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THE CORPUS LUTEUM: AN OVARIAN STRUCTURE WITH MATERNAL INSTINCTS AND SUICIDAL TENDENCIES

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1. ABSTRACT

The corpus luteum is a unique hormone-regulated, transient reproductive gland that produces progesterone, a required product for the establishment and maintenance of early pregnancy. In the absence of pregnancy the corpus luteum will cease to produce progesterone and the structure itself will regress in size over time. Although the process of luteal regression has been studied for several decades, many of the regulatory mechanisms involved in loss of function and involution of the structure are incompletely understood. More importantly, we are far from understanding how these complex mechanisms function in unison. The factor or factors responsible for initiating and mediating luteolysis are no doubt more complex than originally envisioned. Further, efforts to elucidate the mechanisms responsible for luteolysis have been complicated by different interpretations of what is 'luteolysis', discrepancies between *in vitro* and *in vivo* studies, and subsequent biases which are associated with the different methods of analyses. Moreover, the complexity of the mechanisms which regulate the life span of the corpus luteum are compounded by the presence of a heterogeneous population of cells which often respond differentially to the same ligand or stimuli. Attempts to isolate specific luteal cell types for the intention of defining intracellular signaling mechanisms have yielded valuable information. However, studies of a specific cell type taken out of context are often subject to criticism. The most obvious being that the cells are no

longer maintained within their three dimensional environment. Evaluation of the corpus luteum *in vivo*, is not without its criticisms either. A subtle change evoked within a subpopulation of cells can be overlooked if measured in whole tissue or in mixed cell preparations. Furthermore, treatment *in vivo* with a single agent/ligand (*i.e.*, prostaglandin F2 alpha) may induce a secondary ligand that is ultimately responsible for the biological response. All arguments are valid and cannot be ignored. There are secondary levels of complexity in the corpus luteum brought about by the pleiotropic actions of specific ligands. For example, one ligand can be luteotropic to a steroid producing cell and cytotoxic to a luteal endothelial cell. Furthermore, a specific cell type within the corpus luteum may respond differentially depending on the developmental stage of the luteal phase (*i.e.*, early, mid, or late luteal phase) suggesting that the intracellular signaling pathways are key to defining ligand-induced biological responses. The purpose of this review is to culminate what is known regarding signal transduction pathways activated by initiator(s) and/or mediators of luteolysis. We recognize that an all-inclusive review describing the molecular mechanisms involved in the development, maintenance and regression of the corpus luteum would be impossible within the context of this review. There are a number of recent reviews that discuss luteal development, luteal maintenance and luteolysis with emphasis on neuroendocrine events (1-3). Consequently, we have focused our review primarily on potential intracellular signaling events of proposed regulators and mediators of luteal regression. Where possible we have attempted to incorporate references that represent rodents, domestic farm animals and primates.

2. INTRODUCTION

2.1. Role of the corpus luteum

The corpus luteum is a transient endocrine gland that evolves from the remnants of the ovulated follicle within the ovary. In response to luteotropins the granulosa and theca cells become luteinized and transform from cells that are responsible for the production of estrogen to cells that primarily produce progesterone. Following their differentiation, these steroid producing cells are often described as small luteal or large luteal cells (SLC and LLC respectively) based on their size. Alternatively, these same cell types are described as theca-lutein or granulosa-lutein based on their recognized origin (4-7). Although both cell types produce progesterone they are regulated by different mechanisms (8-10). Evidence for the two steroidogenic cell types is not limited to a single species and has been reported in the primates, rodents, domestic farm animals and lagamorphs (7, 10-17). Regardless of cellular origin, the steroidogenic cells of the corpus luteum produce progesterone to initiate uterine quiescence and glandularization in preparation for the establishment of pregnancy. Additionally, progesterone serves as a negative feedback mechanism to the hypothalamus to suppress further follicular development (18, 19). In the absence of a successful pregnancy, or at the end of the pregnancy the corpus luteum will cease to produce progesterone and the tissue mass will decrease in size accompanied by a loss cellular integrity.

2.2. Luteolysis

Luteolysis is generally defined as loss of function and the subsequent involution of the luteal structure. However, the luteolytic process is typically subdivided, whereby the decline in progesterone is described as functional luteolysis and the structural involution is described as structural luteolysis. Unfortunately the terms are often misused or over interpreted. Therefore we would like to re-introduce the word luteolysis. The root of the word, luteal is derived from the Latin word *luteolus* meaning yellow and the suffix of the word luteolysis, lysis, is derived from the Greek word *lyo* meaning to loose, dissolve, or break up (20). Luteolysis is defined in medical dictionaries as something that promotes death of the corpus luteum (21) or degeneration of corpus luteum (22). It is interesting to note that the term luteolysis does not refer to a loss of function. It is unclear whether or not the original definition of luteolysis was meant to include loss of function. If loss of function was included what term should be used if only function is lost? This is not to say that the loss of function is less important than structural regression or vise versa. Whether these two facets are independent events or are interdependent is often argued. There are those that would consider the functional and structural components of luteolysis a continuum with loss of function required for structural involution to proceed (23). Much of the controversy could be attributed to the multiple definitions that have evolved to describe the process of luteolysis. For example, in a review by Greenwald and Rothchild in 1968, a luteolysin was described as a substance, which causes a corpus luteum to regress in size or to stop secreting progesterone or related steroids, or both (24). This definition was focused on the rodent models and implied that loss of function or regression of the tissue or the two events combined could be considered luteolysis. Anderson and colleagues (25) described the term luteolytic (and the equivalent nouns) to mean destroying luteal structure and (or) function. Irving Rothchild (26) defined the luteolytic process in all its forms (luteolysis, luteolysin, *etc*.) as the exact opposite of luteotropic. More specifically, the luteolytic process is the quality of stopping the secretion of progesterone by the corpus luteum. Furthermore, the subcategorization of functional and structural helps only to describe differences between the regression of progesterone and corpus luteum size (26). No matter how a particular investigator may define luteolysis it must be made clear within the context of the report so that others can interpret the results accurately. To some this may be a mute point, however when trying to discern the specific mechanisms involved in luteal regression it becomes important to identify the distinct players and their signaling components which lead to reduced or altered steroidogenesis and those involved in the physical regression of the tissue.

In general, the dichotomy of loss of function and/or involution of the luteal structure may be attributed to species-differences. For example, the hamster corpora lutea undergoes loss of function on day 2 of the natural estrous cycle and near complete structural luteal regression occurs within one cycle (27, 28), while the corpora lutea of the rat and mouse undergo loss of function in the

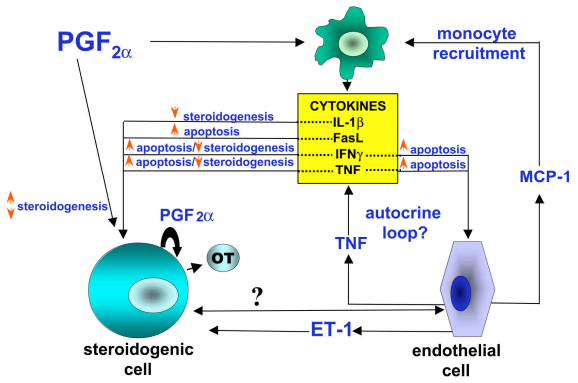

Figure 1. PGF2 alpha exerts direct and indirect influences on steroidogenesis and the recruitment and activation of immune cells. PGF2 alpha treatment indirectly alters endothelial cell function. This cartoon depicts the multiple opportunities for cytokines (TNF alpha, IFN gamma, FasL, IL-1 beta) and other peptides (ET-1, MCP-1) to alter steroidogenesis and influence the fate of ovarian steroidogenic and endothelial cells.

immediate cycle with the luteal mass involuting/regressing over several cycles (29). Three or more generations of corpora lutea are present within the ovary of a normal cycling adult mouse or rat. There is also evidence to suggest that the corpora lutea which continually regresses over a number of cycles also maintains some level of function (*i.e.*, steroid production) during the subsequent luteal phases (30, 31). A similar finding was reported in the baboon (32). This would argue that the loss of function observed at the termination of the luteal phase is only temporary or incomplete and may involve only a subset of cells. Those luteal cells that survive the initial regression appear to maintain the ability to be hormonally rescued (30, 31). Another level of complexity can be added when comparing the hallmark(s) of luteolysis in the corpus luteum of the luteal phase, pregnancy and pseudopregnancy. Structural involution of the corpus luteum following pregnancy occurs at a slower rate than that of a corpus luteum of the estrous cycle in the ewe (33). In addition, the corpora lutea of the nonpregnant rat undergoes apoptosis whereas the corpora lutea of pregnancy does not undergo apoptosis (34). Together these studies suggest there are differences in the luteolytic program among the differential physiological paradigms, which must be taken into consideration when making general conclusions about luteolysis or luteolysins.

2.3. Identifying a physiological luteolysin

Hysterectomy prolonged the lifespan of the corpus luteum in the guinea pig (35) and the rabbit (36) providing some of the first evidence that a luteolytic signal could be of a uterine origin. Subsequent studies in domestic farm animals (37) supported this notion. In 1969 Pharriss and Wyngarden (38) hypothesized that PGF2 alpha, a uterine secreted vasoconstrictor could restrict blood flow to the ovary lowering ovarian perfusion and induce luteolysis. Their hypothesis was based on the knowledge that PGF2 alpha was a potent vasoconstrictor and it was present in the endometrium (39). To test the hypothesis the investigators infused PGF2 alpha into pseudopregnant rats, which resulted in reduced progesterone levels and shortened the length of pseudopregnancy. This must have been somewhat of a surprise since an *in vitro* study performed a year earlier suggested that PGF2 alpha had no toxic effect on steroidogenic tissue and instead stimulated progesterone synthesis (40). Supporting experiments demonstrated that injection of PGF2 alpha caused luteolysis in the guinea pig, rabbit, rat, hamster, sheep and cow (38, 41-45). Further confirmation that PGF2 alpha had luteolytic actions was established by inhibition of prostaglandin synthesis with indomethacin which blocked spontaneous luteal regression in cows, sheep, and guinea pigs (46, 47). Consequently, PGF2 alpha is accepted as a primary luteolysin in most of the domestic species and the luteolysin during pseudopregnancy in the rat. PGF2 alpha may serve as a mediator of prolactin-induced luteal regression since indomethicin will prevent prolactin-induced luteolysis in the rat (48).

