luteal phase heifers 24 hours following PGF<sub>2α</sub> administration (Carlson et al., 1982). Luteal tissue collected from mid-luteal phase heifers which was also treated with PGF<sub>2α</sub> in vitro demonstrated similar liquid to gel phase transitions (Carlson et al., 1982). These data are supported by the studies of Benhaim et al. (1987) which demonstrated PGF<sub>2α</sub> inhibition of LH-stimulated progesterone production by small bovine luteal cells. No such changes were observed in luteal membranes from gilts undergoing natural luteal regression or at 24 hours following injection of day 13 gilts with PGF<sub>2α</sub> (Buhr et al., 1986).

Changes in the fluidity of the lipid bilayer such as those described in CL in the rat and the cow during natural and induced luteal regression may explain the observed uncoupling of the LH receptor from adenylate cyclase. Similar changes in the activity of other membrane bound enzymes such as Na<sup>+</sup>-K<sup>+</sup>-ATPase have also been shown to be decreased in pseudopregnant rats following PGF<sub>2α</sub>-induced luteolysis (Kim and Yeoun, 1983). The failure to observe similar changes in membrane fluidity in pigs is consistent with the relatively smaller decline in luteal LH receptor numbers seen during luteolysis in pigs (Barb et al., 1984). Indeed, Buhr et al. (1986) did not find significant reductions in LH receptor levels in gilts during prostaglandin-induced luteal regression in that study. Apart from disturbances in receptor coupling, lipid turnover in the cell
membrane (Glew et al., 1985) is important in maintaining cellular integrity and membrane repair processes (Dawson, 1973; Dawidowicz, 1987).

**Calcium and protein kinase C: overview**

Considerable evidence is accumulating on, and increasing attention is being directed towards other aspects of membrane function. In addition to activation of adenylyl cyclase, receptor-ligand binding of many types in many tissues and cell types leads to increased phospholipid turnover. Because of the vast literature in this area (Abdel-Latif, 1986) and the immense variety of systems, cells and tissues, in which these events have been studied, the following brief summary draws on information largely referred to by reviews the emphasis of which provide more detail on specific points. For the sake of brevity, no attempt will be made to discuss examples or circumstances under which particular phenomena have been observed. Rather, a general view is given with the intention of providing insight into the possible complexities and physiological implications of activation of the calcium/protein kinase C system. A discussion of literature pertinent to luteal function will follow.

By an as yet poorly defined mechanism probably involving a specific G protein (Abdel-Latif, 1986; Stryer and Bourne, 1986; Casperson and Bourne, 1987; Jeremy and Dandona, 1987), phosphatidyl-inositol 4,5-bisphosphate (PIP$_2$) is hydrolysed to produce inositol, 1,4,5-triphosphate (IP$_3$) and 1,2-diacyl glycerol (DG) in the plasma membrane by the activation of phospholipase C (Farese, 1983; Abdel-Latif, 1986). Release of IP$_3$ subsequent to hydrolysis is considered the primary event in initiating the cellular response (Berridge, 1987) through release of intracellular calcium stores (Berridge and Irvine, 1984) and eventually stimulation of calcium entry from the extracellular matrix (Berridge, 1987; Irvine, 1987). Transient increases in the cytosolic calcium concentration are thought to activate the cellular response in a transitory fashion (Berridge, 1987).

At least two possible fates exist for the DG generated by PIP$_2$ hydrolysis, either phosphorylation by diacylglycerol kinase generating phosphatidic acid or deacylation by membrane lipases yielding arachidonic acid, stearic acid and glycerol (Abdel-Latif, 1986), and the rate of each of these reactions will affect the level of DG in the membrane. Diacylglycerol formed transiently at the
plasma membrane stimulates or activates protein kinase C (Kaibuchi et al., 1981) a calcium dependent kinase involved in the phosphorylation of as yet poorly defined regulatory intracellular proteins (Nishizuka, 1986). Protein kinase C is largely isolated from the soluble fraction of most tissues in its inactive form (Nishizuka, 1986) and is therefore thought to translocate to the cell membrane following extracellular calcium uptake (McArdle and Conn, 1986; Wiltbank et al., 1988) where it is first activated then subsequently degraded (Nishizuka, 1984; Nishizuka et al., 1984). In addition to calcium and DG, a third component-phosphatidylserine, is also important in the activation of protein kinase C. Although the majority of phosphatidyl serine is located on the inner surface of the plasma membrane, further supporting the translocation hypothesis for enzyme activation, this is not the only possible site of location of either phosphatidylserine or activation of protein kinase C (Niedel and Blackshear, 1986). In this regard, Vilgrain et al. (1984) demonstrated that protein kinase C was associated with the inner intramitochondrial membrane of bovine adrenal cells and that protein kinase C activity resulted in the phosphorylation of the cytochrome P450 side-chain cleavage enzyme.

The importance of DG in the activation of protein kinase C lies in its modification of the requirement for Ca++ in enzyme activation. It is well known that intracellular cytosolic calcium concentrations are tightly regulated (Carafoli, 1987) because of the potential toxic effects of this element on cellular function (Faber, 1981; Campbell, 1987). A reduction in the requirement of protein kinase C enzymatic activity on calcium concentration allows a rapid return of cytosolic calcium levels to normal while cellular activation continues, so called sensitivity modulation (Rasmussen and Barrett, 1984; Rasmussen et al., 1985; Berridge, 1987). In fact, one consequence of activation of protein kinase C is a rapid stimulation of calcium extrusion (Nishizuka, 1986) by turning on membrane calcium pumps (Berridge, 1987).

Various pharmacological agents have been used as probes to investigate the role of these systems in cellular function and the interaction between changes in cytosolic calcium levels, activation of protein kinase C and even the adenylate cyclase system. Foremost among these has been the group of compounds known as phorbol esters which are effective analogues of DG (Castagna et al., 1982). These compounds have been used to stimulate protein
kinase C in a variety of experimental settings (Abdel-Latif, 1986). Phorbol esters have also been used to demonstrate the paradoxical down-regulation of protein kinase activation (Niedel and Blackshear, 1986) and phosphorylation of membrane receptors leading to inactivation of the receptor-activated response (Berridge, 1987). In addition, phorbol esters have been shown to enhance Gs protein binding to adenylyl cyclase promoting elevations of cAMP (Berridge, 1987). Protein kinase C can also be activated directly by large increases in cytosolic calcium concentrations (Nishizuka et al., 1984) while smaller elevations in response to calcium ionophores can induce cellular responses independent of protein kinase C activity (Nishizuka, 1984).

Calcium and protein kinase C in luteal function

Early studies in the rat suggested that increases in cytosolic calcium induced with calcium ionophores mimicked the inhibitory effects on PGF2α on LH-stimulated cAMP accumulation by luteal cells in vitro (Thomas et al., 1978; Dorflinger et al., 1984). The inhibition by PGF2α of cAMP accumulation following LH stimulation of rat luteal cells was not inhibited in either calcium free media or in the presence of calcium channel blockers (Lahav et al., 1983) suggesting that it may involve the mobilization of intracellular calcium. Raymond et al. (1983) demonstrated that within 2 to 5 minutes following the in vitro exposure of rat luteal cells to PGF2α, there was a stimulation of phosphatidyl inositol turnover, and the calcium ionophore A23187 mimicked this response (Leung, 1985). These data suggest that PGF2α and A23187 may stimulate the release of calcium from intracellular stores and that PGF2α may induce intracellular calcium release through an increase in phosphatidylinositol hydrolysis and the generation of IP₃ (Leung et al., 1986).

The possible involvement of protein kinase C in the control of steroidogenesis in rat luteal cells as suggested by the above data on increased membrane phospholipid turnover, was investigated by Baum and Rosberg (1987). Luteal cells from immature rats treated with PMSG and HCG were collected on day 7 following HCG administration. Progesterone secretion was determined following incubation with the phorbol ester phorbol myristate acetate (PMA, equivalent to tetradecanoyl phorbol acetate or TPA), A23187 and
PGF$_{2a}$. Dose response curves were generated for both the phorbol ester and the calcium ionophore and cAMP was measured in homogenates following incubation. PMA had no effect on basal cAMP or progesterone secretion but decreased both cAMP accumulation and progesterone secretion stimulated by LH at concentrations of PMA up to 5 μM. Calcium ionophore similarly decreased cAMP and progesterone secretion by luteal cells in a dose dependent manner when added up to concentrations of 100 μM. Analogues of cAMP were ineffective in reversing the inhibitory effects of either agent (Baum and Rosberg, 1987).

Davis et al. (1981) first reported that dispersed luteal cells from 2 to 6 month pregnant cows responded to LH by increased radioactive labeling of membrane phospholipids indicating a stimulation of phospholipid turnover and both LH and PGF$_{2a}$ stimulated IP$_3$ production in bovine luteal cells (Davis et al., 1987). Subsequent studies demonstrated the presence of a phospholipid and Ca$^{2+}$ sensitive protein kinase in cytosolic fractions of bovine CL (Davis and Clark, 1983). Identification of this protein kinase as protein kinase C was suggested by both its Ca$^{2+}$ dependence, stimulation by 1,2 diolein and the increased enzyme activity in the presence of lipids particularly phosphatidylserine (Davis and Clark, 1983). The ability of phorbol esters to alter steroidogenesis of mid-luteal phase bovine luteal cells has been reported by Brunswig et al. (1986) and Hansel and Dowd (1986). PMA caused a dose dependent increase in both basal and LH-stimulated progesterone secretion in the absence of changes in cAMP (Brunswig et al., 1986). Another phorbol ester 1-oleoyl-2-acetyl glycerol (OAG) also stimulated progesterone production (Brunswig et al., 1986) and Hansel and Dowd (1986) reported a similar effect of PMA on luteal cells from cows on day 16 to 17 but not day 4 to 6 of the estrous cycle. Furthermore, the effects of PMA appear to be a result of stimulation of small cell production rather than large (Alila et al., 1988). The stimulatory effects of both PMA and HCG were abolished by cycloheximide and PMA failed to affect the rate of pregnenolone synthesis from several hydroxylated cholesterol substrates (Brunswig et al., 1986). These data suggest that PMA may have stimulated the rate of cytochrome P450 side-chain cleavage activity probably by inducing phosphorylation of this enzyme (Vilgrain et al., 1984) through the synthesis of an intermediate protein. Hansel and Dowd (1986) also reported that the calcium ionophore A23187 enhanced LH
stimulated but not basal progesterone secretion. No additivity was observed
between A23187 and PMA (Hansel and Dowd, 1986).

Protein kinase C activity has been identified in porcine CL collected from
pigs at unknown stages of the estrous cycle (Noland and Dimino, 1986). More
activity was found in luteal than follicular (minced follicles devoid of fluid)
cytosol but less was associated with the luteal than the follicular mitochondrial
fraction (Noland and Dimino, 1986). LH has also been shown to increase the
turnover of porcine luteal membrane phospholipids and increase the
concentrations of DG within 15 secs of addition of LH to incubates of luteal
plasma membranes (Allen et al., 1988).

The effect of phorbol esters on steroidogenesis by porcine granulosa cells
has been studied by Veldhuis and Demers (1986). These investigators reported a
dose dependent inhibition of progesterone secretion stimulated by FSH which
was not dependent on changes in levels of cAMP. This effect persisted in the
presence of substrates for side-chain cleavage suggesting that the effect was
mediated through changes in the activity of cytochrome P450 scc (Veldhuis and
Demers, 1986). In a subsequent investigation, Veldhuis et al. (1987) reported
that PMA stimulated the synthesis of PGF2α, an effect that was enhanced by
A23187 but blocked by treatment with indomethacin. Prostaglandin synthesis
enhanced by phorbol esters has been demonstrated in rat liver cells (Levine et al.,
1987) and urinary bladder (Jeremy and Dandona, 1987) and was attributed to
the liberation of arachidonic acid by the action of phospholipase C.

Luteal membrane phospholipid turnover and the effects of PMA on luteal
steroidogenesis in other domestic or laboratory animal species are limited to
preliminary reports at this time. McCann and Flint (1987) reported that the
PGF2α inhibition of LH-stimulated progesterone secretion by day 10 ovine luteal
slices was not mimicked by PMA. PGF2α induced an increase in the generation of
IP3, and pertussis toxin, which presumably blocked the G protein induced
hydrolysis of GTP, also inhibited IP3 production (McCann and Flint, 1987). Some
controversy exists when examining preliminary reports on the effects of phorbol
esters on progesterone secretion by large and small ovine luteal cells. It is
generally agreed that phorbol esters inhibit progesterone secretion by large
ovine luteal cells (Hoyer et al., 1988; Knickerbocker et al., 1988; Wiltbank et al.,
1988). However, some investigators have reported inhibitory effects of phorbol
esters on progesterone secretion by small ovine luteal cells (Knickerbocker et al., 1988; Wiltbank et al., 1988) while others have reported no effect at all (Hoyer et al., 1988). Protein kinase C activity has been detected in large and small luteal cells (Hoyer et al., 1988) and treatment with phorbol esters has been shown to cause translocation of the enzyme to the plasma membrane (Wiltbank et al., 1988). Finally, Rodgers et al. (1983b) found no effect of 100uM of A23187 on progesterone secretion by ovine luteal cells although treatment of cell preparations with the calcium channel blocker verapamil decreased progesterone secretion. It is clear that a considerable amount of work is required to elucidate the role of the calcium/protein kinase second messenger system in luteal function in the ewe and other species.

The above discussion emphasizes that considerable species differences exist in the action of phorbol esters and therefore probably the role played by protein kinase C in luteal steroidogenesis. The demonstration in all the above mentioned species, rat, cow, pig and sheep, of PGF2α induced increases in phospholipid turnover suggest that the interaction between PGF2α and the luteal membrane is similar among species. However, the differences in response to phorbol esters suggests that phospholipid turnover in luteal cell membranes may not always lead to activation of protein kinase C and/or that activation of protein kinase C itself induces different cellular responses. Further, studies are required to clarify these differences.
SECTION I: DIRECT LUTEOTROPIC EFFECT OF ESTROGEN ON PORCINE CORPORA LUTEA

Abstract

The direct effects of estradiol-17β (E2) on porcine corpora lutea (CL) were investigated using E2-releasing silastic implants placed inside individual CL on an ovary in 15 normal cycling gilts. Three groups (5 gilts/group) received intra-luteal implants releasing about 4.4 ± 1.1 (Group 1), 15.0 ± 1.1 (Group 2) or 22.0 ± 1.0 (Group 3) µg E2/d, respectively, as determined by in vitro incubation of implants of similar wt and E2 contents. On day 11 of the estrous cycle, (day 0 = first day of estrus), 3 CL on one ovary received E2-implants, 3 CL on the other received vehicle implants while all other CL on both ovaries served as uninjected control CL. An additional group of 6 animals served as non-E2 implanted controls and included 2 sows and 2 gilts receiving bilateral vehicle implants on day 11 and 2 unoperated gilts. All animals were slaughtered on day 19 of their estrous cycle and the wt, progesterone content and concentration of each CL were measured. In Group 3 gilts, luteal wt, progesterone content and concentration were greater (P<.01) in E2-implanted (345.0 ± 20.7 mg, 22.99 ± 2.65 µg and 66.20 ± 5.52 ng/mg, respectively) than in vehicle implanted CL (276.4 ± 18.7 mg, 16.45 ± 2.20 g and 58.7 ± 5.6 ng/mg, respectively) which were similar to uninjected control CL. Further, all CL of Group 3 gilts were heavier and contained a greater content and concentration of progesterone (P<.01) than gilts in Groups 1 and 2, as well as non-E2 implanted controls whose values were similar. These data demonstrate that E2 can directly stimulate the growth of an individual CL, however, the E2 release rate must be high enough to bilaterally maintain all CL before this effect is seen.

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

Introduction

Luteal maintenance in pigs is dependent on continued pituitary luteotropic support (Anderson et al., 1967) and the abrogation of the luteolytic influence of the uterus (Anderson et al., 1965). Kidder et al. (1955) first reported that
exogenous estrogen extended luteal function in pigs and since then many studies have investigated the mechanism involved in this effect. Bazer et al. (1986) reviewed data which indicate that estrogen decreases the release of prostaglandin F\textsubscript{2\alpha} into the uterine vein thereby reducing the luteolytic signal, but estrogen may also have a direct effect on corpora lutea (CL) in the pig. It has been reported that estrogen prevented PGF\textsubscript{2\alpha}-induced luteolysis in hysterectomized gilts (Kraeling et al., 1975) and increased luteal LH receptor numbers in both pregnant and hysterectomized animals (Garverick et al. 1982). Neither these nor other experiments performed so far, however, have been able to exclude the possibility of estrogen induced changes in pituitary luteotropic support (Chakraborty et al. 1972) or uterine mediated effects in the same animal. In addition, most experiments to date have utilized high levels of estrogen without regard to what might be a minimally effective dose by the route of administration chosen. Therefore, the following experiment was performed to investigate the direct luteotropic effect of estradiol-17\beta (E\textsubscript{2}) in gilts by the constant unilateral exposure of 3 CL on an ovary to E\textsubscript{2} released from a silastic implant inside each CL while 3 CL on the opposite ovary received vehicle control implants. This model assumes that both luteotropic and luteolytic influences will be similar for both the E\textsubscript{2}-treated and vehicle control ovary. Furthermore, the level of E\textsubscript{2} exposure was varied to determine the lowest level which would induce luteal maintenance.

**Materials and Methods**

**Experimental protocol**

Various aspects of the protocol were determined on the basis of results obtained in a preliminary study (Conley and Ford, 1988). Accordingly, 15 gilts, 6-8 months of age weighing 120-150 Kg which had exhibited two estrous cycles of normal duration (19-21 days), were assigned to surgery on day 11 (day 0 = first day of estrus). Anesthesia was induced with Surital (Parke-Davis, Morris Plains, NJ) and maintained with Halothane (Halocarbon Laboratories Inc., Hackensack, NJ) and oxygen (Magness and Ford, 1982).
Ovaries were exposed by mid-ventral laparotomy and numbers of CL were recorded. Three randomly selected CL on one ovary received estrogen implants while 3 CL on the opposite ovary received vehicle (silastic only) implants. All other remaining CL on each ovary were left as uninjected control CL. Therefore within each gilt 4 sets of CL could be recognized consisting of E₂ implanted and uninjected control CL on one ovary and vehicle implanted and uninjected control CL on the other (Figure 1). Each injected CL was marked with 3-0 silk for identification and ovaries were replaced in the body cavity. At a subsequent laparotomy on day 19, all gilts were ovariectomized. All CL were dissected from the ovaries and weighed. Implanted CL were weighed before and after implant removal. Each CL was homogenized separately in saline and stored at -70°C for radioimmunoassay of progesterone.

Control animals

Four animals were used to investigate the effects of bilateral vehicle implants on luteal wt, progesterone content and concentration. Procedures were identical to those described above except that vehicle implants were placed into 3 CL on each ovary of each animal. Two additional gilts were slaughtered at day 19 of the estrous cycle as nonsurgical controls for comparison.

Implant preparation

Silastic compound was prepared by mixing equal quantities of Medical Grade Elastomer #382 and Medical Fluid #360 (Dow Corning Corporation, Midland, MI) with catalyst (stannous octoate, Dow Corning Corporation, Midland MI) in an ethanol-rinsed test tube which resulted in curing of the compound within 3 min. During this period the compound remained fluid enough to pass through an 18 G needle, enabling the injection of 25-50 μl of silastic into individual CL exposed at surgery and the in situ formation of solid intraluteal implants weighing approximately 34 mg. Estrogen implants were prepared by combining crystalline E₂ with silastic compound at the following concentrations: 5, 15 and 50 mg of estrogen/ml (groups 1, 2, & 3 respectively)
and therefore, implants of 34 mg were estimated to contain a total of about 150, 500 and 1500 µg of E2, respectively.

**Implant release rate in vitro**

Estrogen release rate from implants of similar wt and E2 contents was determined by in vitro culture at 37°C in plasma pooled from castrated male pigs. Implants at each level of estrogen were incubated in 2 ml of plasma for 7 days. Plasma was changed daily, frozen and stored at -70°C until assayed for E2 by radioimmunoassay. All samples were included in a single assay.

The release rate of E2 from intraluteal implants cultured in vitro remained constant over the 7 day incubation period (P > .5). Implants containing 150, 500 and 1500 µg of E2 released 4.4 ± 1.1, 15.0 ± 1.1 and 22.4 ± 1.0 µg/d, respectively (P < .01).

**Assays**

Both progesterone and E2 radioimmunoassays were run as previously described and validated in this laboratory for porcine luteal tissue (Ford and Christenson, 1979) and plasma (Magness and Ford, 1982). Sensitivity of these assays were defined as that amount of steroid that yielded 95% of the cpm in the buffer control tubes; this amount was 2 pg and 50 pg for E2 and progesterone, respectively. Recovery rates averaged over 90% for both steroids. Intraassay coefficients of variation (CV) for estrogen (13.7 ± 5.1 pg/ml) and progesterone (27.22 ng/ml) pools were 9.2 and 11.3% respectively, and interassay CV for progesterone was 10.0%. All samples from a gilt were assayed for progesterone together in a single assay.

**Statistical analysis**

Arithmetic means were calculated for each set of CL within each gilt (Figure 1). The variable number of remaining uninjected control CL among gilts resulted in greatly unbalanced data so only means and standard errors have been included in tables for comparative purposes. Differences between E2 and vehicle
implanted CL were evaluated by analysis of variance of a completely randomized split plot where level of E2 was the main plot and luteal treatment (E2 or vehicle implants) was the subplot. The in vitro release of E2 from implants was analyzed by one way analysis of variance to determine differences among levels, and linear regression was conducted to test linear and quadratic trends of release over time. Vehicle implant effects on luteal wt, progesterone content and concentration of control sows and gilts with bilateral vehicle implants were tested by analysis of variance for a randomized block where animals were considered to be blocks.

Results

Weight, progesterone content and concentration of all CL were significantly elevated (P<.01) in Group 3 gilts when compared to those in either of the other two groups which were similar (Table 1).

Further, in Group 3 gilts, E2 implanted CL were heavier (P<.01) and had higher (P<.01) progesterone content and concentration than vehicle implanted CL (Table 1). When a frequency distribution of luteal wt was compiled among Groups 1, 2, & 3 a bimodal distribution appeared to be obtained suggesting that CL were either maintained (Group 3) or not (Groups 1 & 2). No linear effect of level of E2 on CL was obtained (Figure 2). In addition, ovaries of gilts in Groups 1 and 2 consistently contained populations of 8 to 12 mm follicles consistent with the approach of estrus. Follicular development on ovaries from Group 3 gilts however, consisted exclusively of 3 to 6 mm follicles, consistent with the presence of functional CL. There were no consistent differences in wt or progesterone content or concentration between vehicle implanted CL and uninjected CL on either ovary, within groups of gilts.

In control pigs there were no differences between vehicle implanted and unimplanted CL in luteal wt, progesterone content or concentration which averaged 152.8 mg, 1.35 µg and 9.64 ng/mg, respectively (P>.05). These values were similar to those observed in the two unoperated control gilts whose average luteal wt, progesterone content and concentration were 115.5 mg, 0.55 g and 4.57 ng/mg respectively. As observed for gilts in Groups 1 and 2, ovarian follicular development indicated approaching estrus. Thus in comparing the
luteal characteristics of gilts in Groups 1, 2 & 3, with those receiving bilateral vehicle implants and nonsurgical control gilts, only CL in Group 3 gilts appeared to have been maintained.