Although PGF2 alpha is considered a luteolysin in domestic farm animals and some laboratory species, there is some controversy as to whether it is effective in the human and non-human primates. Hysterectomy, in the human or non-human primates does not prolong the luteal phase suggesting that if PGF2 alpha is luteolytic it is not of a uterine origin. Short-term infusion of PGF2 alpha during the luteal phase can temporarily reduce progesterone levels (49, 50). Injection of PGF2 alpha directly in to the corpus luteum of the human results in a decease in progesterone and involution of the tissue (51, 52). These studies provide limited evidence that intra luteal PGF2 alpha may also contribute to the demise of the CL in primates.

In the sequence of luteolytic events initiated by exogenous PGF2 alpha it was determined that the endothelial cells and small luteal cells were the first to undergo cell death in the sheep corpus luteum. This is of particular interest since in sheep the large luteal cells contain the receptors for PGF2 alpha (53). This raises the possibility that PGF2 alpha elicits its death response indirectly. Prostaglandin F2 alpha is an accepted initiator of luteolysis yet, the available evidence from *in vitro* and *in vivo* experiments suggest that it is not the sole mediator of luteal regression. These studies would include but are not limited to studies that show that 1) if the corpus luteum is removed from the cow immediately following administration of PGF2 alpha and the cells are placed in culture the luteolytic signal is terminated and the cells remain viable (54), 2) treatment with PGF2 alpha does not induce death of the luteal steroidogenic cells *in vitro* (55), 3) treatment of luteal tissue with PGF2 alpha *in vitro* resulted in an acute increase progesterone production (8, 56, 57), 4) PGF2 alpha does not appear to prevent the interaction of LH or IGF-1 (luteotrophic hormones) with their receptors when reductions in serum levels of progesterone are observed (1, 2). Consequently, PGF2 alpha's primary role during luteolysis may be to disrupt steroidogenesis, initiate a cascade of signaling events involving hormones and cytokines, and possibly disrupt intracellular growth factor signaling (see figure 1). Cell death may happen as an indirect effect. Studies which have

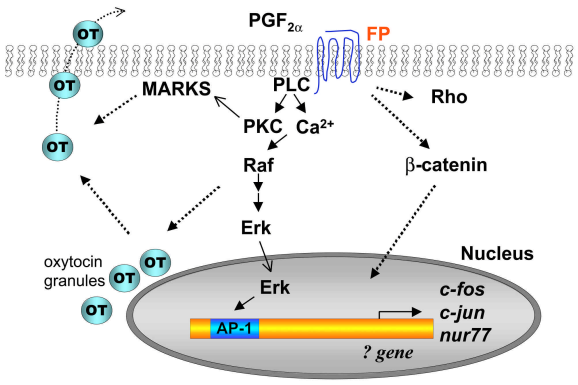

Figure 2. PGF2 alpha exerts pleiotropic actions on the steroidogenic cells of the corpus luteum. PGF2 alpha activates intracellular signaling pathways that are responsible for regulating cytoskeletal organization, transcription factor activation, gene expression, and secretion of peptide and steroidogenic hormones.

provided data to support this argument include the demonstration that infusion of phorbol myristate acetate (PMA) in the ovarian artery caused a dramatic decline in progesterone (58). The decline in progesterone was not associated with a dramatic increase in cell loss. In addition, the progesterone levels returned to normal after the infusion of PMA was ceased and by all appearances the cycle resumed. This study was further corroborated by Juengel *et al* (59) who demonstrated a reduction of progesterone followed administration of sub-lytic concentrations of PGF2 alpha (3 mg) in the ewe without a dramatic increase in cell death or loss in luteal weight. Prostaglandin F2 alpha may also regulate steroidogenesis by down regulating sterol carrier protein 2 (60), tubulin (61) and steroid acute regulatory protein (StAR) expression (62-64). Each of these proteins plays a role in transporting cholesterol to the mitochondria where it can be used for progesterone. However, there is no evidence to suggest that inhibition of one or more of these proteins will lead to cell death. Together these studies provide evidence that PGF2 alpha may be responsible for loss of function, however its role as a direct initiator of cell death may be overstated.

3. SIGNALING VIA THE PGF2 ALPHA RECEPTOR

3.1 Prostaglandin F2 alpha Receptors

The PGF2 alpha receptor (FP) is a member of the guanine nucleotide binding (G) protein-coupled receptor (GPCR) family containing seven-membrane spanning regions (65)(see figure 2). PGF2 alpha receptors have been identified in ovaries and isolated luteal cells of many species by ligand binding and immunohistochemistry techniques, as well as by *in situ* hybridization and Northern analysis. Immunoreactive FP sites are present in the luteal cells, theca cells of Graafian follicles, and some interstitial cells of the rat ovary (34). Based on studies of corpora lutea from mice, rats, sheep, and cows (66-70), and studies on isolated bovine luteal microvascular cells (71-76), the microvascular endothelial cells of the corpus luteum do not possess significant amounts of FP, although a rare subset of luteal endothelial cells has been reported to possess FP (74) and respond to PGF2 alpha (77). Prostaglandin F2 alpha binding sites (78) and mRNA (79) have been identified in both bovine large and small luteal cells. However, levels of binding sites in large and small cells are not consistent with levels of FP mRNA. Sakamoto *et al* (69, 80) using in situ hybridization reported that levels of FP mRNA are abundant in large but not small luteal cells. Based on the documented presence of FP binding sites (78) and responsiveness of bovine small cells to PGF2 alpha (8, 81), it seems likely that PGF2 alpha receptor mRNA and protein are not coordinately expressed in bovine small cells. In sheep, the large luteal cell possesses FP, whereas the small cells do not possess FP and are unresponsive to PGF2 alpha (1, 82). These differences can be possibly explained by methodologies that have used size exclusively to characterize luteal cells, versus consideration of cellular origins or other characteristics of luteal cells.

In most species studied to date, FP mRNA levels are elevated following ovulation, increase during the luteal phase, and decrease during natural or PGF2 alpha-induced luteolysis. The rat (67) and mouse (83) are different, however, in that treatment with PGF2 alpha elevates levels of FP mRNA in the corpus luteum. These differences may provide clues to the role of PGF2 alpha in regulating functional or structural regression of the corpus luteum. In FP-deficient mice ovulation, fertilization and implantation occur (84, 85). However, in FP-deficient mice, the animals do not undergo parturition because the prepartum decrease in progesterone does not transpire. This strongly suggests that FP-initiated signals that reduce progesterone are required at this critical period in mice. Since the structure of the mouse corpus luteum remains intact for an extended period following the reduction in progesterone (24, 86), it appears that signals in addition to those provided by PGF2 alpha are required to initiate luteolysis.

Investigations utilizing PGF2 alpha radioligand binding approaches have demonstrated high- and low-affinity binding sites in rat (87), ovine (65, 88), porcine (89) and bovine (90) corpora lutea. The physiological significance of these sites is presently unknown, but McCracken *et al* (3) have suggested that activation of high affinity binding sites with low levels of PGF2 alpha may be coupled to the secretion of oxytocin from ovine large luteal cells without affecting the secretion of progesterone. In contrast, high levels of PGF2 alpha are required to activate low affinity receptors and results in both oxytocin secretion and a reduction in progesterone secretion. It is unclear whether these *in vivo* experiments reflect the ability of high levels of PGF2 alpha to activate additional signaling mechanisms by one or more receptor types.

Recent studies present evidence for the presence of FP receptor subtypes that may either exert inhibitory actions or activate additional intracellular signals. The newly identified FP isoforms are generated by alternative mRNA splicing. This is very similar to other prostanoid receptors (*e.g.*, EP and thromboxane A2 receptors) in which alternative mRNA splicing gives rise to a variety of isoforms in humans and in other species (91, 92). The novel FP isoform identified by Ishii and Sakamoto (93) is

spliced in the middle of the sixth transmembrane segment resulting in a truncated protein lacking the VII transmembrane segment and the carboxyl-terminal intracellular tail. The levels of mRNA for the normal FP and the splice variant were similarly expressed in bovine corpora lutea during the estrous cycle and during pregnancy (93). Using COS-7 cells in which transiently expressed FP isoforms were introduced, these authors provided data suggesting that the alternatively spliced FP isoform acts as negative regulator to attenuate normal FP function. This observation raises a question, does the FP normally function as a dimer as has been suggested for other G-protein coupled receptors (94)?

Other studies demonstrate that alternative mRNA splicing gives rise to a novel ovine FP receptor that possess additional signaling capabilities (95). The amino acid sequences of the ovine FP and slice variant (FPB) prostanoid receptor isoforms are the same throughout their amino termini and seven-membrane-spanning domains, but the FPB isoform is truncated and lacks the last 46 carboxyl-terminal amino acids normally present in the cytoplasmic tail (96). The physiological significance of these receptor isoforms is not clear, although differences have been shown to exist with respect to some aspects of second messenger coupling and receptor desensitization (95, 97). A brief summary of these differences is presented in the following sections. Future studies designed toward understanding the factors and mechanisms responsible for regulation of FP isoform expression and function during luteal development and regression will greatly expand our understanding of the physiology of corpus luteum regression.

3.2. Phospholipase C and Adenylyl Cyclase

An initial intracellular event following PGF2 alpha binding to FP involves the activation of a G-protein-sensitive phospholipase C that causes the hydrolysis of phosphatidylinositol 4,5 phosphate (PIP2). Activation of phospholipase C generates the second messengers diacylglycerol and inositol trisphosphate (98) (see figure 2). Inositol trisphosphate (InsP3) binding to receptors in the endoplasmic reticulum stimulates the release of calcium ions, resulting in an elevation in the concentration of cytoplasmic calcium. PGF2 alpha–induced activation of phospholipase C involves the coupling of Gq/11 to phospholipase C beta (91, 99-101). The ability of PGF2 alpha to activate phospholipase C has been reported in rat, bovine, ovine, porcine, primate, and human luteal cells (65, 66, 99, 102). In the cow, both large and small luteal cells respond to PGF2 alpha with increases in InsP3 and intracellular calcium (103-105). In keeping with studies on the selective distribution of PGF2 alpha receptors to ovine large luteal cells, in the sheep only large luteal cells respond to PGF2 alpha with increases in InsP3 (82) Ca^{2+} (106, 107). The ovine FP and FPB receptor isoforms have similar pharmacological properties and PGF2 alpha stimulates phospholipase C to a similar extent in cells expressing these isoforms (96).