Discussion

This study is the first to demonstrate in vivo a direct luteotropic effect of estrogen on pig CL. These data show that intraluteal implantation of E2 selectively increased luteal wt and progesterone content when compared to other CL on the same ovary or on the opposite ovary in gilts exhibiting luteal maintenance. Luteal hypertrophy is detectable by day 24 to 26 post-estrus in pregnant and hysterectomized gilts which have been unilaterally ovariectomized early in the luteal phase (Rathmacher et al., 1967; Staigmiller et al., 1972, 1974). Since compensatory ovarian hypertrophy in the pig is accompanied by increases in follicular size and ovarian venous estrogen concentrations (Redmer et al., 1984), it is tempting to speculate that a similar local exposure of CL to these increases in estrogen may result in an increase in luteal wt. Although no changes in systemic LH concentrations occur following unilateral ovariectomy (Redmer et al., 1984) or during estradiol benzoate induced pseudopregnancy (Flowers et al., 1987), Garverick et al. (1982) reported that estradiol increased LH receptor levels in CL of pregnant and hysterectomized gilts. Therefore, it is possible that the increase in luteal wt observed in the present study may be due in part to increased LH sensitivity and response, perhaps through increased luteal LH receptor populations. Whether or not this reflects hyperplasia or simply luteal hypertrophy is not presently known; however, both LH and HCG have recently been shown to induce increases in luteal cell size in mid-luteal phase ewes (Farin et al., 1988).

Two other important points emerged from the present study. Firstly, a minimally effective luteotropic dose of intra-luteal E2 was determined which, even when administered unilaterally, still resulted in bilateral luteal maintenance. Saunders et al. (1983) failed to observe any increased potency of E2 in inducing luteal maintenance when 100 μg of E2/d was infused into the uterine lumen as compared to the same daily treatment administered subcutaneously. However, Ford et al. (1982a) demonstrated bilateral luteal maintenance and
reduced utero-ovarian venous PGF$_{2\alpha}$ concentrations in gilts following intrauterine infusion of just 1.5 µg of E$_2$/d. Therefore, it is unlikely that Saunders et al. (1983) could have detected any such sensitivity difference at the levels of estrogen used in that study even though they were 50 fold less than those commonly employed to induce pseudopregnancy in pigs (Geisert et al., 1987).

The minimally effective luteotropic level of E$_2$ used in the present study (67 µg/ovary/d) was also much higher than that found to be luteotropic when administered by an intrauterine route (Ford et al. 1982a). Therefore, it seems probable that the systemic effect of E$_2$, inducing bilateral luteal maintenance in this study, is due to an effect on the uterus, possibly through a bilateral reduction of PGF$_{2\alpha}$ release into the utero-ovarian vein such as that noted by Ford et al. (1982a) and as first described by Frank et al. (1977). This supports the conclusions of Bazer et al. (1986) that the primary effect of estrogen which results in the maintenance of luteal function in pigs is a decrease of the luteolytic signal.

At present there seems to be few published reports refuting the notion that estrogen maintains luteal function by an effect on the uterus. However, some evidence does suggest that the embryo may exert a direct luteotropic effect. This evidence includes the high incidence of unilateral maintenance (Christenson and Day, 1971) and elevated progesterone concentrations (Ford and Christenson, 1979) of CL adjacent to the gravid horn in unilaterally pregnant pigs. In addition, Ford et al. (1982b) observed transient increases in luteal blood flow in gilts at the time of maternal recognition of pregnancy. More importantly, Ball and Day (1982) used intraluteal implants containing porcine embryonic extract to maintain the function of individual CL on an ovary. In contrast to the results of the present study, no systemic luteotropic effect seemed to be necessary to elicit this response suggesting that embryonic extract directly prevented the luteolytic effects of the uterus at the level of the CL. Furthermore, the active principle(s) was heat stable and charcoal extractable indicating that it was of low molecular wt, possibly a steroid. The data obtained in this study would suggest that, if involved, estrogen is not the only luteotropic factor produced by the porcine embryo. The identity of additional factors such as prostaglandin E$_2$ (Akinlosotu et al., 1986) as embryonic luteotropins remains to be determined.
The second important point to emerge from these studies is that these data clearly demonstrate the all-or-none effect of estrogen on luteal maintenance (Figure 2) previously described by Gardner et al. (1963). Similarly, an identical study to that reported here was performed in sows and included the same estrogen treatments plus an additional higher level, but still no linear effect of level of estrogen exposure of CL was seen on any luteal characteristic measured (Conley and Ford, 1988). At present, no explanation can be offered for the mechanism by which this event occurs.

Finally, the results of this study suggest that implantation of CL as described had no measurable effect on luteal wt, progesterone content or concentration. It was suggested by Ball and Day (1982) that the implantation procedures utilized in their study may have caused some luteal damage. Luteal implants used in this study weighed 10% of average CL wt but still release adequate levels of E₂ to maintain luteal function and release rate was relatively constant. Therefore, this procedure seemed to provide an effective means by which to investigate the effects of local exposure of CL to steroids and possibly other compounds on porcine luteal function.

In conclusion, this study demonstrates both direct and indirect effects of estrogen on luteal maintenance in pigs. Further studies will be necessary to determine the physiological relevance of the local exposure of CL to estrogen and other blood-borne factors released by the porcine conceptus.

Literature Cited


Table 1. Means ± standard errors of luteal weight (CLWT), luteal tissue progesterone content (CONT) and luteal tissue progesterone concentration (CONC) of groups of CL within gilts given intraluteal estradiol-17β (E2) implants

<table>
<thead>
<tr>
<th>Treatmentsa</th>
<th>CL groupb</th>
<th>Luteal characteristic</th>
<th>Group 1 (4 µg E2/d)</th>
<th>Group 2 (15 µg E2/d)</th>
<th>Group 3 (23 µg E2/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2 implanted OVARY 1</td>
<td>CLWT (mg)</td>
<td>119.9 ± 10.9</td>
<td>164.7 ± 47.7</td>
<td>345.0 ± 20.7x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CONT (µg)</td>
<td>.4 ± .2</td>
<td>3.0 ± 2.7</td>
<td>23.0 ± 2.7x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CONC (ng/mg)</td>
<td>3.3 ± 1.3</td>
<td>10.1 ± 7.4</td>
<td>66.2 ± 5.5x</td>
<td></td>
</tr>
<tr>
<td>Vehicle implanted OVARY 2</td>
<td>CLWT (mg)</td>
<td>130.2 ± 5.5</td>
<td>172.3 ± 43.4</td>
<td>276.4 ± 18.7y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CONT (µg)</td>
<td>4.8 ± 1.1</td>
<td>3.8 ± 3.0</td>
<td>16.5 ± 2.2y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CONC (ng/mg)</td>
<td>3.7 ± .9</td>
<td>14.7 ± 8.6</td>
<td>58.7 ± 5.6y</td>
<td></td>
</tr>
<tr>
<td>Unimplanted control OVARY 1</td>
<td>CLWT (mg)</td>
<td>103.3 ± 9.4</td>
<td>151.0 ± 41.0</td>
<td>278.6 ± 19.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CONT (µg)</td>
<td>.4 ± .2</td>
<td>2.5 ± 2.2</td>
<td>18.4 ± 1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CONC (ng/mg)</td>
<td>3.7 ± 1.6</td>
<td>9.4 ± 6.9</td>
<td>65.6 ± 4.7</td>
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</tr>
<tr>
<td>Unimplanted control OVARY 2</td>
<td>CLWT (mg)</td>
<td>92.3 ± 6.9</td>
<td>139.8 ± 51.9</td>
<td>266.4 ± 14.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CONT (µg)</td>
<td>.4 ± .1</td>
<td>3.5 ± 2.9</td>
<td>18.4 ± 1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CONC (ng/ml)</td>
<td>4.1 ± 1.1</td>
<td>15.5 ± 8.6</td>
<td>68.9 ± 3.7</td>
<td></td>
</tr>
</tbody>
</table>

aLuteal characteristics of Group 3 gilts were significantly different (P<.05) from those of Group 1 and Group 2 which were similar.

bUnimplanted control CL from ovary #1 and ovary #2 not included in statistical analysis.

x,yFor each luteal tissue characteristic means within a column, with different super scripts differ (P<.05).
Figure 1. Schematic diagram depicting implant placement in estrogen-implanted gilts
• ESTROGEN IMPLANTED

• VEHICLE IMPLANTED

□ UNINJECTED CONTROL
Figure 2. Frequency distribution of individual luteal weights of estrogen-implanted gilts in groups 1, 2 and 3 obtained at slaughter on d 19 of the estrous cycle.
SECTION II: EFFECT OF INTRALUTEAL ESTRADIOL-17\(\beta\) IMPLANTS ON WEIGHT AND PROGESTERONE SECRETION OF PORCINE CORPORA LUTEA

Abstract

Previous studies have demonstrated that estradiol-17\(\beta\) (E\(_2\)) locally increases corpus luteum (CL) wt in pigs, suggesting a direct luteotrophic effect. This study examined the effect of intraluteal E\(_2\) implants on utero-ovarian vein (UOV) progesterone (P\(_4\)), E\(_2\), PGF\(_{2\alpha}\) and CL wt in cyclic gilts. Eight gilts were laparotomized on day II (day 0 = first day of estrus), catheters were placed in both UOV and gilts were then divided into 2 equal groups (E\(_2\)-treated and controls). Each CL on one ovary of E\(_2\)-treated gilts was injected with a quick curing silastic implant containing E\(_2\) while each CL on the contralateral ovary received a vehicle implant. Control gilts received bilateral vehicle implants. Blood was collected at 10 min intervals for 1 hour beginning at 0600 and 1800 h from day 11 to day 18. Gilts were ovariectomized on day 19. Corpora lutea were weighed, homogenized and assayed for P\(_4\). Utero-ovarian venous plasma was assayed for P\(_4\), E\(_2\), and PGF\(_{2\alpha}\) by validated RIA. No side differences were observed in CL wt or UOV P\(_4\) within groups. CL were maintained in E\(_2\)-treated, but not control gilts as evidenced by CL wt (333 ± 20 vs 154 ± 12mg), CL P\(_4\) content (21.8 ± 2.1 vs .3 ± .1g), and CL P\(_4\) concentration (65.7 ± 5.5 vs 2.3 ± .6ng/mg), respectively. Utero-ovarian venous P\(_4\) values were similar between groups up to 1800 h on d 15, declining from 346 ± 122ng/ml at 1800 hour on day 11 to 147 ± 20ng/ml on day 12, then remaining constant to 1800 hour on day 15. Thereafter UOV P\(_4\) in E\(_2\)-treated gilts returned progressively to day 11 values by 1800 hour on day 18 (364 ± 97ng/ml) while values of control gilts declined to low levels (5.4 ± .6ng/ml). In E\(_2\)-treated gilts in UOV plasma E\(_2\) was elevated 13 fold ipsilateral, and 4 fold contralateral, to the E\(_2\)-treated ovary in comparison to levels in control gilts. Uterine PGF\(_{2\alpha}\) release was suppressed in E\(_2\)-treated compared to control gilts. E\(_2\) (\(\mu\)g quantities) failed to prevent the initial decline in UOV P\(_4\) between day 11 and day 15, but subsequently returned CL function to normal, as evidenced by increasing UOV P\(_4\), through day 18.

(Key Words: Estrogen, Pig, Corpus Luteum, Prostaglandin F\(_2\), Progesterone.)
Introduction

Despite intensive efforts, the mechanism whereby estrogen extends luteal function in the pig and its role in pregnancy maintenance in this species is still unclear. Several studies support the idea that estrogen decreases uterine prostaglandin F2a (PGF2a) release in pigs (Bazer et al., 1986). However, most previous studies of estrogen-induced luteal maintenance in pigs have used relatively high doses of this hormone (Kidder et al., 1955; Gardner et al., 1963; Geisert et al., 1987) including those studies additionally investigating uterine PGF2a release following estrogen administration (Frank et al., 1978; Guthrie and Rexroad, 1981; Flowers et al., 1987).

Recent studies in this laboratory have conclusively demonstrated, for the first time, both direct (local) and indirect (systemic) luteotropic effects of estradiol-17β (E2) on luteal function in gilts (Conley and Ford, 1988). These investigations established a minimally effective level of E2 which had direct luteotropic effects on corpora lutea (CL) but these effects were only observed if CL were already maintained by a systemic mechanism. However, in these studies, luteal function was evaluated solely by obtaining luteal tissue measurements on day 19. The functional activity of E2-implanted versus other maintained CL (vehicle implanted and control) were not investigated at any time post-implantation. Additionally, daily E2 release rates, in these studies, were estimated from in vitro incubation of E2-containing implants maintained in porcine plasma at 37°C for 7 d, no attempt was made to determine release rates in vivo (Conley and Ford, 1988).

Therefore, the following experiment was designed to measure ovarian progesterone secretion in pigs with luteal maintenance induced by E2 releasing intraluteal implants. Concentrations of E2 released into the utero-ovarian vein were also determined to evaluate the in vivo hormone release rate over several days and utero-ovarian venous PGF2a was quantified to monitor uterine PGF2a release under conditions of E2 administration approximating minimally effective doses.
Materials and Methods

Experimental protocol

Eight Yorkshire gilts weighing approximately 160 kg and exhibiting estrous cycles of normal duration (19 to 21 days) were randomly assigned to (E2) treatment or control groups for surgery on day 11 of the estrous cycle (day 0 = first day of standing heat). Anesthesia was induced with thiamylal (Surital, Parke-Davis, New Jersey), and maintained with halothane and oxygen (Magness and Ford, 1982). The reproductive tract was exposed by midventral laparotomy, numbers of CL on each ovary were counted and heparinized polyvinyl catheters were placed 15 cm into each utero-ovarian vein through a branch of the tubal vein adjacent to each ovary. Catheters were secured in place and all CL on an ovary received intraluteal implants containing E2 or vehicle as described previously (Conley and Ford, 1988). Briefly medical grade elastomer and fluid (#480 and 382 respectively, Dow Corning Corp, Michigan) were mixed in an ethanol-rinsed test tube. Catalyst was added and the mixture was drawn into a 1 ml syringe. Approximately 50 μl was injected into each CL on the ovary through a sterile 18 gauge needle. Estrogen implants contained approximately 1500 μg of crystalline E2 and each implant released approximately 20 μg/day for 7 days (Conley and Ford, 1988), while vehicle implants consisted of silastic only. Estrogen-treated gilts received E2 implants into all CL on one ovary and vehicle implants into all CL on the other. Control gilts received vehicle implants into all CL on both ovaries.

Utero-ovarian venous blood was collected twice daily at 0600 and 1800 hour each day from 1800 on day 11 until 0600 h on day 19. Collection consisted of seven 2 ml samples of blood drawn with 1 cc syringes every 10 minute for 1 hour. Blood was centrifuged, plasma was collected, frozen immediately and stored at -88°C until assayed. Following blood collection at 0600 hour on day 19, gilts were laparotomized as before, ovaries were removed, and CL dissected, weighed and homogenized.
Assays

Progesterone in plasma (Magness and Ford, 1983) and luteal tissue (Ford and Christenson, 1979) was measured by radioimmunoassay as previously described. Samples were extracted with a mixture of benzene-hexane (1:2; >90% recovery) and assayed with antibody #GDN-337 (G. D. Niswender, Colorado State University, Colorado). The sensitivity of the assay as defined as the amount of steroid yielding 95% of the counts in the buffer control tubes was approximately 50 pg/tube. Luteal samples were assayed in a single assay. Pooled luteal tissue samples measured 15.7 ± 1.3 µg/CL with an intraassay CV of 19%. Plasma was assayed along with standards which measured 29.14 ± .80 ng/ml. The intraassay coefficient of variation was 9.8% and the interassay coefficient of variation was 3.9%.

Plasma was pooled within side, hour, and time of day for each gilt (28 samples • gilt-1 • day-1) prior to assay for PGF2α according to the method of Van Orden and Farley (1973). Briefly, samples were extracted with ethyl acetate and chromatographed on silicic acid columns to separate PGF2α using benzene, ethyl acetate and methanol solvent. The dried eluate was assayed in cold phosphate buffer (.1 M) with bovine gamma globulin (2 mg/ml). Characterization of the antibody used has been published (Van Orden & Farley, 1973) and cross-reactivities are as follows: PGF1α, 77%, PGE1, 1%; PGE2, .25% and PGA1, 0%. Recoveries averaged 68%. Separation of bound from unbound PGF2α was achieved with polyethylene glycol (25%) and counted in a Packard Tri-Carb liquid scintillation counter. Standard curves ranging from 1.25 to 1000 pg were diluted in assay buffer and 5 pg was significantly different from zero (P < .05). The intra- and interassay coefficients of variation were 3.5 and 10.5%, respectively.

Estradiol-17β was quantified in UOV plasma by a previously described and validated radioimmunoassay (Magness and Ford, 1983). Samples from each side were pooled within day for E2-treated gilts, but across sides within day in control gilts. Plasma was extracted three times with benzene, and the extract was subsequently washed with sterile water. E2 was separated on sephadex LH-20 (Sigma Chemical Co., St. Louis, Missouri) chromatographic columns. Internal recoveries were used to determine procedural losses in each sample. Radioim-
munoassay was performed using the antiserum S-310 No.5/G. Abraham with previously characterized cross-reactivities (Tulchinsky and Abraham, 1971). The sensitivity of the assay, defined as the amount of E2 displacing 95% of the counts in the buffer control tubes, was 2 pg. The intra- and inter-assay coefficient of variation were 8.6 and 4.9%, respectively.

Statistical analysis

Statistical significance was determined by analysis of variance for a completely randomized design, and by using a split plot analysis and linear regression for time effects.

Results

No differences were seen due to effects of side of estrogen or vehicle implants in UOV plasma progesterone concentrations (P4) or luteal characteristics and therefore, UOV P4 data were pooled across sides. Utero-ovarian venous P4 was similar and decreased (P < .05) in both E2-treated and control groups from 1800 hour on day 11 to 1800 hour on day 12, then remained constant through 0600 hour on day 14. Thereafter, UOV P4 in estrogen implanted gilts to values that were similar to those on day 11. Concentrations of progesterone in UOV plasma of control gilts continued to decline through day 18 (Fig. 1).

Prostaglandin F2α concentrations in UOV plasma (Fig. 2) were higher on day 12 in control gilts than in E2-treated gilts (495 ± 121 and 175 ± 26 pg/ml respectively, P < .05). Thereafter, PGF2α concentrations progressively increased in control gilts to reach a maximum of 1980 ± 1047 pg/ml on day 16, then decreased to 588 ± 124 pg/ml on d 18. Utero-ovarian venous PGF2α concentrations remained constant (P > .5) in E2-treated gilts throughout the experiment averaging 166 ± 11 pg/ml (Fig. 2).

Luteal weight, progesterone content and concentration were higher in E2-treated versus control gilts averaging 333.3 ± 20.1 and 153.7 ± 11.7 mg, 21.8 ± 2.1 and .3 ± .1 µg and 65.7 ± 5.5 and 2.3 ± of ng/mg respectively. No significant correlations were found between any luteal characteristics and implant weight.
which averaged 38.5 ± 2.8 mg and no significant correlations were found between any luteal parameter and UOV progesterone concentrations within the E2-treated group.

E2 concentrations in the UOV of E2-treated gilts were constant from day 12 to day 18 (Figure 3), but were higher on the side ipsilateral to the ovary receiving E2 implants compared to the side receiving vehicle implants (P < .01), averaging 815.5 ± 70.4 and 180.5 ± 13.0 pg/ml, respectively. In contrast, the level of E2 in the UOV of control gilts was lower than that in E2-treated gilts (P < .05) remaining below 50 pg/ml until day 15 when levels increased linearly (P < .01) to reach 250.6 ± 111.7 pg/ml on day 18.

Discussion

Estrogen synthesis associated with embryonic elongation and the maternal recognition of pregnancy in pigs, occurs around day 12 (Perry et al., 1976). Peaks of prostaglandin metabolite (PGFM) are seen in both pregnant and nonpregnant pigs on day 12 but in pregnant animals uterine PGF2α release is suppressed thereafter (Shille et al., 1979). Parenteral administration of E2 to pigs between day 11 and day 15 also results in luteal maintenance (Geisert et al., 1987) and inhibits uterine PGF2α release (Frank et al., 1977). Therefore it has been suggested that estrogen is the embryonic signal inducing luteal maintenance by reducing the uterine luteolytic signal (Thatcher et al., 1986).

However, other evidence suggests that both the conceptus and estrogen have direct luteotropic effects in pigs. Unilaterally pregnant pigs have a high incidence of unilateral maintenance of CL and heavier luteal weights on the side ipsilateral to the gravid uterine horn (Niswender et al., 1970; Christenson and Day, 1971). Recent studies in this laboratory have demonstrated an increase in the weights of CL implanted with E2-releasing silastic implants (Conley and Ford, 1988). No similar, preferential increase in luteal weights of E2-implanted CL was observed in the present study possibly due to the higher exposure of all CL to circulating E2 because of the greater number of implants utilized in the present study (6.5 ± .5, present study versus 3, Conley and Ford, 1988). Ford et al. (1982b) observed locally elevated concentrations of P4 in UOV of sows infused unilaterally with 1.5 g E2/d into an isolated uterine horn. No corresponding
unilateral effects were noted with regard to luteal weight or uterine PGF2α release which was equally suppressed on both sides (Ford et al., 1982b). Therefore, both pregnancy and estrogen treatment appear to have local effects on luteal function not adequately explained by a reduction of uterine PGF2α release.

Intraluteal E2 implants elevated the levels of E2 in UOV plasma several fold over those of control gilts. From day 12 to 15, E2 concentrations in the UOV ipsilateral and contralateral to E2 intraluteal implants were 13 and 4 times respectively, greater than controls. PGF2α was not quantified in the UOV separately by side in E2-treated gilts. However, the very low levels of PGF2α measured in UOV plasma pools from E2-treated gilts suggests that if the suppression of uterine PGF2α release was different by side, that the difference was minor in comparison to the levels of UOV PGF2α in control gilts during this period. By day 18 E2 in UOV plasma of control gilts had increased to levels similar to that in UOV plasma draining the vehicle-implanted ovary of E2-treated gilts, suggesting that increases in UOV E2 concentrations equal to proestrous levels may be adequate to prevent uterine PGF2α release. How such levels relate to the amount of E2 produced and released from the uterus by pig embryos is difficult to assess. Ford et al. (1982a) reported 3 pg/ml as the arterial-venous difference in E2 across the uterus in pigs on day 13 post-mating. Although the levels of UOV E2 contralateral to the E2 implant were similar to those found toward the end of the luteal phase, these are still excessive in comparison to those induced by porcine conceptuses at the time of maternal recognition of pregnancy in this species.

The results of the present study also demonstrate a delay in the luteotropic effects of estrogen which were not evident until day 15. Ford et al. (1982b) also reported a delay of 2-3 days from the time on initiation of E2 treatment and elevations of UOV P4 concentrations on the side ipsilateral to the E2-treated as opposed to the vehicle-treated uterine horn. In neither experiment were these increases in luteal progesterone secretion related to changes in uterine PGF2α release. Estrogen has been shown to increase luteal receptors numbers in hysterectomized gilts (Garverick et al., 1982) and luteal cells collected from estrogen-induced pseudopregnant sows demonstrate increased sensitivity to LH (Grazul et al., 1986). The delay in the onset of increase in UOV P4 concentrations
in these experiments may result from the time taken for CL in the pig to develop an increase in responsiveness to pituitary luteotropins.