Although PGF2 alpha elevates cAMP levels in NIH3T3 cells (108), PGF2 alpha does not stimulate Gs and the adenylyl cyclase/cAMP pathway in ovarian cells. However, PGF2 alpha has been shown to inhibit (109) LH-stimulated cAMP accumulation in rat luteal cells by a mechanism independent of elevations in cytosolic calcium (110). Similarly, PGF2 alpha inhibits gonadotropin-stimulated cAMP accumulation in human granulosa-luteal cells (81). Michael and Webley (111) reported that in human luteal cells PGF2 alpha reduces agonist-stimulated accumulation of cAMP by a protein kinase C-dependent activation of phosphodiesterase. In contrast, PGF2 alpha has no effect (112, 113) or amplifies (114) gonadotropin-stimulated cAMP accumulation in bovine luteal cells. The ability of PGF2 alpha to amplify agonist-induced cAMP accumulation in bovine luteal cells is thought to be mediated by protein kinase C (114, 115). Considerable progress has been made in the study of adenylyl cyclase isoforms in other tissues (116), but little is known about the expression adenylyl cyclase in luteal cells. One report indicates that adenylyl cyclase types three and six are present in the bovine corpus luteum (114). Given the important role for cAMP in luteal function (117) and the differential sensitivities of this family of enzymes to elevations in cytosolic calcium, additional studies are needed to identify the adenylyl cyclase isoforms present during the development and regression of the corpus luteum to determine their contribution to luteal physiology.

3.3. Calcium Signaling

Luteal cells respond to PGF2 alpha with a rapid transient increase in calcium, as a result of InsP3 action, followed by a secondary sustained increase in intracellular calcium concentrations, apparently due to influx of extracellular calcium ions (103, 104, 106, 118, 119). The increase in intracellular calcium not only promotes translocation of some protein kinase C (PKC) isozymes to the plasma membrane, but in concert with DAG, is essential in activating the conventional isoforms of protein kinase C (120). Manipulations of extracellular and intracellular calcium have been shown to have detrimental effects on basal and gonadotropin-induced luteal steroidogenesis (107, 112). Thus, calcium appears to be required for optimal steroidogenesis in luteal cells.

Calcium activation of calcium-calmodulin (CaM) -dependent enzymes regulates vital process in all cells (121). Some actions of PGF2 alpha require activation of calcium-CaM-dependent enzymes. Duncan and Davis (122) demonstrated that a CaM-sensitive enzyme, InsP3-3-kinase, is apparently activated following treatment with PGF2 alpha. This enzyme phosphorylates InsP3 to produce an InsP4 isoform that may regulate calcium influx across the plasma membrane in bovine luteal cells. Other inositol phosphates play important roles in regulating ion flux (123, 124). Upon agonist stimulation calcium CaM signals activate a calcium-activated chloride channel in many cell types. Sustained activation of phospholipase C, as observed in response to PGF2 alpha (99), results in the gradual elevation in cytoplasmic levels of Ins 3,4,5,6-P4 which serve to inhibit calcium-triggered opening of the channel (125).

Calmodulin-dependent protein kinases also play a role in ovarian function. Mice deficient in CaM kinase IV

(126) exhibit numerous reproductive defects. Recent studies by Stocco *et al* (127, 128) demonstrate that PGF2 alpha activates a CaM-protein kinase-dependent process that leads to the rapid induction of the orphan nuclear receptor nerve growth factor inducible protein-B (NGFI-B also referred to as Nur77) in rat corpora lutea. This group reports that Nur77 (a nuclear transcription factor) is required for the induction of 20 alpha-hydroxy steroid dehydrogenase, an enzyme that converts progesterone into an inactive metabolite that cannot support pregnancy. The observation that the early response gene Nur77 is involved in PGF2 alpha action is intriguing because in studies on T-cells, the calcium-dependent phosphatase calcineurin mediates the induction of Nur77 (129) and Nur77 is implicated in T-cell apoptosis (129, 130). Furthermore, Nur77 appears to be a substrate for the anti-apoptotic protein kinase Akt (131), and phosphorylation of Nur77 by Akt reduces the transcriptional activity of Nur77 (132). A role for nuclear receptors like Nur77 in the regulation of ovarian cell death is presently unknown, but has been suggested in other tissues (133). It is tempting to speculate that luteotropic factors such as gonadotropins (134, 135) and IGF-I (136, 137), which can activate Akt, may prevent or reduce PGF2 alpha-induced Nur77-mediated transcription of 20 alpha hydroxysteroid dehydrogenase. It seems likely that Nur77 may also be involved in the regulation of other steroidogenic enzymes as it is up regulated by LH in rat ovarian follicles (138) and testicular Leydig cells (139), and is thought to regulate genes for androgen (140), estrogen (141, 142) and cortisol (143, 144). These reports indicate that the Nur77 / NGFI-B transcription factor may be important for discerning the physiologic role of calcium signals in the induction of specific genes responsible for regulating steroidogenesis and other metabolic processes (145).

3.4. Protein Kinase C

Protein kinase C is involved in the mechanism of PGF2 alpha action. Studies in isolated luteal cells have demonstrated PGF2 alpha is capable of inducing translocation of protein kinase C isoforms from the cytoplasm to the plasma membrane by measurement of enzyme activity and protein content in cytosolic and particulate cellular fractions (146). Of interest is the observation of Orwig *et al* (147) who demonstrated that PGF2 alpha treatment *in vivo* resulted in an increase in the activity of calpastatin. This enzyme could conceivably inhibit the action of proteolytic enzymes (*e.g.*, calpain (148)) and reduce proteolytic degradation of protein kinase C to maintain the pool of active protein kinase C. Elevations in cellular calpastatin could also result in controlled proteolytic activity during corpus luteum regression. The understanding of the role protein kinase C in the corpus luteum is made more complex by the expression of multiple protein kinase C isoforms in luteal cells (99, 147, 149-151) and a newly described family of receptors for activated C-kinase (RACKs) which serve as anchoring proteins for specific protein kinase C isoforms and other signaling molecules (152).

Treatment of ewes with PGF2 alpha *in vivo* results in regression of the corpus luteum characterized by reductions in serum progesterone, luteal weight, levels of mRNA for 3-beta hydroxysteroid dehydrogenase (58), LH receptors (153), and the steroidogenic acute regulatory (StAR) protein (154) and increases in oligonucleosome formation (58). These *in vivo* studies also demonstrated that protein kinase C activating phorbol esters can mimic only some of the inhibitory effects of PGF2 alpha (*i.e.*, reductions in progesterone and levels of mRNA for 3-beta hydroxysteroid dehydrogenase and the steroidogenic acute regulatory protein) but did not cause a reduction in luteal weight or induction of apoptosis. Phorbol esters are also known to target other cellular proteins and exert actions that are unrelated to protein kinase C (155). It seems likely therefore that the complete process of luteolysis may involve additional responses to PGF2 alpha (*i.e.*, calcium mobilization) and interactions among the various luteal cells types (*i.e.*, endothelial cells, immune cells, macrophage and the steroidogenic large and small cells) (156, 157).

A specific protein kinase C isoform has been identified and studied during luteal development in the rat (158-160). Protein kinase C delta is dramatically increased in rat corpora lutea in the latter portion of pregnancy, a period of time dependent on rat placental lactogens for the maintenance of corpus luteum function and pregnancy. The expression of this isoform is induced by estrogen and placental lactogen-1 (158), and prolactin activates protein kinase C delta in rat corpora lutea during this period of pregnancy (159). Furthermore, Peters *et al* (160) demonstrated that protein kinase C delta is required for prolactin to induce the expression of relaxin. These results demonstrate that specific protein kinase C isoforms are required for maintenance and normal function of the corpus luteum.

It is well established that the corpus luteum secretes PGF2 alpha. Recent studies clearly demonstrate that PGF2 alpha and protein kinase C activators can induce the expression of cyclooxygenase 2, a rate-limiting enzyme for prostaglandin synthesis, and up-regulate the synthesis of PGF2 alpha in luteal cells (161, 162). Thus, PGF2 alpha may activate an autocrine signaling mechanism that provides PGF2 alpha in sufficient quantities to ensure that corpus luteum steroidogenesis is interrupted, and the cascade of events leading to luteolysis is initiated. Wu and Wiltbank (161) reported that PGF2 alpha and protein kinase C activation of cyclooxygenase gene expression required a functional E-box response element. The upstream stimulatory factors 1 and 2 (USF-1 and –2), basic-helix-loop-helix-leucine zipper transcription factors with homology to Myc oncoproteins, were found to be present in luteal cells and bind to the E-box. Surprisingly, the levels of the USF transcription factors were not altered in response to PGF2 alpha or protein kinase C activators. It may be that other co-activators or co-repressors are regulated by PGF2 alpha and are required to provide full activation of E-box-mediated transcription of the cyclooxygenase gene.

Prostaglandin F2 alpha also stimulates the secretion of oxytocin *in vivo* and *in vitro*. This ability of

PGF2 alpha to increase oxytocin secretion is likely to be mediated by activating the phospholipase C/protein kinase C signaling system. Bovine large luteal cells contain high levels of vesicles carrying dense granules of neurophysin/oxytocin between Days 7 and 14 of the estrous cycle (163). Administration of PGF2 alpha during the midluteal phase of the cycle causes rapid degranulation of bovine large luteal cells (164), and an increase in blood levels of oxytocin (147). These secretory granules in luteal cells exist as a large paranuclear cluster. The clustered pattern of secretory granules in the bovine luteal cell differs from the more diffuse distribution of granules observed in luteal cells of the sow (165) and ewe (166).

Stormshak *et al* (146) provide an interesting model for the involvement of the protein kinase C substrate myristoylated alanine-rich C-kinase substrate (MARCKS) in the exocytosis of oxytocin. The effector domain of the MARKS protein allows cross-talk with many signaling systems; it is phosphorylated by protein kinase C, binds calmodulin and enhances polymerization of actin filaments *in vitro* (167). The MARCKS protein is associated with the actin filaments of bovine luteal cells and is phosphorylated in response to both PGF2 alpha and protein kinase C activators (146, 168, 169). The proposed sequence of signaling events begins with the activation of phospholipase C leading to protein kinase C-induced phosphorylation of MARCKS (see figure 2). The phosphorylated MARCKS protein is released from cytoskeletal actin binding sites which then leads to changes in the cytoskeleton that promote the exocytosis of oxytocin. It has been suggested that MARCKS may bind a significant fraction of PIP2 in a cell, sequestering a portion of the cellular pool of PIP2 for use in other cellular responses involving calcium or protein kinase C (170). Therefore, it seems likely that phosphorylation of MARCKS in conjunction with reductions in levels of PIP2 and increases in calcium levels may all individually contribute to actin depolymerization and promote the exocytosis response. Activation of the Rho family of small G-proteins may also participate in regulating the cytoskeletal changes and exocytosis (171).