In addition to the observed luteotropic effects of E2, gilts receiving E2 implants also experienced a transient decline in UOV\textsubscript{4} concentrations. The 24 to 36 hour period following surgery during which UOV\textsubscript{4} concentrations were stable in control gilts suggests that the implantation procedure itself did not affect luteal P\textsubscript{4} secretion, and the subsequent decline coincided with elevations in UOV PGF\textsubscript{2\alpha} concentrations marking the onset of luteolysis. Others have observed a similar decrease in systemic progesterone concentrations following E\textsubscript{2} treatment (Guthrie and Rexroad, 1981; Ziecik et al., 1986). Estradiol-17\textbeta\ treatment on day 11 to day 15 of the estrous cycle in gilts has been shown to decrease LH concentrations (Flowers et al., 1987). However, Ziecik et al. (1986) administered twice the daily dose over the same interval as that used by Flowers et al. (1987). No decrease in LH was observed that might explain the decline in systemic progesterone concentrations (Ziecik et al., 1986). Further studies will be required to elucidate the mechanism whereby estrogen transiently inhibits luteal function in pigs.

In conclusion, this study demonstrates that E\textsubscript{2} can have both inhibitory and trophic effects on luteal function in pigs when administered during the midluteal phase of the estrous cycle. Neither the trophic nor the inhibitory effects can be attributed solely to modification of the release of PGF\textsubscript{2\alpha} from the uterus and may involve the action of other as yet unidentified factors. Although the levels of administered E\textsubscript{2} were considerably less than most previous studies, they are still in excess of those produced by the conceptus and therefore may not relate directly to the events occurring at the time of maternal recognition of pregnancy.

Literature Cited


Figure 1. Utero-ovarian venous concentrations of progesterone in estrogen and vehicle implanted gilts from day 11 to 18 of the estrous cycle
Figure 2. Utero-ovarian venous concentrations of prostaglandin F₂ in estrogen and vehicle implanted gilts from day 12 to 18 of the estrous cycle. Each point represents the value for a pool of 28 individual samples.
Figure 3. Utero-ovarian venous concentrations of estradiol-17 (E2) ipsilateral and contralateral to the E2-treated ovary in E2-treated gilts, and pooled across sides in control gilts
UTERO-OVARIAN VEIN ESTRADIOL
-17β CONCENTRATION (pg/ml)

DAY OF ESTROUS CYCLE

ESTROGEN TREATED GROUP (n = 4)
ESTRADIOL -17β IMPLANTED SIDE

ESTROGEN TREATED GROUP (n = 4)
VEHICLE IMPLANTED SIDE

BILATERAL VEHICLE CONTROL
POOLED BOTH SIDES (n = 4)
SECTION III: EFFECTS OF PGF\(_{2\alpha}\) ON PORCINE CORPORA LUTEA FOLLOWING ADMINISTRATION ON DAY 9 OF THE ESTROUS CYCLE

Abstract

During the period prior to day 12 of the estrous cycle, pig corpora lutea (CL) are apparently refractory to the luteolytic effects of prostaglandin F\(_2\) (PGF\(_{2\alpha}\)). This study investigated functional and structural effects of PGF\(_{2\alpha}\) on pig CL during this refractory period. Eight gilts were unilaterally ovariectomized on day 8 (first day of estrus = day 0) and femoral arterial (FA) catheters were inserted. Gilts received 20 mg PGF\(_{2\alpha}\) or saline at 0700 hour on day 9. Following PGF\(_{2\alpha}\) or vehicle treatment, blood was collected hourly for 6 hours, then twice daily (0700 and 1900 hour) until the remaining ovary was removed at 0700 to 800 hour on day 12. Progesterone had declined markedly in the FA by 3 hour following PGF\(_{2\alpha}\), but had returned to pretreatment levels by 1900 hour on day 11. Increases in luteal weight, protein and DNA were observed (P < .05) from day 8 to day 12 and were similar for both PGF\(_{2\alpha}\) and vehicle groups averaging 23.5 ± 6.6, 29.2 ± 9.5 and 46.0 ± 10.0% respectively. The protein to DNA ratio decreased similarly in both groups during this same period by 12.5 ± 3.2% (P < .01). Progesterone content increased more (P < .05) in control (9.8 ± 2.5 μg/CL) than in PGF\(_{2\alpha}\)-treated gilts (3.2 ± .7 μg/CL). Luteal progesterone concentration increased in control (.814 ± .256 μg/mg protein, P < .05) but not in PGF\(_{2\alpha}\)-treated gilts (.104 ± .39 μg/mg protein, P > .1). These data indicate that PGF\(_{2\alpha}\) administered to pigs on day 9 of the estrous cycle transiently inhibits luteal function without detectable effects on luteal composition or growth which continues from day 8 to day 12 possibly by increases in the numbers of small, non-steroidogenic cell types.

(Key Words: Porcine, Corpus Luteum, Prostaglandin, Luteolysis.)

Introduction

In most domestic animal species, the first 4 to 6 days of the estrous cycle represent a refractory period during which PGF\(_{2\alpha}\) is unable to significantly alter
luteal life span. After this time, PGF$_{2\alpha}$ effectively induces luteolysis which is characterized by a rapid reduction in luteal progesterone secretion and a rapid return to estrus (Horton and Poyser, 1976; Hansel and Convey, 1983). The pig is thought to be unusual in that PGF$_{2\alpha}$ is unable to induce luteolysis prior to day 10 to 12 of the estrous cycle (Diehl and Day, 1974; Hallford et al., 1975). Even so, prior to day 10 of the estrous cycle in pigs, exogenous PGF$_{2\alpha}$ has been shown to cause significant although transient reductions in systemic progesterone concentrations (Gleeson, 1974; Connor et al., 1976), and histologically recognizable luteal degeneration (Krzymowski et al., 1978). However, these studies did not determine quantitatively the effects of PGF$_{2\alpha}$ administration on luteal tissue. Luteal degeneration would be expected to result in reductions in luteal weight, progesterone concentration and/or possibly changes in luteal cell numbers which might be reflected in DNA content of corpora lutea (CL). The following study was performed to determine the effect of administration of PGF$_{2\alpha}$ on dy 9 of the estrous cycle of pigs on these luteal characteristics measured 3 day after injection.

Materials and Methods

Experimental protocol

Eight gilts exhibiting estrous cycles of normal duration (18 to 22 days) were assigned to surgery on day 8 of the estrous cycle (day 0 = first day of estrus). A blood sample was obtained from the anterior vena cava immediately prior to anesthesia which was induced with thiamylal (Surital, Parke-Davis, New Jersey) and maintained with halothane as previously described (Magness and Ford, 1982). After exposure via mid-ventral laparotomy, numbers of CL on both ovaries were determined and one randomly selected ovary was removed. Further, a polyvinyl catheter was inserted into the femoral artery via the saphenous artery and exteriorized in the flank for chronic blood sampling. On day 9, the day following surgery, gilts were randomly assigned to receive an intramuscular injection of 4 ml saline or 20 mg of PGF$_{2\alpha}$ at 0700 hour. Ten ml blood samples were collected into heparinized tubes (100 IU heparin/ml blood) from the femoral artery at 1900 hour on the day of surgery, then hourly from
0600 hour to 1300 hour and again at 1900 hour on day 9. Blood sampling was continued twice daily (0700 and 1900 h) thereafter until 0700 hour on day 12, immediately prior to the removal of the remaining ovary.

Following removal of each ovary, CL were immediately dissected free of the ovarian stroma, weighed, and frozen individually on dry ice in 3 ml of .9% saline for storage at -88°C until homogenized and assayed for progesterone, protein and DNA content.

Assays

Progesterone in plasma and luteal tissue was measured by radioimmunoassay previously validated in this laboratory (Magness and Ford, 1982; Ford and Christenson, 1979). Samples were extracted with a mixture of benzene-hexane (1:2; >90% recovery) and assayed with antibody #GDN-337 (G. D. Niswender, Colorado State University, Colorado). The sensitivity of the assay as defined as the amount of steroid yielding 95% of the counts in buffer control tubes was approximately 50 pg/tube. All plasma and luteal samples were each included in a single assay with a plasma (n = ) and luteal tissue (n = ) pool. Intraassay coefficients of variation were 11% for the plasma pool (22.0 ± .7 ng/ml) and 19% for the luteal tissue pool (15.7 ± 1.3 μg).

Protein concentration in luteal tissues was determined in a single assay by the method of Bradford (1976). Samples were diluted 1:50 with distilled water, and following addition of Coomassie Brilliant Blue G-250 (Biorad, Richmond, CA), absorbance was measured on a Gilford spectrophotometer at a wavelength of 595 Å. A standard curve prepared from bovine serum albumen ranged from 1 to 25 μg/ml and 1 g/ml was found to be different from zero (P < .05). Samples of a luteal tissue pool included in the assay measured 7.71 ± .15 μg/ml (n = 12) on the standard curve with a co-efficient of variation of 2.6%.

Luteal tissue DNA content was measured by the method of Labarca and Paigen (1980) using the DNA specific dye H 33258 (Calbiochem, San Diego, California) at a concentration of .1 μg/ml of unextracted luteal tissue homogenate which was diluted 1/100 with Tris/EDTA buffer (NaPO4 .05M, NaCl 2.0M, EDTA .002M) and assayed along with standard curves (.05, .10, .25, .50, 1.00, 1.20, 2.0 μg/ml) prepared from calf thymus DNA. Equal aliquots of tissue
homogenate or standards were combined with dye and fluorescence was measured at excitation and emission wavelengths of 358 and 456 nm, respectively, in a Spex fluorimeter. Serial dilutions of unextracted homogenates of a luteal tissue pool (1:50, 1:100, 1:150, and 1:200) which measured 1.78 ± .01, .99 ± .02, .64 ± .01 and .48 ± .01 μg, were parallel to the standard curve. Coefficients of variation, determined by quantifying DNA in quadruplicate samples within an assay were less than 5%.

**Statistical analysis**

Each luteal tissue measurement was averaged for all CL on an ovary and used as a single data point for analysis. Differences between treatment groups were assessed by split plot analysis of variance for a completely randomized design, where gilts were considered the experimental units nested within treatment groups and measurements over time were the subplot. Within group differences were tested by paired T-test. Correlations were calculated among luteal weight and content of progesterone, protein and DNA.

**Results**

Systemic progesterone concentrations which were similar on day 8 in PGF₂α-treated and control gilts averaged 29.0 ± 2.8 ng/ml at 0700 hour then decreased (P < .01) by 62.6 ± 5.3% following unilateral ovariectomy to 10.7 ± 1.3 ng/ml at 1900 hour. Corpora lutea number was reduced by unilateral ovariectomy from 13.8 ± 1.4 to 7.3 ± 1.1 in PGF₂α-treated gilts and from 13.8 ± .5 to 6.8 ± 1.6 in control gilts, respectively. No significant correlation was observed between the post-operative decline in systemic plasma progesterone concentration and the decrease in CL number in either group. Thereafter, systemic progesterone concentrations remained constant throughout the experimental period in control gilts. In treated gilts, however, following PGF₂α administration at 0700 hour on day 9, progesterone concentrations had declined to 50% of pretreatment levels by 1200 hour on day 9 (Fig. 1). Plasma progesterone concentrations in PGF₂α-treated gilts remained lower (P < .05) then those of control gilts through 1900 hour on day 9, but recovered during day
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10 and day 11 to reach pretreatment levels by 0700 hour on day 12 (Fig. 1). Overall, significant (P < .05) main effects of time were detected for increases in luteal weight, protein and DNA, from day 8 to day 12 which averaged 23.5 ± 6.6, 29.2 ± 9.5 and 46.0 ± 10.0%, respectively (Table 1). The ratio of luteal protein to DNA content decreased from d 8 to d 12 similarly for both PGF2α-treated and control groups averaging 12.5 ± 3.2% (P < .01). Significant treatment by time interactions were detected for luteal progesterone content and concentration per mg protein (P < .05). Luteal progesterone content increased by 9.8 ± 2.5 μg/CL and 3.2 ± .7 μg/CL in control and PGF2α-treated gilts (P < .05), respectively. Progesterone concentration increased in control gilts (8.14 ± 2.56 μg/mg protein, P < .05) but not in PGF2α-treated gilts (1.04 ± 3.91 pg/mg protein, P > .1). Luteal weight and progesterone content on dy 12 were significantly correlated in control gilts (r = + .61, P < .001) but not in PGF2α-treated gilts.