3.5. Mitogen-Activated Protein Kinase

Whereas it seems clear that the initial event in PGF2 alpha action involves the activation of phospholipase C, other downstream signaling pathways are activated by PGF2 alpha. One such pathway is the mitogen-activated protein (MAP) kinase pathway. Four distinct MAP kinase cascades have been described in yeast and three in vertebrates (172). The MAP kinases in vertebrates are the extracellular signal-regulated kinases (Erks), the Jun N-terminal kinases (JNKs) or stress-activated protein kinases (SAPKs) and the p38 MAP kinases. Each of these MAP kinases is activated by dual-specificity MAP kinase kinases (MAPKK or MEK) which phosphorylate MAP kinases or threonine and tyrosine residues. The MAPKK or MEKs are distinct for each pathway and are phosphorylated by several MAPKK kinases (MAP3K) or MEK kinases including Raf, c-Mos and MEKK1. These upstream regulators of MAP kinases are activated by receptor tyrosine kinases, soluble tyrosine kinases, G protein-coupled receptors and other mechanisms (172). All the components of the ras-Raf, MEK, ERK pathway are present in luteal cells (99, 173-176). Recent studies demonstrate that PGF2 alpha activates Erk MAP kinase signaling in rat, pig, cow and human luteal cells.

The exact mechanisms that lead to activation of MAP kinase signaling in the corpus luteum have not been firmly established. In bovine luteal cells (173, 174) and human granulosa-luteal cells (176-177) PGF2 alpha and phorbol esters rapidly increase phosphorylation and activation of Erk1 and Erk2. Chen *et al* (173-174) proposed that PGF2 alpha activates phorbol ester-sensitive protein kinase C isoforms which phosphorylate and activate Raf-1 or B-Raf to initiate the Erk MAP kinase signaling cascade. Activated Raf then phosphorylates MEK1 which leads to the phosphorylation and activation of Erk1 and Erk2. In contrast, in a luteinized granulosa cell line derived from the rat, Stocco *et al* (127, 128) proposed that PGF2 alpha induces activation of Erk signaling by a CaM dependent mechanism, apparently independent of protein kinase C. Although the studies by Stocco *et al* (127, 128) did not identify the signaling pathways upstream of Erk, in many systems ligand-induced calcium signals have been shown to regulate the activation of Ras (178). Given the complexities of various cell models and multiple mechanisms available for activating Raf/MEK/Erk signaling it seems unlikely that a single initiating mechanism will emerge that is responsible for Erk signal transduction. However, identification of these signaling pathways is a prerequisite for understanding the processes regulated in luteal cells of various species. One emerging theme is that activation of Erk MAP kinase signaling represses the ability of gonadotropins to elevate the expression of StAR mRNA, StAR protein and progesterone secretion in ovarian cells (176, 179, 180). However, the role of Erk signaling in the control of steroidogenesis is likely to be more complex, as a stimulatory role for Erk MAP kinase activity was found in cAMP regulation of steroidogenesis and StAR expression in adrenal Y-1 cells (181).

Prostaglandin F2 alpha has been shown to activate calcium-dependent tyrosine kinase signaling pathways that promote the growth of NIH 3T3 cells (182). Subsequent reports have linked the actions of PGF2 alpha to hypertrophy of A7r5 vascular smooth muscle cells (183) and rat ventricular myocytes (184-187). Adams *et al* (184) suggested that the PGF2 alpha-induced myocyte hypertrophy occurs independent of protein kinase C, as well as p38 and Erk MAP kinases. However, in those studies the actions of PGF2 alpha were coupled to the activation of Jun N-terminal kinase. Chen *et al* (188) demonstrated that PGF2 alpha was also capable of activating p38 MAP kinase and Jun N-terminal kinase in bovine luteal cells. It is well established that granulosa cells undergo hypertrophy during their differentiation into the so-called large luteal cells of the corpus luteum (4, 8). The observation that PGF2 alpha receptors are highly expressed in the granulosa cells following ovulation (2, 3) raises the possibility that PGF2 alpha-initiated MAP kinase signaling may contribute to luteal cell hypertrophy during

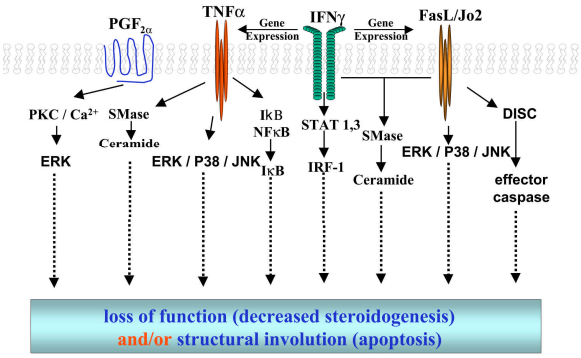

Figure 3. Illustration of the complexity involved in delineating the signaling pathways and their potential roles as regulators of steroidogenesis and/or cell death.

corpus luteum formation. Although little is known about the regulation of the MAP kinase cascade during corpus luteum development and regression, a report by Maizels *et al* (175) demonstrated that p38 MAP kinase and its upstream activator MAP kinase kinase-6 (MKK6) were chronically activated during the maturation of rat corpora lutea. Furthermore, a downstream target of p38 MAP kinase, MAP kinase-activated protein kinase-3 was induced and active during corpus luteum formation. The activation of p38 MAP kinase and MAP kinase-activated protein kinase-3 were linked to the phosphorylation of the cAMP regulatory element binding protein CREB. This study clearly provides evidence that components of MAP kinase signaling are active and may support corpus luteum development maturation.

3.6. Early Response Genes

Activation of MAP kinase results in its translocation to the nucleus where it phosphorylates and activates the ternary complex factor $p62^{TCF}$ other wise known as ELK-1 (172, 189). This transcription factor is part of a ternary complex that interacts with the serum response element (SRE) which promotes the expression of the c-fos gene. Members of the JNK or SAPK family can also phosphorylate and activate ELK-1 (172). The JNK/SAPK enzymes are also capable of phosphorylating N-terminal serine residues on another transcription factor, c-jun. These events appear to be responsible for stimulation of transcriptional activity of genes containing AP-1 sequence elements. The ability of PGF2 alpha to induce the transcription of the early response gene c-jun *in vivo* was reported by Khan *et al* (190) and Bertrand and Stormshak (191) in rat and bovine corpora lutea, respectively. Chen *et al* (173, 174) recently reported that activation of PLC/protein kinase C is correlated with Erk activation, translocation of Erk to the nucleus, and rapid induction of both c-fos and c-jun mRNA in cultures of bovine luteal cells. Stocco *et al* (128) demonstrated in luteinized granulosa cells that Erk signaling was required for the PGF2 alpha-induced phosphorylation of jun-D and the induction of the early response gene Nur77.

3.7. Additional signaling mechanisms

Cellular interactions with the matrix and with neighboring cells profoundly influence a variety of signaling events (192) including those involved in mitogenesis, vesicle trafficking, survival, cell motility and differentiation. Pierce *et al* (193) have recently demonstrated that stimulation of 293EBNA cells expressing FP or FPB with PGF2 alpha activates Rho leading to the formation of actin stress fibers, phosphorylation of p125 focal adhesion kinase, and cell rounding. Stimulation of Rho in response to PGF2 alpha is thought to require the activation of the G-proteins G12 and/or G13, although other mechanisms involving the calcium-dependent thiol protease calpain has been implicated in Rho activation (148). Of interest are recent reports demonstrating that another lipid, lysophosphatidic acid, (lysoPA) activates Rho in bovine luteal cells (194). The actions of Rho were correlated with the ability of lysoPA to inhibit LH-induced stellate morphology in primary cultures of luteal cells. It is well established that Rho proteins regulate the actin cytoskeleton, secretory events, and cell migration (171, 195). These observations provide fertile ground for investigation into the role of Rho GTPase signaling in the control of corpus luteum development and regression.

Recent studies demonstrate that the PGF2 alpha signaling pathway may cross-talk with the Wnt signaling pathway. Wnt gene family members (over 13 in mammals) encode glycoprotein hormones that bind to members of the Frizzled family of cell surface receptors (196). Activation of Wnt receptors results in an increase in the concentration of beta-catenin in the nucleus where it acts to promote the expression of Wnt-responsive genes. In both FP and FPB expressing 293 cells, PGF2 alpha-stimulated increases in cytosolic beta-catenin. In FP-expressing cells this was accompanied by increased beta-catenin phosphorylation. In FPB-expressing cells this was accompanied by decreased beta-catenin phosphorylation. In FPB-expressing cells PGF2 alpha also stimulated T-cell factor/lymphoid enhancer factor (Tcf/Lef) reporter gene activity that was not present in FP-expressing cells. A key control point could be in the differential phosphorylation of beta-catenin after agonist stimulation of FP or FPB. It seems possible that Rho may also be involved in this response since constitutively active mutants of G12 and G13 interact with E-cadherin resulting in a release of beta-catenin and stimulation of Tcf/Lef reporter gene activity (197). Since the Tcf/beta-catenin signaling pathway is known to mediate the actions of Wnt acting via Frizzled receptors it will be important to clarify the crosstalk between PGF2 alpha signaling and this little understood signaling pathway in the ovary. The possible role of FPB receptors in corpus luteum function is intriguing and awaits future studies.

4. MEDIATORS OF LUTEAL REGRESSION

Whether or not one single factor is responsible for initiating both the functional and structural aspects of luteolysis has not been conclusively demonstrated (see figure 3). Moreover, the potential luteolytic agents vary among the mammalian species. Consequently, a number of factors have been implicated in the luteolytic process including prolactin (31, 198-200), prostaglandin F2 alpha (44), elevated levels of reactive oxygen species (201-208),

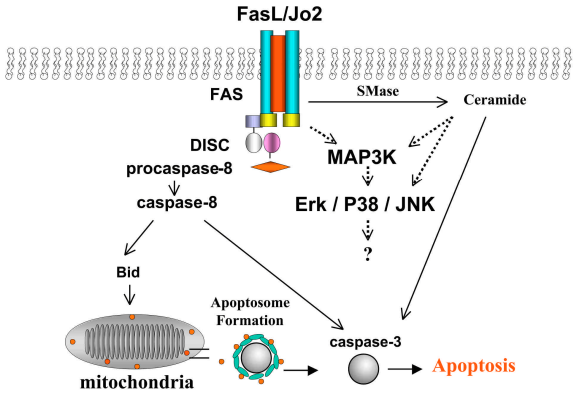

Figure 4. FASL activates multiple pathways in non-ovarian cells. The known pathways include activation of sphingomyelinase with the accumulation of ceramide, activation of the mitogen-activated protein kinase cascade, and/or activation of the caspase cascade leading to apoptosis. Current understanding of FASL actions in whole luteal tissue, preparations of mixed luteal cells, or specific luteal cell types is limited to the accumulation of ceramide, onset of cell death and the regulation of steroidogenesis.

tumor necrosis factor alpha (TNF alpha) (209-211) interferon gamma (IFN gamma) (210-215) Fas ligand (FasL) (216, 217), nitric oxide (207), endothelin -1 (218-221), heat shock protein 70 (222-226), steroid withdrawal or receptor inhibition (227, 228), inadequate gonadotropin and/or receptor (229), and inhibition of cell survival pathways (230), to name a few. It is also important to realize that not all potential mediators of steroidogenesis have the capacity to directly regulate apoptosis. Likewise, mediators of apoptosis may not directly inhibit steroidogenesis associated with loss of function, again suggesting that the processes of structural regression and loss of progesterone synthesis are separately regulated entities.

4.1. Cytokine-involvement in regression of the corpus luteum

It is becoming increasingly clear that loss of function and the involution of the luteal structure is mediated in part by a number of cytokines including FasL, TNF alpha and IFN gamma. The cytokines, FasL and TNF alpha are members of the TNF superfamily (a.k.a. TNSF) which consists of 18 genes encoding 19 type II transmembrane proteins (231). Members of the TNF superfamily interact with members of the TNF receptor superfamily (a.k.a. TNFRSF) of which there are 29 known members in the human (231). Despite the continually expanding number of ligands and receptors within these two families, studies involving members of TNF and TNF receptor superfamilies in the ovary are limited and are essentially restricted to FAS (a.k.a. TNFRSF6)/FasL (a.k.a. TNFSF6), TNFR (a.k.a. TNFRSF1)/TNF alpha (a.k.a. TNFSF1) and TRAIL (a.k.a. TNFSF 10) (209, 232-243). The majority of these studies focus on correlative expression of the receptor and/or ligand or binding studies. Essentially no detailed information is available on the signaling events, which occur in luteal cells upon receptor activation.

Within the TNF alpha superfamily there are a handful of cytokines which bind a specific subclass of TNF superfamily receptors known as death receptors (231). The death receptor classification is based on either function and/or the presence of a homologous amino acid segment located within the carboxy-terminal of the cytoplasmic region of the receptor known as the death domain (DD) (244-246). For example, activation of FAS results in the recruitment of cytoplasmic proteins (*i.e.*, FAS associated death domain protein, FADD) which bind and interact via the DD and the DED (death effector domain). The death effector domain of FADD recruits caspase-8 (a.k.a. FLICE). Collectively, this complex is named the death inducing signaling complex or DISC propagating the death-signaling cascade (247). Although it has not been demonstrated, it is not unrealistic to depict the death receptors as integral active mediators of luteal cell death via apoptosis since FasL or TNF alpha have the potential to stimulate death via apoptosis. Many factor(s) implicated as primary or secondary luteolysins include ligands that would bind members of the death receptor family.

4.1.1 FAS/ FasL

The temporal and spatial expression of FAS and FasL mRNA and/or protein in the human (248), bovine (249), rat (242, 250) and mouse (250) corpora lutea are strongly correlated with luteolysis. Based on immunohistochemical analysis Kondo *et al* (248) demonstrated the presence of FAS in the granulosa-lutein cells of the human corpus luteum during the early luteal phase and the levels increase during mid-luteal phase. The theca-lutein cells do not express FAS in the early luteal phase, however the receptor is evident in the mid-luteal phase. During the late luteal phase the overall expression of the Fas antigen increases. The levels of FAS diminish only when the corpus luteum undergoes transition to the corpus albicans. Similar results were found in the mouse with the highest levels of FAS being observed during luteal regression (250).

From a mechanistic standpoint, FasL or FAS activating antibodies induce luteal cell death in the human (217), mouse (216), rat (237, 242) and cow (251) (see figure 4). These *in vitro* studies are supported by *in vivo* experiments in which IV or IP administration of FAS-activating antibody induces luteolysis in the mouse (250). FAS-mediated cell death results in the activation of caspase-3, a central effector caspase (Carambula and Rueda unpublished results). More interestingly, the onset of FAS mediated cell death was attenuated in caspase-3 deficient mice when compared to wild type mice (Carambula and Rueda unpublished data). Further support for a functional role of FasL or FAS in luteolysis is evident in the homozygous gld (non-functional FasL) and lpr (reduced FAS expression) mice (250). The corpora lutea of these mice undergo luteolysis but at irregular intervals (250). Together these studies provide evidence to suggest that FAS mediated events are critical to the cyclicity of the female mouse.

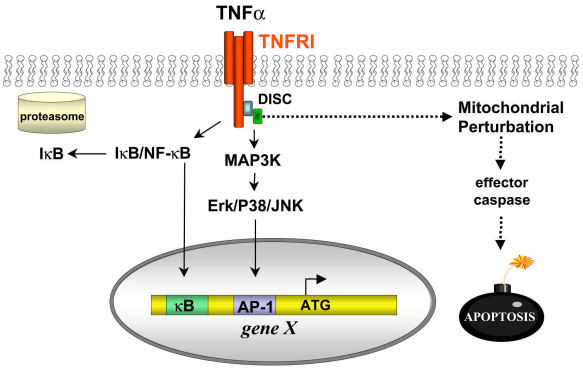

Figure 5. TNF alpha activates NF kappaB and mitogen-activated protein kinase signaling cascades in luteal cells. These events are known to regulate steroid and luteal PGF2 alpha secretion. In combination with IFN gamma, TNF alpha can induce apoptosis in the steroidogenic cells of the corpus luteum. The exact signaling mechanisms by which these events occur are unknown. However, we do know that the pathways activated in response to TNF alpha are likely to be influenced by the presence of other cytokines (*i.e.*, FasL and IFN gamma).

4.1.2. Tumor necrosis factor alpha

Other cytokines have the capacity to either inhibit steroid synthesis or induce cell death including IFN gamma and TNF alpha (209, 215-217, 252-254) (see figures 5 and 6). Tumor necrosis factor alpha mRNA or protein has been detected in the corpus luteum of the rabbit, rat, pig, cow and human (232-235, 255-258). It's origin is typically thought to be that of monocytes or macrophages, however, a significant concentration of TNF alpha is found within the endothelial cell component of the corpus luteum (232, 257). The message encoding TNF alpha does not change in the corpus luteum during the bovine estrous cycle (234, 259). However, at the protein level TNF alpha concentrations in the bovine corpus luteum are low in the early to mid luteal phase and increase dramatically during the late luteal phase and decline after regression (234). Although TNF alpha mRNA was undetectable by Northern analyses, TNF alpha bioactivity increased in after the progesterone levels declined (260). This increase in activity was believed to correspond to an increase in immune cells (260).

Tumor necrosis factor alpha normally can bind one of two TNF receptors, TNFRI or TNFRII (261, 262). To date the majority of what has been published with respect to TNF receptors subtypes within the corpus luteum has been focused on TNFR1 (209, 233-235, 263). An increase in the levels of mRNA encoding the TNFR1 subtype is observed in luteal tissue derived from natural or PGF2 alpha-induced luteolysis (209). These results, however, contrast an earlier study by Sakumoto *et al* (234), who provided evidence that TNFR mRNA levels remained unchanged throughout the luteal phase. These results were similar to those reported by Petroff *et al* (259). The reason for the discrepancy is unknown. Tumor necrosis factor alpha binding sites are present in luteal cells of multiple species (234, 235, 240) and are present throughout the luteal phase in the cow (234). Interestingly, Okuda *et al* (233) provided evidence of low and high affinity binding sites in the microvascular endothelial cells of the bovine corpus luteum.

Tumor necrosis factor alpha can initiate luteotropic or luteolytic effects that are likely stage, species or environment dependent (see figure 5). Tumor necrosis factor alpha can increase human chorionic gonadotropin (hCG) binding and hCG-stimulated progesterone in steroidogenic cells derived from human preovulatory follicles (264). Tumor necrosis factor alpha can also stimulate proliferation of human granulosa luteinized cells taken prior to ovulation following a gondotropic stimulation regime (265). However, TNF alpha has opposing effects in luteal cells derived from mid to late luteal phase of the human, mouse and pig. In cells derived from these stages, TNF alpha inhibits the response to luteotropins *in vitro* (264-268). The TNF alpha initiated response in the pig is somewhat complex in that it can inhibit the luteotropic effect of estradiol (269), yet in the absence of TNF alpha, PGF2 alpha can stimulate progesterone synthesis in young corpora lutea of the pig. Alternatively, pretreatment with TNF alpha and subsequent treatment with PGF2 alpha will further reduce progesterone levels than that of TNF alpha alone (270). The effect of TNF alpha is not limited to regulating steroid producing cells and it has been proposed to play a role in the vascular development of the pig corpora lutea and serve as a growth factor or regulator for epithelial cells and fibroblasts (257). Interestingly, TNF alpha is cytotoxic to endothelial cells derived from the bovine corpus luteum (209, 271).

Further evidence for the involvement of TNF alpha in corpus luteum function is provided by an investigation using knockout mice. Roby *et al* (272) demonstrated that TNFR1 deficient mice become 'locked' into a diestrous phase and do not proceed through the estrous cycle. This study implicates TNF alpha as a critical regulator of luteal regression. These results are supported by an earlier study in which anti-thymocyte antiserum was injected in rats to inhibit immune function (273). Similar to the TNFR1 null mice these rats failed to progress past the diestrous phase. Although this study does not directly implicate TNF alpha, it does support the notion that the immune system plays an integral role in luteal regression.