Discussion

Unilateral ovariectomy or a step-wise reduction in CL number in gilts during pregnancy only results in transient reductions in systemic progesterone concentrations following CL removal, with progesterone levels returning to pretreatment values within a few days or weeks (Webel et al., 1975; Martin et al., 1977; Thomford et al., 1984). Martin et al. (1977) concluded that the remaining CL were able to compensate for those CL removed by increasing their progesterone synthesis, but a contribution from an extraovarian source such as the gravid uterus could not be excluded. In the present study, unilateral ovariectomy resulted in a rapid decline in systemic progesterone concentration, however, no significant correlation was observed between the reduction in CL number and the decrease in circulating progesterone. Because of differences in weight among individual CL, the change in CL number may not accurately reflect the proportional reduction in luteal mass. Nevertheless, these data suggest that luteal progesterone synthesis is not closely related to luteal weight and that luteal progesterone production is controlled by as yet poorly defined mechanisms.
Prior to day 12 of the estrous cycle, the porcine CL is remarkably resistant to the luteolytic effects of PGF$_{2\alpha}$ (Diehl and Day, 1974). However, several studies have demonstrated that prior to this time, PGF$_{2\alpha}$ induces reductions in systemic progesterone concentrations lasting for up to 96 h (Gleeson, 1974; Connor et al., 1976; Guthrie and Polge, 1976). A similar transient decline also occurred in the present study following PGF$_{2\alpha}$ treatment. In addition, luteal progesterone content was lower on day 12 in PGF$_{2\alpha}$-treated versus control gilts even though circulating progesterone concentrations were similar between groups. Torday et al. (1980) demonstrated that PGF$_{2\alpha}$ decreased mitochondrial availability of cholesterol of pig CL possibly through inhibition of transport mechanisms. Regardless of the mode of action, the present study demonstrates that PGF$_{2\alpha}$ has an inhibitory effect on luteal progesterone synthesis and/or secretion in pigs on day 9 of the estrous cycle. Furthermore, the reduction in luteal progesterone content on dy 12 in PGF$_{2\alpha}$-treated gilts compared to controls suggests that this luteal characteristic does not acutely reflect the functional capacity of luteal tissue in pigs.

Little evidence suggests that PGF$_{2\alpha}$ has structural effects on CL in pigs prior to day 12 of the estrous cycle. Krzymowski et al. (1978) infused PGF$_{2\alpha}$ unilaterally into a single uterine horn of sows over a 12 hour period on days 6, 8 or 10 of the estrous cycle. Histology of CL collected on days 15, 17 or 18 revealed signs of fatty degeneration in CL from PGF$_{2\alpha}$-treated gilts which was more prominent in CL ipsilateral to the PGF$_{2\alpha}$-infused horn. The results of the present study provide no evidence to suggest that administration of PGF$_{2\alpha}$ to gilts on day 9 of the estrous cycle induced alterations in luteal composition to day 12. These data indicate that if present, degenerative changes in CL induced by PGF$_{2\alpha}$ administered to pigs prior to dy 12 of the estrous cycle are relatively minor. In sheep, Denamur et al. (1973) demonstrated a significant reduction in luteal DNA content within 3 days of the onset of luteal regression. No evidence of even partial luteolysis was found in the present report.

Morphological (Bjersing, 1967; Cavazos et al., 1969) and other studies of the pig CL during the estrous cycle (Masuda et al., 1967) suggest that the CL is fully developed by day 8 to day 10 following estrus. In contrast, Corner (1920) and Burger (1952) observed a continued increase in luteal size in pigs until day 14. In the present study no increase in systemic progesterone concentration
occurred in control gilts from day 8 to day 12 suggesting that maximum functional activity, was achieved by day 8. Despite constant systemic progesterone concentrations, luteal weight, DNA, protein and progesterone content increased to day 12 in control gilts indicating continued luteal development up to this time. Luteal growth during this period is unlikely to be a result of unilateral ovariectomy because several studies in which unilateral ovariectomy of pigs from day 1 to day 7 of the estrous cycle failed to show luteal hypertrophy in the remaining ovary until after day 15 (Brinkley et al., 1964; Short et al., 1965; Brinkley and Young, 1969; Staigmiller et al., 1972, 1974).

It is impossible, from the present data, to determine the components involved in the growth of CL between day 8 to day 12. However, the ratio of protein: DNA decreased slightly, though significantly, in both groups up to day 12 suggesting that the size of cells in CL had on average actually decreased (Baserga, 1985). Kineman et al. (1987) observed no change in the proportion of large luteal cells per CL in dispersed cell preparations from hysterectomized pigs between day 10 and day 14. In view of the low mitotic rate of porcine lutein cells (Corner, 1920) and the decrease in the ratio of luteal protein: DNA content it seems most likely that the increase in DNA content or cell numbers in the present study was due to increases in the number of smaller, nonsteroidogenic elements.

In conclusion, these data indicate that PGF$_{2\alpha}$ administration on day 9 of the estrous cycle in pigs has transient inhibitory effects on luteal function without effects on luteal composition. Luteal growth continues in pigs from day 8 to day 12 and is not affected by a luteolytic dose of PGF$_{2\alpha}$ on day 9. Finally, unknown mechanisms operate to control the functional activity of CL in pigs so that only a poor relationship exists between luteal mass and progesterone production.

**Literature Cited**


Table 1. Luteal weight and content of progesterone, protein and DNA in PGF$_{2\alpha}$-treated and control gilts on day 8 and day 12

<table>
<thead>
<tr>
<th></th>
<th>Day 8</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Luteal weight (mg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGF$_{2\alpha}$</td>
<td>376.3 ± 26.2</td>
<td>435.0 ± 22.3</td>
</tr>
<tr>
<td>Control</td>
<td>389.9 ± 33.5</td>
<td>500.7 ± 43.8**</td>
</tr>
<tr>
<td><strong>Progesterone content (μg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGF$_{2\alpha}$</td>
<td>13.9 ± 1.6</td>
<td>17.1 ± 1.9*</td>
</tr>
<tr>
<td>Control</td>
<td>12.5 ± 2.0</td>
<td>22.2 ± 2.8*</td>
</tr>
<tr>
<td><strong>Protein content (mg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGF$_{2\alpha}$</td>
<td>5.28 ± .31</td>
<td>6.52 ± .56</td>
</tr>
<tr>
<td>Control</td>
<td>5.76 ± .73</td>
<td>7.40 ± .76*</td>
</tr>
<tr>
<td><strong>DNA content (pg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGF$_{2\alpha}$</td>
<td>523 ± 30</td>
<td>740 ± 106*</td>
</tr>
<tr>
<td>Control</td>
<td>497 ± 78</td>
<td>747 ± 114*</td>
</tr>
</tbody>
</table>

*P < .05.

**P < .01, indicates difference, day 12 from day 8 by paired T test.
Figure 1. Femoral arterial progesterone concentrations in gilts treated with 20 mg of prostaglandin F₂α or saline on day 9 of the estrous cycle.
SECTION IV: EFFECTS OF A PHORBOL ESTER (TPA), CALCIUM IONOPHORE (A23187) AND PROSTAGLANDIN F\textsubscript{2}\textalpha\ (PGF\textsubscript{2}\textalpha) ON PROGESTERONE SECRETION BY DISPERSED OVINE LUTEAL CELLS

Abstract

The possible roles of protein kinase C, intracellular calcium and oxygen environment in luteal progesterone (P\textsubscript{4}) production and their interaction with PGF\textsubscript{2}\textalpha were investigated in dispersed ovine luteal cells. The following experiments were performed: 1) dose response to TPA and A23187, 2) interactions between TPA and PGF\textsubscript{2}\textalpha at 5\% or 18\% O\textsubscript{2}, 3) effects of TPA and PGF\textsubscript{2}\textalpha on basal and luteinizing hormone (LH)-stimulated P\textsubscript{4} secretion, 4) interaction of submaximal inhibitory concentrations of TPA with PGF\textsubscript{2}\textalpha and the effect of indomethacin (IN) on the TPA response. Day 9 (day 0 = first day of estrus) corpora lutea (CL) from ewes exhibiting estrous cycles of normal duration (15 to 17 days) were dispersed and 50,000-150,000 cells were cultured for 4 h in Delbeccos Modified Eagles Medium. The proportion of luteal cells > 22 \(\mu\) in these preparations averaged 17.8 ± 2.1\%. P\textsubscript{4} in medium was measured by radioimmunoassay (RIA). Both TPA and A23187 inhibited P\textsubscript{4} secretion in a dose dependent manner. Maximum inhibition (500 nM) was greater for TPA than A23187 (66.4 ± 3.4 and 83.2 ± 7.2\% of controls, respectively; \(P < .05\)), and the two were not additive in their effects. Reducing O\textsubscript{2} did not affect P\textsubscript{4} secretion with or without TPA, PGF\textsubscript{2}\textalpha or both. Basal P\textsubscript{4} secretion was inhibited 30\% by TPA and 10\% by PGF\textsubscript{2}\textalpha, but no additivity was seen. IN reduced the TPA inhibition of basal P\textsubscript{4} secretion from 30.2 ± 4.4 to 18.7 ± 2.6\% (\(P < .05\)), but failed to affect PGF\textsubscript{2}\textalpha inhibition, suggesting an action involving prostanoid synthesis. In the presence of LH, P\textsubscript{4} secretion was increased above baseline by 74.1 ± 5.2\%, and this increase was not affected by TPA, PGF\textsubscript{2}\textalpha or a combination of the two. These results suggest that TPA and PGF\textsubscript{2}\textalpha may inhibit basal P\textsubscript{4} secretion by related mechanisms that are not enhanced by lower O\textsubscript{2}. The lack of effects of TPA and PGF\textsubscript{2}\textalpha on LH-stimulated P\textsubscript{4} secretion is consistent with an effect of these compounds on the large luteal cell component of these dispersed luteal cell populations.

(Key Words: Corpus Luteum, Progesterone, Protein Kinase C, Prostaglandin F\textsubscript{2}, Ovine.)
Introduction

Prostaglandin F2a (PGF2α) is a potent luteolytic agent in the ewe (McCracken et al., 1970). Although little is known of the mechanism whereby it induces luteolysis, it has been suggested that, both in rats and in ewes, luteolysis may result from hypoxia secondary to the vasoconstrictor effects of PGF2α (Pharriss and Wyngarten, 1969; Niswender et al., 1976). Alternatively, a direct effect of PGF2α on luteal cells in sheep is suggested by the demonstration that PGF2α inhibits basal and luteinizing hormone (LH)-stimulated progesterone secretion by dispersed luteal cells and luteal slices (Evrard et al., 1978; Evrard-Herouard et al., 1981; Fletcher and Niswender, 1982).

More recently PGF2α has been shown to increase phospholipid turnover in luteal cell membranes of the rat (Raymond et al., 1983) and cow (Davis et al., 1987). Phospholipid turnover involving the membrane protein, phospholipase C, results in the generation of diacyl glycerol and inositol triphosphate (IP3) (Nishizuka, 1984). Diacyl glycerol is involved in the activation of protein kinase C, a calcium-dependent protein involved in phosphorylating regulatory proteins and, hence, regulating the function of a number of different endocrine tissues. IP3 is thought to stimulate release of stored intracellular calcium and raise cytoplasmic calcium concentrations (Nishizuka, 1984). Investigations of the role of protein kinase C have utilized specific activators of protein kinase C, such as the phorbol ester TPA. Calcium ionophores, such as A23187, have been utilized to increase cytosolic calcium concentrations. Studies with rat and bovine luteal cells indicate that TPA and A23187 have effects similar to PGF2α on luteal progesterone (P4) secretion (Brunswig et al., 1986; Baum and Rosberg, 1987).