Few studies have focused on delineating the various signaling pathways activated in the corpora lutea or in dispersed luteal cells in response to TNF alpha (see figure 5). Tumor necrosis factor receptor I, like FAS, contains the DD segment and activation of the receptor results in the recruitment of TNF receptor associated death domain (TRADD) protein. TRADD can serve as an adapter protein to link the TNF alpha death signal to FADD and the formation of the DISC (274-276) which propagates the signal through the same downstream pathway as has been described for FAS. The formation of this complex activates downstream pro-caspase zymogens propagating the apoptotic signaling. Direct evidence for cytotoxic effects of TNF alpha treatment is limited to endothelial cells in culture derived from the corpus luteum of the cow (209). However, TNF alpha can augment IFN gamma-

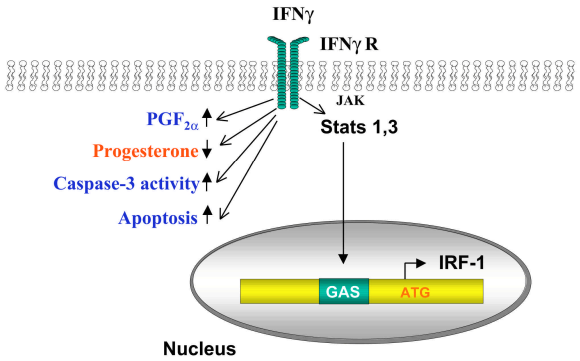

Figure 6. Treatment with IFN gamma can increase PGF2 alpha synthesis, decrease progesterone secretion, and elevate caspase-3 activity which corresponds to an increase in cell death via apoptosis. The specific mechanism(s) by which these events occur has not been demonstrated. IFN gamma can activate the JAK-STAT pathway and elevate the cytokine-specific transcription factor IRF-1. Whether or not this pathway is critical for the regulation of steroidogenesis and/or luteal cell fate remains to be determined.

induced cell death in luteal steroidogenic cells derived from the cow (215, 249, 252). To date, the utilization of the death receptor paradigm in the cytotoxic actions of TNF alpha or FasL in the ovary has not been conclusively demonstrated.

It is important to recognize that the limited number of cytokine receptors that are members of the death receptor family do not necessarily always fall within the 'death' signaling category. The binding of TNF alpha to its receptors commonly causes activation of two major transcription factors, nuclear factor-kappa B (NF kappa B) and activator protein–1 (AP-1), that in turn induces genes that are typically involved in inflammatory responses. In fact some of these genes serve to inhibit TNF alpha-induced apoptosis (277-281). This anti-apoptotic process is initiated by the recruitment of yet another cytoplasmic protein, TNF-receptor-associated factor 2 (TRAF2)(282). The formation of the TNFR1-TRADD-TRAF2 complex recruits an additional protein described as receptor-interacting protein 1 (RIP) (283). The NF kappa B activation step is regulated primarily by phosphorylation of inhibitory proteins, the I kappa Bs, which retain NF kappa B in the cytoplasm of unstimulated cells. In response to TNF alpha, the receptor protein complex initiates the phosphorylation of I kappa B and its degradation in a proteosome (274, 280). Upon its separation from I kappa B, the freed NF kappa B translocates to the nucleus where it initiates transcription, and translation of new gene products; presumably those which are anti-apoptotic in nature. Often times both pathways are activated simultaneously. In mixed luteal cells derived from the cow, treatment with TNF alpha results in rapid I kappa B degradation and translocation of NF kappa B (215) suggesting that this pathway may be functional, however, its role in luteal function remains to be determined.

Activation of TNFR also leads to the stimulation of the mitogen-activated protein (MAP) kinases, p38 MAP kinase and jun-n-terminal kinase (172, 284) (see figure 5). The mechanisms leading to the activation of these MAP kinases are complex and involve multiple upstream (*e.g.*, ASK1, MEKK1). However, studies indicate that overexpression of TRAF2 is sufficient to activate signaling pathways leading to NF kappa B and MAP kinase (285, 286). Recent results demonstrate that treatment with TNF alpha stimulates the phosphorylation and activation of p38 MAP kinase and jun-n-terminal kinase with minimal activation of ERK 1 or 2 (287). Based on the above *in vitro* studies, is seems clear that TNF alpha can activate multiple signaling pathways, whether or not these events occur *in vivo* have yet to be determined.

4.1.3. Interferon gamma

Interferon gamma, a type II interferon (and not a member of the TNF superfamily) can inhibit basal (266) and gonadotropin-stimulated progesterone production in human luteal cells (211, 266, 288); yet it has no effect on progesterone production in dispersed luteal cells derived from normally cycling cynomolgus monkeys derived from different days of the cycle (289). Treatment with interferon gamma can induce cell death in human (213), bovine (215, 249, 252) and mouse luteal cells (216). In contrast to IFN gamma-induced cytotoxic effects in human, murine and bovine, IFN gamma has a luteotrophic effect in the porcine corpora lutea (269). It is not clear why there are dissimilar effects. It is possible that the difference can be attributed to species variability. Alternatively, and perhaps more importantly, the origin of the recombinant IFN gamma protein may play a factor since the recombinant protein is based on IFN derived from different species in each of these studies. Therefore, it is possible that the biological activity of the compounds is different, consequently, the outcome is different. It was initially proposed that IFN gamma may exert its deleterious effects by increasing prostaglandin levels since an increase in PGF2 alpha was observed in response to IFN gamma in cultured bovine luteal cells (288). Subsequent experiments demonstrated that treatment with indomethacin did not reduce the capacity of IFN gamma to reduce gonadotropin-stimulated progesterone (288) suggesting that PGF2 alpha did not mediate the antisteroidogeneic action of IFN gamma. In contrast to the bovine model, no increase in PGF2 alpha was observed in response to IFN gamma in the luteal cells derived from the normally cycling cynomolgus monkey (290).

The majority of what is known with respect to IFN gamma signaling in the corpus luteum is related to cause and effect. There is little information on the cellular mechanisms used by IFN gamma to elicit its responses in ovarian cells. It is known that IFN gamma can signal via the janus activating kinase (JAK)-signal transducers and activators of transcription (STAT) pathway in non-ovarian cells (291, 292). Similarly, IFN gamma treatment results in an increase in nuclear levels of phosphorylated STAT-1 in the steroidogenic cells of the cow corpora lutea (215) (see figure 6). The increase in STAT-1 phosphorylation in other cell types results in the formation of STAT-1 dimers and translocation to the nucleus. STAT-1 dimers are known to

bind GAS sites on the promoters of interferon responsive genes (293, 294). Suter *et al* (215) demonstrated that IFN gamma treatment also resulted in a rapid induction of the cytokine-specific transcription factor interferon regulatory factor 1 (IRF-1). The expression of IRF-1 is driven predominately by cytokines that utilize STAT-1 or NF kappa B signaling pathways (293, 294). In bovine luteal cells, IRF-1 protein levels were elevated for up to 48 hours following treatment with IFN gamma. The significance of IRF-1 to luteal cell physiology is unclear at present, but in other systems, IRF-1 has been implicated in processes which include induction of cyclooxygenase, nitric oxide synthase, major histocompatibility antigens, p21waf/cip inhibitor of cell cycle progression, caspases 1 and 7, and cellular apoptosis susceptibility genes (293, 294). Alternatively, IFN gamma, like many of the other cytokines, can activate alternative signaling pathways, *e.g.*, the sphingomyelin pathway.

4.2. Vascular contributions to luteal regression

The corpus luteum is a highly vascularized organ with roughly 50 % of its cellular make up being endothelial cells (295-298). The highly vascular nature of the mature corpus luteum corresponds to the volume of blood flow that passes through this structure (299). Luteal regression has been attributed in part to changes in luteal blood flow. Whether the changes observed are causal or secondary events and contribute to luteolysis remains controversial. What is known is that acute changes in blood flow in response to luteolysins have been measured. Administration of PGF2 alpha can decrease blood flow to the rat and rabbit ovaries (300, 301). Subsequent studies in the ewe, supported the ability of PGF2 alpha to reduce blood flow concomitantly with progesterone levels (302, 303). These data, combined with morphological studies, led Nett, Niswender and colleagues to suggest that PGF2 alpha affected the vascular component of the corpus luteum (302, 303). More recent studies in the ewe utilizing the autotransplanted ovarian model (3) provide evidence to suggest that low levels of PGF2 alpha are sufficient to reduce progesterone prior to decreases in blood flow. The differences in the results between the two studies have been attributed to the different concentrations of PGF2 alpha (3). Depending on how an individual defines luteolysis could dictate how this discrepancy is interpreted. Reduction of circulating progesterone independent of any evidence of reduced blood flow implies loss of function is independent of luteal regression. However, if luteolysis is defined as the loss of function and cellular involution then a decrease in progesterone would not be directly attributed to a structural regression. The decrease in blood flow could however, be associated with structural involution of the luteal mass (304).

Prostaglandin F2 alpha reportedly acts directly on microvascular endothelial cells within the corpus luteum (77, 79). This information must be interpreted with caution since recent evidence indicates that the cells utilized for this study are considered a rare luteal endothelial cell type that has a phenotype similar to endothelial cells derived from the bovine aorta, a large blood vessel (71, 74, 75). Endothelial cells display variable morphology and function, which may be attributable to tissue specificity. At least five different microvascular endothelial cell phenotypes have been described in the bovine corpus luteum (76, 305-307). The more predominate luteal endothelial cell type which lack prostaglandin E or F receptors is cytokeratin 18 negative, whereas the rare cells with morphology similar to large vessels are responsive to PGF and are cytokeratin 18 positive (71, 74). The functional significance of the other individual endothelial cell phenotypes and their potential response to luteotrophic or luteolytic stimuli is yet to be determined. Moreover, the number, phenotype and response observed by the varied endothelial cell types could vary with the stage of development, the stage of the estrous or menstrual cycle or in response to pregnancy. However, no evidence for the expression of FP in endothelial cells has been reported in studies using in situ hybridization to identify luteal cell types expressing FP mRNA. Additionally, no PGF2 alpha receptors were evident in the blood vessels within the rat corpora lutea of the estrous cycle or pregnancy as measured by immunohistochemistry (34). It seems possible that species differences and cell type specific responses will have to be considered until further studies are available to argue convincingly for the endothelial cell as a direct target of PGF2 alpha.