Few reports have appeared describing the effects of these compounds on ovine luteal cells, and few studies have investigated in vitro luteal cell responsiveness under conditions of a reduced oxygen environment. Therefore, using dispersed ovine luteal cells, we investigated 1) the effects of and interaction between TPA and A23187 on basal P4 secretion, 2) the interactions between TPA and PGF2α at 5% or 18% O2, 3) the effects of TPA and PGF2α on LH-stimulated P4 secretion, 4) the interaction of submaximal inhibitory concentrations of TPA with PGF2α, and 5) because indomethacin has been shown to reduce the inhibitory effects of TPA on P4 secretion by swine granulosa cells (Veldhuis et al., 1987), we
treated cultures with TPA in the presence or absence of indomethacin to see if
the effects of TPA were linked with prostanoid synthesis.

Materials and Methods

Experimental protocol

Multiparous crossbred ewes were checked twice daily for estrus with a
vasectomized ram. Ewes were assigned to the study after exhibiting at least two
cycles of normal duration (15 to 17 day). On day 9 (day 0 = first day of estrus),
corpora lutea (CL) were collected by midventral laparotomy under pentobarbi­
tone anesthesia and transported to the laboratory in sterile medium within 30
minutes of collection. The medium used for transportation, cell dispersion and
culture, unless otherwise stated, was sterile Dulbecco's Modified Eagles Medium
(DMEM, Gibco, Grand Island, NY) supplemented with .1% bovine serum
albumen (BSA) and antibiotics (100 units/ml penicillin G and 100 µg/ml strep­
tomycin sulphate; Gibco).

Cell dispersion

Techniques used were essentially as described for the dispersion of porcine
luteal tissue by Taylor and Clark (1987). Corpora lutea were washed 3 times with
5 ml of medium in a glass petri dish. Approximately 300 mg of tissue, finely
minced with scalpel blades was placed into a Spinner's Flask (Belco Glass, Inc.,
Vineland, New Jersey) containing 15 ml of medium with .1% collagenase (Type
III, Cooper Biomedical, Freehold, New Jersey) and incubated for 1 to 2 h at 37°C.
At 20-min intervals tissue fragments were drawn gently in and out of a sterile 5-
ml pipette to facilitate cell dispersion. The supernatant was subsequently
centrifuged at low speed for 3 to 5 min, and the pellet was resuspended and
rewashed twice by using Spinner's Minimum Essential Medium (Gibco) with .1%
BSA. After filtering through 75 µ nylon mesh (Tetco, Inc., Elmsford, New York),
the cell suspension was evaluated in a hemocytometer grid by utilizing an
Olympus IM-2 inverted-stage microscope with an ocular micrometer for 1) luteal
cell concentration, 2) proportion of cell > 22 µ in diameter (large luteal cells)
and 3) cell viability (trypan blue exclusion). Cells were washed twice more in medium and resuspended at a concentration of 50,000 to 150,000 cells/ml. Cell yield averaged approximately 30 x 10^6 cells/CL with 17.8 ± 2.1% large luteal cells and 90 to 95% viability. Viability of these cell preparations has been previously demonstrated by the linear accumulation of progesterone during a 4-h culture period (Conley et al., 1987).

Cultures

One milliliter aliquots of medium containing drugs were added in triplicate to wells (24 well culture plates, Corning Glass Works, Corning, New York) containing 1 ml of the cell suspension and cultured for 4 h at 37°C. Final drug concentrations in medium are reported. Dimethyl sulphoxide (DMSO) was used as the solvent for TPA, A23187 and indomethacin. In separate experiments, concentrations of .01 to 1.0% DMSO had no effect on progesterone accumulation, and all subsequent experiments utilized .1 to .2% DMSO in treatments and controls. The inactive phorbol (4α-phorbol 12,13-didecanoate; PDD) which was included in several experiments (500 nM) to test the specificity of TPA response had no effect on P₄ secretion. Culture at reduced oxygen tension was achieved by using N₂ in a Model #3159 water-jacketed, tri-gas incubator (Forma Scientific, Marietta, Ohio), and simultaneous cultures under normoxic conditions were carried out in a Wedco, twin chambered incubator (Wedco, Silver Spring, Maryland). CO₂ was held at 5% in all experiments.

Drugs

Phorbol esters, A23187 and indomethacin were purchased from the Sigma Chemical Co. (St. Louis, Missouri). PGF₂α was the THAM salt purchased from Upjohn Co., (Kalamazoo, MI). Highly purified ovine LH (LER-1455-1A) was obtained from Dr. L. E. Reichert (Department of Biochemistry, Albany Medical College, Albany, New York).
Assay

Progesterone was measured by radioimmunoassay previously validated in this laboratory (Conley and Ford, 1987). All samples from a single experiment were run in a single assay. The sensitivity, defined as the amount of steroid displacing 95% of the binding in buffer control tubes was 50 pg/tube. Samples of a medium pool to which 20 ng/ml of $P_4$ had been added were included in each assay and averaged $18.48 \pm .59$ ng/ml ($n = 11$). Intra-assay CV = 9.0% and interassay CV = 10.7%. Results are reported as ng/ml of culture media or percentage of control well after correction for procedural loss (92% recovery).

Statistical Analysis

Data were analyzed by analysis of variance. Comparisons among means were made by Duncan's multiple range test unless otherwise specified.

Results

Experiment 1

This study investigated the effects of TPA and A23187 and their interaction on $P_4$ secretion by luteal cells. Nanomolar concentrations (10, 50, 100, 500 and 1000) of TPA and A23187 were added alone or in equimolar amounts to luteal cell suspensions. Results, depicted as a percentage of control (vehicle) $P_4$ secretion ($23.14 \pm 3.37$ ng/ml) are presented in Figure 1. A clear dose-related inhibition of $P_4$ secretion was observed with TPA, which ranged from $3.42 \pm 6.41\%$ at 10 nM to $34.92 \pm 5.37\%$ at 1000 nM. At the lowest concentration (10 nM), A23187 significantly ($P < .05$) stimulated $P_4$ secretion ($12.17 \pm 6.67\%$), but was inhibitory ($P < .01$) at 500 and 1000 nM concentrations (-$16.78 \pm 7.24\%$ and -$23.35 \pm 3.44\%$, respectively). When luteal cell suspensions were incubated with equimolar concentrations of TPA and A23187, $P_4$ secretion was inhibited in a manner similar to that obtained with TPA alone (Fig. 1). Inhibition of $P_4$ secretion was greater ($P < .05$) at 500 and 1000 nM for both TPA and TPA + A23187, which were similar, than for A23187 alone at the same concentrations.
Experiment 2

The effects of TPA (500 nM) and PGF$_{2\alpha}$ (10 ng/ml) on LH (20 ng/ml) stimulated P$_4$ secretion by luteal cells were investigated. Results in Table 1 are reported as a percentage of the P$_4$ secretion by vehicle controls (36.72 ± 5.49 ng/ml). TPA reduced (P < .05) P$_4$ secretion to 72.42 ± 2.70% of control values. PGF$_{2\alpha}$ inhibited (P < .05) P$_4$ secretion by all luteal cell suspensions to which it was exposed, but this reduction averaged less than 10%. LH increased (P < .05) P$_4$ secretion by 70.72 ± 18.8%. This increased P$_4$ secretion in response to LH was not reduced by PGF$_{2\alpha}$, but a marked decrease (P < .05) was observed when LH-treated cells were coincubated with TPA or TPA + PGF$_{2\alpha}$. Both TPA and TPA + PGF$_{2\alpha}$ treatment produced similar inhibitory effects, averaging 30%.

Experiment 3

This study examined the effects of differing levels of O$_2$ (5% and 18%) and PGF$_{2\alpha}$ (10, 100 and 1,000 ng/ml) on TPA (500 nM) induced alterations in P$_4$ secretion by luteal cells. No effect of differing O$_2$ environment was observed in this study; therefore, results were pooled and are presented in Table 2. As observed in Exp. 2, TPA inhibited (P < .05) P$_4$ secretion by luteal cells to 77.8 ± 4.1% of control values. PGF$_{2\alpha}$ inhibited P$_4$ secretion at all three concentrations; however, only the 10 ng/ml concentration reached statistical significance (75.8 ± 4.1%; P < .05). When 10 ng/ml PGF$_{2\alpha}$ was coincubated with TPA, no further inhibition of P$_4$ secretion was observed over that seen after incubation of luteal cells with each compound alone. Similarly, incubation of 100 or 1,000 ng/ml PGF$_{2\alpha}$ with TPA resulted in an inhibition of P$_4$ secretion (P < .05), which was similar to that induced by TPA alone. LH (20 ng/ml) significantly increased (P < .05) P$_4$ secretion by luteal cells when incubated alone (190.5 ± 12.9%) or when coincubated with TPA (147.5 ± 8.0%).
Experiment 4

This experiment investigated the interaction of PGF$_{2\alpha}$ with sub-maximal inhibitory concentrations of TPA, and the possible role of synthesized prostanoids in the TPA response by cultures including indomethacin. Less inhibition of P$_4$ secretion (P < .05) was obtained with TPA at 50 nM as compared with the maximal inhibitory level of 500 nM (14.80 ± 1.88 vs 30.15 ± 4.40%, respectively, Fig. 2). Two levels of PGF$_{2\alpha}$ were chosen that exhibited similar and maximal inhibitory effects on P$_4$ secretion (10.84 ± 2.67 vs 9.27 ± 1.70%, respectively). No additive effects of PGF$_{2\alpha}$ and TPA on inhibiting P$_4$ secretion were observed when PGF$_{2\alpha}$ and TPA were coincubated at either low (1 ng/ml PGF$_{2\alpha}$ + 50 nM TPA) or high levels (10 ng/ml PGF$_{2\alpha}$ + 500 nM TPA). Inhibition of P$_4$ secretion in both groups was similar to that observed after exposure to TPA alone. Indomethacin (10 µg/ml) alone did not influence P$_4$ secretion, nor did it alter the response to 10 ng/ml PGF$_{2\alpha}$. However, indomethacin decreased the inhibitory effect of 500 nM TPA from 30.15 ± 4.40 to 18.17 ± 2.62 (P < .05).

Discussion

These data demonstrate that, at the concentrations used in this study, TPA causes a consistent reduction in basal P$_4$ release from dispersed ovine luteal cells. LH increased progesterone secretion of TPA treated cells by 66% (120/72, Table 1) and 90% (148/78, Table 2) over TPA alone in Exp. 2 and 3, respectively. This represents a similar increase in progesterone secretion induced by LH over vehicle in each experiment (70% and 90%, Tables 1 and 2, respectively). This suggests that the sensitivity of luteal cells to LH was not affected, rather that the accumulation of P$_4$ in medium during LH stimulation in the presence or absence of TPA is dependent only on the baseline production of P$_4$ under these conditions.

Much has been done to define two populations of luteal cells in the ovine corpus luteum (for recent review see Niswender et al., 1985; O'Shea, 1987). It has been suggested that LH-enhanced P$_4$ secretion from sheep CL is attributable to small luteal cells, which possess more LH receptors, whereas basal P$_4$ secretion
is predominantly of large luteal cell origin (Fitz et al., 1982; Niswender et al., 1985). Furthermore, large luteal cell P4 secretion seems to be independent of increases in cAMP (Fitz et al., 1982). Whether or not differences in the effects of TPA on basal and LH stimulated P4 secretion relate to effects or lack thereof on different luteal cell types remains to be investigated.

PGF2α, has previously been shown to affect both basal P4 secretion from large ovine luteal cells (Fitz et al., 1982) and LH-stimulated P4 secretion from minced luteal tissue (Evrard et al., 1978; Evrard-Herouard et al., 1981; Fletcher and Niswender, 1982). Data obtained in the present study demonstrated no effect of PGF2α on LH-stimulated P4 secretion and only minimal inhibition of basal P4 release at levels ranging from 1 to 1000 ng/ml. Lowering atmospheric oxygen had no effect on the degree of PGF2α inhibition or its consistency, nor did it affect the response to TPA or the interaction between the two. Although a decrease in atmospheric oxygen has previously been shown to inhibit LH-stimulated P4 secretion from rat luteal slices (Hermier et al., 1971), no difference was found in this study. Other steroidogenic tissues have not been found to be detrimentally affected by reduced oxygen environments, indeed increases in steroidogenesis by rat leydig cells have been demonstrated at 5% vs 19% O2 (Abney and Myers, 1987). Swann and Bruce (1987) suggested that less than 2% of total ovarian oxygen consumption is required for side chain cleavage of cholesterol in the rat ovary. Therefore, reduced luteal blood flow would be expected to have minor effects on luteal steroidogenesis, and other components of luteal function may be responsible for the high metabolic rate of the corpus luteum.