Sawyer *et al* (308) demonstrated that there was a sequential pattern of cell type specific death within the corpus luteum during luteolysis. The endothelial cells are first to succumb to cell death, followed by the parenchymal cells. These results supported previous studies in the ewe where luteal endothelial cells were shown to undergo apoptosis and sloughed into the lumina of small blood vessels during luteolysis *in vivo* (309, 310). The rapid decrease in blood flow could result in luteolysis. Even today there is still supportive evidence to suggest that hypoxia induced by decreased blood flow could elevate reactive oxygen species and possibly lead to luteal cell death. This idea was first reviewed by Phariss *et al* (300), however, whether or not alterations in blood flow can directly affect luteal function or regression is a topic of debate (3).

4.3. Reactive oxygen species

Elevation of reactive oxygen species (ROS) can result in damage to DNA and RNA, damage to proteins, and initiate lipid peroxidation which can compromise cellular signaling and/function. Therefore, it is logical to expect that an increase in ROS may contribute to the loss of function and/or the demise of the luteal structure. It is well known that the PGF2 alpha-initiated decrease in progesterone in rats is associated with an increase in reactive oxygen species including superoxide radicals (311). Likewise, Sugino and colleagues (312) demonstrated that that ischemic reperfusion of the ovary of day 15 pregnant rats results in a decrease in progesterone in the corpus luteum and a decrease in superoxide dismutase activity. Treatment with PGF2 alpha also results in an increase in hydrogen peroxide (201, 202, 313). Hydrogen peroxide can inhibit LH and cAMP - stimulated progesterone but does not effect basal progesterone production (314). Since progesterone synthesis can be

restored by cell and mitochondrial membrane permeable cholesterol analogs it was proposed that hydrogen peroxide disrupted substrate availability for steroidogenesis (314). Subsequent studies by Musicki *et al* demonstrated that hydrogen peroxide inhibits rapidly inducible proteins that are known to mediate cholesterol within the mitochondria (315).

Nitric oxide (NO), another free radical is synthesized by oxidation of L-arginine by nitric oxide synthase (316, 317). NO levels are stimulated by TNF alpha or IFN gamma or the combination of the two (318). NO is synthesized by luteal cells of the rat and mouse (253, 319) and has been implicated as a mediator of luteal regression (319, 320). Interestingly, others have suggested that cytokine-induced luteal cell death in culture is independent of NO (253, 321). However, a recent *in vivo* study demonstrated that administration of L-NAME, an inhibitor of NOS, extended the life span of the corpus luteum in the cow (320). Moreover, L-NAME attenuated the luteolytic actions of exogenous administration of PGF2 alpha in the cow. *In vitro* studies on isolated corpora lutea of pseudopregnant rabbits demonstrated that PGF2 alpha treatment resulted in an increase in NOS and reduced progesterone synthesis (101, 322, 323). It is not clear why there are such dramatic differences between the in vitro and in vivo studies unless the culture conditions are such that a particular cell type is missing or deficient, or alternatively, NO is not obligatory but may actually serve to make luteal regression more efficient. Only future studies will be able to address this in more detail.

4.4. Non-traditional lipid signaling (sphingomyelin pathway)

Prostaglandin F2 alpha can increase lipid peroxidation and initiate a change in membrane fluidity (324, 325) presumably by reorganization of phospholipids. An increase in lipid peroxidation and alteration in membrane fluidity can alter ROS levels. Carlson and colleagues used wide angle X-ray defraction and fluorescence polarization techniques to describe events which occur at the membrane level of steroidogenic cells derived from the cow (326) and the rat (324, 327-329) during luteal regression. In response to PGF2 alpha there was a change from a liquid-crystalline to a gel phase transition state in phospholipid molecules of the cellular membranes prepared from regressing corpora lutea (326). The authors concluded that membrane proteins contribute to the ordering of lipids in membranes of the regressing corpora lutea. These results were further supported by Hansel and colleagues (324, 328, 330) who concluded that the plasma membrane may be the main source of this gel phase. Furthermore, the overall lipid composition of the microsomal preparations from these cells indicates a role for sphingomyelin, in the presence of cholesterol, for the generation of a gel phase. More importantly, the change in membrane composition was associated with a loss in luteal function (325, 326). Subsequent, studies have demonstrated that there is a decrease in membrane fluidity during induced luteolysis which could be involved in the mechanisms that inhibits LH-stimulated steroidogenesis during luteolysis (328). This change in membrane characteristics occurred in accordance with fluctuations in the sphingomyelin levels. The number of reports investigating alterations in membrane composition and lipid ordering in non-ovarian cells are increasing exponentially. The increased interest in signaling within membranes is paralleled by the increased interest in microdomains and other members of the sphingomyelin pathway which may serve as a platform for downstream signaling events.

The sphingomyelin pathway is a ubiquitous, evolutionarily conserved signal transduction system (see reviews 331-335). Although not as well defined as other signaling pathways (*e.g.*, the adenylate cyclase and phospholipase C pathways), the second messenger ceramide and other intermediates (*e.g.*, sphingosine or sphingosine-1-P) of the sphingomyelin pathway have been shown to transmit signals initiated on the cell surface to the nucleus and likely elsewhere within the cell. Sphingomyelin, a phospholipid, was originally found concentrated in the outer leaflet of the plasma membrane of mammalian cells (335). Sphingomyelin is hydrolyzed via a sphingomyelinase (SMase) generating ceramide. There are three known sphingomyelinases, commensurate with their pH (acid, neutral and basic) (334-337).

The specific enzyme responsible for generating increased intracellular ceramide may be secondary to the subcellular location in which the ceramide is generated. The physical location of the elevated levels of ceramide may be the critical determinant of how the cell is going to respond. It is also possible that cytokines can stimulate de novo synthesis of ceramide by the enzyme ceramide synthase that is localized to mitochondria and the endoplasmic reticular membranes (338). It is clear ceramide can be generated at multiple levels within the cell including the outer and inner leaflet of the plasma membrane as well as in the mitochondria.

The significance of the sphingomyelin pathway in reproductive tissues has only been recently recognized as evidenced by the following examples. ASMase null female mice are born with an over-endowment of germ cells (339). Sphingosine-1-phosphate, the natural inhibitor of ceramide-induced cell death, suppresses developmental, radiation- and drug-induced apoptosis in oocytes (339-341). Exogenous SMase and/or ceramide have been shown to mimic TNF alpha inhibition of gonadotropin-induced progesterone production (342-344). Ceramide mediates TNF alpha inhibition of P450 side-chain cleavage enzyme (342), 3 beta-hydroxysteroid dehydrogenase isomerase and p450 aromatase activity (342, 343). Finally, ceramide is involved in the FAS-mediated apoptotic processes of mouse granulosa cells (345-346). With regard to the corpus luteum, few studies have focused directly on the role of the sphingomyelin pathway. Sphingosine, an intermediate product of the sphingomyelin pathway can inhibit LH- and forskolin-induced cAMP in luteal cells (107, 347, 348). Furthermore sphingosine treatment of non-stimulated luteal steroidogenic cells can decrease progesterone production and reduced cell viability (107). It is important, however, to realize that ceramide can be metabolized to sphingosine

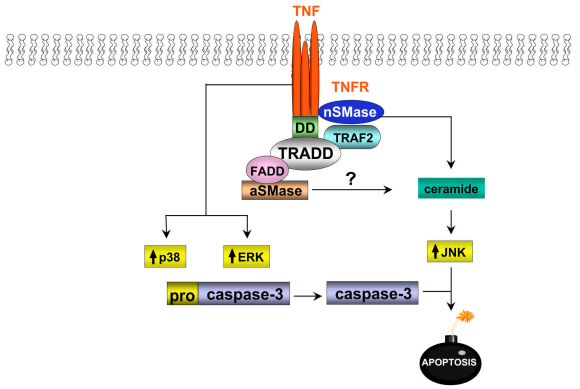

Figure 7. TNF alpha has been shown to elevate ceramide, a lipid byproduct resulting from sphingomyelin hydrolysis. Ceramide is considered to be a second messenger and can activate members of the mitogen-activated protein kinase family, induce cell death and alter steroidogenesis.

by ceramidase and sphingosine can be converted to ceramide by a yet unknown enzyme. Therefore to implicate ceramide or sphingosine as a direct mediator of luteal function will require additional studies.

More recently, cytokines have been shown to elicit their response by signaling through the sphingomyelin pathway (see figure 7). For example, FasL signaling via the sphingomyelin pathway has been demonstrated in a number of cell lines (Jurkat, BAEC, HeLa, *etc*.) and more recently in primary cell models including hepatocytes, lymphocytes, and splenocytes (334, 349, 350). Investigations into the involvement of FasL or TNF alpha activated ceramide in luteal function is ongoing (251, 271). At this time it is not known if the cytokine induced inhibition of steroidogenesis or luteal cell death involves sphingosine *in vivo*.

4.5. Endothelin-1 (ET-1)

Endothelin-1, a potent vasoconstrictor, has been implicated as a mediator of PGF2 alpha-induced steroid inhibition in luteal cells (219, 221). The actions of endothelin-1 in non-ovarian tissues resemble those initiated by PGF2 alpha. Endothelin-1 activates Gq and initiates calcium and protein kinase C signals involving the activation of Erk, p38, and JNK (186, 351). The levels of preproET-1 mRNA and/or ET-1 are elevated in the mid luteal phase and increase during spontaneous or PGF2 alpha-induced luteal regression (77, 218, 352, 353). The increase is believed to be sustained by an autoregulatory loop (218, 354). This increase of ET-1 mRNA at the mid luteal phase is not without controversy, Wright *et al* (355) reported no significant change in ET-1 levels under similar conditions. Endothelin-1 reportedly binds to ETA and ETB receptors with equal affinity however a majority of the effects are believed to be mediated by ETA in domestic farm animals (218, 355) and humans (356), but ETB appears to be involved in ET-1 actions in the rat (357-359). The levels of ETA mRNA do not change during the mid luteal phase in the cow corpus luteum in response to PGF2 alpha as reported by Wright *et al* (355). However, an earlier study by Levy and coworkers provided evidence to the contrary (353). Regardless of whether PGF2 alpha regulates the levels of mRNA encoding the receptor or ligand, the ET-1 peptide is elevated during loss of function (221, 354).