These data demonstrate that the inhibitory effects of TPA on luteal cell P4 secretion, whether maximal or submaximal, were not increased by maximally inhibitory the levels of A23187 or PGF2α, suggesting a common final or shared mechanism of action of these compounds. However, because TPA induced a greater suppression of P4 secretion than either A23187 or PGF2α, a single common mechanism does not adequately explain the TPA effect. A possible interpretation of the biphasic response to A23187 has been provided by Nishizuka et al. (1984), who suggested that low concentrations of A23187 stimulated rises in cytoplasmic Ca+++, whereas in higher concentrations, it stimulated the activation of protein kinase C. Thus, the ionophore-induced
inhibition of $P_4$ accumulation in media observed in the present study may have been due, at least in part, to some stimulation of protein kinase C. In turn, the reduction of the inhibitory potency of TPA induced by indomethacin strongly suggests that part of the TPA response is mediated by prostanoid synthesis. Ovine luteal cells have been shown to be capable of prostaglandin $F_{2a}$ production (Rexroad and Guthrie, 1978), and a similar stimulation of prostaglandin synthesis by TPA has been demonstrated in swine granulosa cells (Veldhuis et al., 1987). Further experiments will be required to identify the presumed prostaglandin involved and whether in fact part of the A23187 response may be sensitive to indomethacin also.

In conclusion, these studies demonstrate that TPA inhibits basal $P_4$ secretion by ovine luteal cells in contrast to the response obtained in the bovine species (Brunswig et al., 1986; Hansel and Dowd, 1986) and therefore emphasizes the existence of species differences in in vitro luteal responsiveness previously noted with PGF$_{2a}$ in the cow and the ewe (Fitz et al., 1984). Both the lack of additivity and the sensitivity of TPA's response in the presence of indomethacin suggest that a common component is involved in the mechanism of action of these compounds. However, the much reduced potency of PGF$_{2a}$ compared with TPA and the lack of dose response to PGF$_{2a}$ suggest that the major component of inhibition induced by TPA is independent of PGF$_{2a}$ or its effects on luteal cells.

Literature Cited


Table 1. Effects of TPA (500 nM) and PGF\textsubscript{2α} (10 ng/ml) under basal and LH-stimulated (10 ng/ml) conditions\textsuperscript{a}

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Response</th>
<th>Treatment</th>
<th>% Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LH</td>
<td>170.72 ± 18.18\textsuperscript{a}</td>
</tr>
<tr>
<td>TPA</td>
<td>72.42 ± 2.70\textsuperscript{d}</td>
<td>TPA/LH</td>
<td>120.16 ± 9.24\textsuperscript{bc}</td>
</tr>
<tr>
<td>PGF\textsubscript{2}</td>
<td>90.66 ± 6.79\textsuperscript{c,d}</td>
<td>PGF\textsubscript{2α}/LH</td>
<td>182.54 ± 22.33\textsuperscript{a}</td>
</tr>
<tr>
<td>TPA/PGF\textsubscript{2α}</td>
<td>73.62 ± 3.02\textsuperscript{d}</td>
<td>TPA/PGF\textsubscript{2α}/LH</td>
<td>140.35 ± 9.83\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Results are expressed as percentage of progesterone secreted by controls (n = 5). Means ± SE with different superscripts differ (P < .05).
Table 2. Effect of increasing concentrations of PGF<sub>2α</sub> (10, 100, and 1000 ng/ml) on basal progesterone secretion and their interaction with TPA (500 nM) at each concentratiion<sup>a</sup>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Response</th>
<th>Treatment</th>
<th>% Response</th>
</tr>
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<tbody>
<tr>
<td>TPA</td>
<td>77.8 ± 4.1*</td>
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</tr>
<tr>
<td>LH</td>
<td>190.5 ± 12.9</td>
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<tr>
<td>TPA/LH</td>
<td>147.5 ± 8.0*</td>
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<td>PG 10</td>
<td>75.8 ± 4.9*</td>
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<td>TPA/PG 10</td>
<td>77.1 ± 6.7*</td>
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<tr>
<td>PG 100</td>
<td>87.4 ± 9.4</td>
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<tr>
<td>TPA/PG 100</td>
<td>79.2 ± 10.2*</td>
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<tr>
<td>PG 1000</td>
<td>84.4 ± 6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPA/PG 1000</td>
<td>74.7 ± 5.2*</td>
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</table>

<sup>a</sup>Results are expressed as percentage of progesterone secreted by controls and represent pooled values from cultures run at 5 and 18% O<sub>2</sub> with 5% CO<sub>2</sub>. No differences were noted between responses at different oxygen tensions (n = 4).

*Significantly different from control, P < .05.
Figure 1. Dose dependent effects of TPA, A23187 and TPA + A23187 on progesterone secretion (ng/ml) by ovine luteal cells. Data are expressed relative to controls. Comparisons among treatments, made by multiple range test, are indicated alphabetically with each bar. Bars with different superscripts differ (P < .05). Asterisks (*P < .05, **P < .01) indicate differences from controls (n = 7)
Figure 2. Percent inhibition of progesterone secretion by ovine luteal cells induced by culture with submaximal and maximal concentrations of PGF$_{2\alpha}$ (1 and 10 ng/ml, respectively), TPA (50 and 500 nM, respectively) and the effect of indomethacin (INDO, 10 μg/ml) on maximally inhibitory levels of TPA and PGF$_{2\alpha}$. (n = 4)
GENERAL DISCUSSION

The results of these experiments provide new information relevant to an understanding of luteal function in pigs. In the first experiment it was shown that estrogen administered to pigs via release from intraluteal silastic implants could result in maintenance of CL. An effective level of implanted estrogen was also determined along with lower doses found not to be effective. Data from this study demonstrated both direct and indirect effects of estrogen on luteal function. The direct stimulatory effects of estrogen on weight of individual implanted CL were seen only when all CL, implanted and unimplanted, were maintained. Therefore, luteal maintenance in response to estrogen in pigs appears to rely critically on an indirect, systemic mechanism of action.

The second experiment investigated the amount of estrogen released from intraluteal implants during luteal maintenance in pigs. Also investigated, was the secretion rate of progesterone from estrogen implanted and nonimplanted CL and uterine secretion of PGF$_2\alpha$. These studies demonstrated a profound, inhibitory effect of estrogen on uterine PGF$_2\alpha$ release at levels of estrogen approximating minimally-effective luteotrophic doses. Together, these experiments suggest that the major luteotrophic effect of estrogen in pigs is on uterine PGF$_2\alpha$ release and that the purported direct luteotrophic effects of embryos on luteal function in pigs cannot be attributed solely to embryonic estrogen. Therefore, these two experiments provide support for the notion that luteal function is maintained during pregnancy in the pig, as in other domestic animal species, by an effect of the conceptus which reduces the luteolytic effect of the uterus (Thatcher et al., 1986). Further studies will be required to identify and characterize the direct effects of embryonic products which inhibit luteolysis at the level of the ovary.

In addition to the luteotrophic effects of estrogen, the luteolytic effects of PGF$_2\alpha$ were investigated in pigs on day 9 of the estrous cycle in order to better understand the apparent resistance of the CL of the pig to PGF$_2\alpha$ exposure early in the luteal phase. The results of these studies demonstrated that PGF$_2\alpha$ administered to pigs on day 9 of the estrous cycle resulted in a transient decline in systemic progesterone concentrations lasting 1 to 2 days, and a resultant decrease in luteal progesterone content on day 12. No changes were detectable
in luteal composition between CL from PGF$_{2\alpha}$-treated or control gilts on day 12, suggesting that the effects of PGF$_{2\alpha}$ on day 9 porcine CL were transient, involving steroidogenetic function, rather than affecting luteal structural components.

In addition to the effects of PGF$_{2\alpha}$ on day 9 CL of pigs, the results of experiment III also demonstrated an unexpected increase in luteal weight and luteal tissue content of progesterone, total protein and DNA, indicating a substantial amount of growth of CL from day 8 to day 12. Luteal growth during this period appears to be due mainly to an increase in the number of smaller, nonsteroidogenic cells, because luteal progesterone secretion did not increase during this period, and the ratio of luteal protein:DNA decreased, suggesting an overall decrease in cell size. Few reports deal with the compositional growth of CL in the pig or other domestic animal species during the estrous cycle, and therefore, it is impossible to make a comparison between species at this time. Nevertheless, CL of all species are refractory to the luteolytic effects of exogenous PGF$_{2\alpha}$ administered during the first few days of the estrous cycle when luteal growth is occurring (Horton and Poyser, 1976).

It is not known whether or not any relationship exists between the process of luteal growth and the development of sensitivity to the luteolytic effects of PGF$_{2\alpha}$ in domestic animal species. However, the presence of an IUD in both sheep and pigs inhibits the normal increase in luteal weight, which occurs during luteal development, in the first stages of the luteal phase (Hawk, 1968). The effects of IUDs on luteal function probably arise as a result of the stimulation of uterine PGF$_{2\alpha}$ release. In pigs it has been shown that uterine venous PGF$_{2\alpha}$ concentration is locally elevated on day 6 following placement of an IUD in one uterine horn on day 2 (Magrini et al., 1978). Therefore, the failure to observe an effect of exogenous PGF$_{2\alpha}$ on luteal growth in pigs in experiment III, may have resulted from too brief a period of PGF$_{2\alpha}$ exposure, and PGF$_{2\alpha}$ may have the potential to alter luteal growth in this, and other species. The importance of a possible relationship between luteal growth and the luteolytic effects of PGF$_{2\alpha}$ in domestic animal species awaits further investigation, particularly with regard to luteal development.

The final experiment, utilizing dispersed ovine luteal cells, provides new data on the effects of phorbol esters on, and the possible role of protein kinase C
in luteal steroidogenesis in this species. The inhibitory effects of TPA and A23187 were more dramatic and consistent than those of PGF$_{2\alpha}$ and therefore the relevance of these observations, in relation to the luteolytic effects of PGF$_{2\alpha}$, may seem to be in doubt. However, Moor et al. (1970) demonstrated that hysterectomy could reverse the decline in luteal function seen in ewes on day 15 or 16 of the estrous cycle. This observation emphasizes the importance of the cumulative effects of PGF$_{2\alpha}$ on CL which are important in the luteolytic process in the sheep and other species. Therefore, even though it is impossible to reproduce in vivo conditions in an in vitro environment, cultures lasting only hours may never demonstrate the luteal inhibitory effects of PGF$_{2\alpha}$ which accumulate over days, under physiological conditions. The only report describing deterioration of ovine luteal cells exposed to PGF$_{2\alpha}$ in vitro, utilized 6 hours of continuous exposure in culture (Fitz et al., 1984b). Finally, it is important to recognize the broader structural consequences of luteolysis, and therefore, the limited information on luteal effects of PGF$_{2\alpha}$ which is provided by the measurement of steroid production alone.

In conclusion, the studies reported here provide some rationale for the hypothesis that luteal function in the pig may be similar, in many respects, to that in other domestic animal species. This is true when it is considered that the major influence of estrogen on luteal function is indirect via uterine PGF$_{2\alpha}$ release. It is also suggested when emphasis is given to the possible relationship between luteal growth and the development of sensitivity to the luteolytic effects of PGF$_{2\alpha}$. In vivo experiments in the pig, and in vitro studies with sheep luteal cells, have also underlined the possibility that progesterone production, regardless of its value as an indicator of luteal function, may be a less important component of luteal regression than is, perhaps, generally believed. Future studies, expanding these concepts, may lead to a better understanding of luteal function, pertinent to all animal species, in which the CL is of importance.


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