Endothelin-1 can inhibit basal and gonadotropin-induced progesterone synthesis in a dose dependent fashion (218). The mechanism by which ET-1 inhibits gonadotropin-induced progesterone synthesis in luteal cells is not yet known. However, ET-1 stimulates an increase in PGF2 alpha levels, which may serve to regulate steroidogenesis. Alternatively, the fact that ET- stimulates prostaglandin E at a much higher rate than PGF2 alpha in human luteal cells (360) can not be ignored since prostaglandin E activates the cAMP pathway and is often considered a luteotropin (360-362). In vivo administration of ET-1 to ewes resulted in an acute increase in progesterone levels within 4 hours of treatment, however the levels of progesterone had decreased when reassessed at 24 hours. Interestingly, in the same experiment PGF2 alpha treatment only transiently decreased progesterone levels and eventually the levels returned to the same as the saline treated animals without reducing the life span of the corpus luteum. Administration of ET-1 in concert with a sub-luteolytic dose of PGF2 alpha resulted in premature luteal regression (363). These data support the finding that ET-1 is not sufficient in of itself to induce luteolysis as defined by decrease in steroid levels and involution of the luteal structure. Juengel and colleagues previously demonstrated that administration of reduced concentrations of PGF2 alpha can decrease progesterone but have no effect on luteal weight (59). Together these data raise some interesting questions. Prostaglandin F2 alpha can increase the levels of ET-1, ROS and decrease blood flow. Additionally, hypoxia or oxidative stress can induce ET-1. It is not clear which event occurs first in the sequence of events.

5. STRUCTURAL REGRESSION

5.1. Apoptosis

The structural involution process reportedly involves apoptosis in the mouse (216, 364), rat (204, 242, 365, 366), hamster (28, 367), rabbit (205, 227), sheep (308, 368-370), cow (371-373), pig (374) and the primate (289, 375, 376).

Apoptosis is a physiological form of cell death distinguishable from necrosis by morphological and biochemical parameters. Apoptosis is typically associated with a growing family of Bcl-2-related proteins (377) that interact forming hetero- and homodimers to mediate function of the mitochondria (377-379). The Bcl-2 related proteins are segregated into three subclasses based on apparent function: the anti-apoptotic multi-domain members, the BH3 multi-domain members that facilitate or induce apoptosis, and the BH3 only domain members. The BH3 only domain members serve to bring together pro-apoptotic multi-domain members of the Bcl-2 family, by multiple mechanisms into heterodimeric units between the inner and outer wall of the mitochondrial membrane (377, 380-383). These components by nature can form pores

resulting in the release of 'apoptogenic' factors (cytochrome c, Smac/Diablo, apoptosis inducing factor; IAP) (377, 384, 385) which can be inhibited by antiapoptotic multi-domain members (377, 378, 386). Cytochrome c is essential for the generation of the apoptosome, a complex containing apoptosis activating factor-1 (APAF-1) and procaspase-9 (387-390). The resulting interaction generates procaspase-9 oligomers, leading to auto- or transcatalytic processing of the precursor enzyme to fully activated caspase-9. Activated, caspase-9 sequentially activates death effector caspases (*e.g.*, caspase-2, -3, -6 and/or -7) in the apoptotic cell death program (387, 389). Caspases regulate selective destruction of key structural and functional proteins in the cell including cytoskeletal proteins, mediators of signal transduction, DNA repair enzymes, RNA repair components, cell cycle regulatory proteins, nuclease activity-modulating factors and nuclear matrix proteins (387, 391, 392).

The belief that luteolysis of human and the non-human primate corpus luteum involves apoptosis, is not without controversy. Whereas some studies demonstrate primate luteal cells are dying by apoptosis (376, 393, 394) others provide evidence that luteal cell death occurs by autophagocytosis (395, 396). In support of apoptosis in the corpus luteum there are numerous reports which utilized a variety of techniques including terminal deoxynucleotidyl transferase-medited dUTP nick end-labeling (known more commonly as TUNEL) and the highly sensitive electrophoretic analysis of low molecular weight DNA fragmentation which suggest apoptosis is occurring (376, 393). Moreover, the incidence of apoptotic cells increases in the regressing corpus luteum when compared to mid-luteal phase human corpus luteum or the corpus luteum of pregnancy (376, 394). In contrast, large vacuoles were observed within the steroidogenic cells of the non-human primate corpus luteum by electron microscopy prompting investigators to suggest that luteal cells were dying by autophagocytosis and not apoptosis (289, 395). The formation of autophagic cells in the corpus luteum is not new and was first described in the 'atretic' human corpus luteum in 1971 (397) has since been supported by Fraser *et al* (395). Although the controversy has not been completely resolved it is possible that both apoptosis and autophagocytosis occur within the same structure. The corpus luteum consists of a heterogenous cell population, and it is not unrealistic that both forms of cell death could be occurring concomitantly and/or maybe specific to one cell type or another.

5.2. Bcl-2 family members

With identification of apoptosis in the corpus luteum, numerous studies were initiated to elucidate the mechanism(s) by which apoptosis was regulated within the corpus luteum. One of the first studies to report evidence of BCL-2 in the human corpus luteum was conducted by Rodger and colleagues (398). The Bcl-2 protein was found to be localized in the granulosa-lutein, theca-lutein and in endothelial cells of some blood vessels by immunohistochemistry. Using Western blotting techniques the authors detected no difference in the levels of Bcl-2 protein in the normal luteal phase or in response to chorionic gonadotropin. In contrast, more recent studies have provided evidence that BCL-2 is, in fact, regulated during the human luteal phase, early pregnancy and in response to human chorionic gonadotropin (207, 394). The controversy over whether Bcl-2 related proteins are truly involved in luteal regression is not limited to Bcl-2.

An early study investigating the levels of Bax protein in the human corpus luteum suggested that the levels of BAX did not vary through the luteal phase (399); yet again, more recent studies have suggested that BAX levels increase in the regressing corpus luteum, remain low in the mid-luteal phase corpus luteum, and are absent in the corpora lutea of pregnancy (394). It is not clear to what the differences in the two studies can be attributed. It is possible that the differences can be the result of different conditions related to fixation, embedding, antigen retrieval methodology or simply different antibody specificity. Whatever the difference(s), it is also important to realize that the level of change of a particular death agonist may not be as important as its location. BAX has been shown to be translocated to the mitochondria and nuclear regions following a death stimuli (400). The mitochondria is considered a pivotal player in steroidogenesis and the apoptotic process. Moreover, marked ultrastructural changes in the mitochondria were described during luteolysis as early as 1966 (401) whereby it was noted that the mitochondria swelled and underwent rarefication of the matrix which preceded the decline in progesterone in sheep luteal cells. This early description is similar to the morphological characteristics used today to describe a cell undergoing apoptosis.

BAX homodimers can form pores in the mitochondria membrane disrupting the membrane potential (386, 402, 403) which can ultimately lead to disruption of steroidogenesis and cell death. Therefore, it may not only be the levels of BAX and BCL-2 which are important but where they are localized. Furthermore, with the ever growing numbers of Bcl-2 family members being identified, there are sure to be an increased number of Bcl-2 family members which are being implicated in the growth, maintenance, and regression of the corpus luteum in the future.

5.3. Caspases

Since members of the Bcl-2 family have been implicated in the regulation of caspases (386), more recent studies have been designed to elucidate the role of caspases in the corpus luteum. Caspase-3 was localized to the human (404) and rat corpus luteum (405). Prostaglandin F2 alpha-induced luteal regression in the rat is associated with cleavage of putative caspase-3 substrates PARP and actin (405). No difference in the levels of caspase-3 in the healthy and the luteolytic rat corpora lutea was detected. This is not unexpected since many of the early antibodies were unable to distinguish between the active and inactive caspases. However, PGF2 alpha treatment in the ewe resulted in an increase in caspase-3-like activity as demonstrated by a substrate cleavage assay (406). Furthermore, cleavage of the pro-caspase 9 and 3 was evident in human luteinized granulosa cells in response to

staurosporine treatment (407) providing support that the enzymes were present and had the potential to be activated. Using an antibody which recognizes the active form of the effector caspase, it was recently shown that stress can activate caspase-3 which is coordinately associated with the decrease in progesterone levels and the onset of apoptosis in bovine luteal steroidogenic cells (287). The importance of caspase-3 to luteal regression is only becoming apparent with recent studies utilizing caspase-3 deficient mice (408). Whereas corpora lutea derived from wild-type mice will undergo apoptosis within 24 hours of culture in a serum free medium, the corpora lutea from caspase-3 deficient remain intact. Eventually, the corpora lutea from caspase-3 deficient mice will succumb overtime to apoptosis, but the onset is much delayed. The same result was observed *in vivo* (408). Corpora lutea derived from gonadotropin-induced luteal phase of caspase-3 deficient mice had a delayed onset of structural involution when compared to their wild-type sisters. Interestingly, regardless of the genotype, the serum levels of progesterone did not differ. This phenomenon provides an interesting clinical question. Can the lifespan of the corpus luteum be extended in a patient who has undergone *in vitro* fertilization to allow time for adequate generation of endogenous hCG levels for continued support of the conceptus? Alternatively, is the corpus luteum of the caspase-3 deficient mice hormonally functional and can it be rescued?

Aside from Bcl-2 and Caspase family members there have been efforts to characterize the role of proto-oncogenes like c-myc or p53. c-myc has been shown in the human corpora lutea, however, its relevance to luteal function or demise has not been fully demonstrated (409, 410). Similar to c-myc, p53 expression has been shown in the corpora lutea at the level of mRNA and protein, nonetheless, the changes detected were not necessarily associated with luteal regression *in vivo* (411). *In vitro* experiments, however, have shown that levels of p53 protein and/or mRNA are elevated in apoptotic human granulosa-lutein cells in response to serum withdrawal (254, 412). More importantly, the increase p53 protein and/or mRNA levels is inhibited by hCG (254, 412).

5.4. Immune cells and phagocytosis

Although apoptosis is often considered a non-inflammatory response, the cells, which undergo apoptosis, are often phagocytized by immune cells. Macrophages and T-lymphocytes are present in the corpus luteum throughout the cycle (413, 414), however, they accumulate in regressing corpora lutea of many species (415-418). Macrophages and T-lymphocytes likely take an active role in luteolysis via phagocytosis of luteal cells (419), degradation of extracellular matrix (23), and secretion of pro-inflammatory mediators that influence luteal steroidogenesis (252). For example, monocyte chemoattractant protein-1 (MCP-1), a chemokine, is produced in the corpora lutea during luteal regression and might aid in its destruction (416, 420-425).

5.5. Monocyte chemoattractant factor-1

MCP-1 has the potential to facilitate the attachment and migration of immune cells, specifically monocytes, macrophages and T-lymphocytes, from the blood stream (426) into the corpus luteum. Whether this occurs in the luteolytic corpora lutea is unclear. However, there is mounting evidence including MCP-1 expression increases during luteal regression (241, 420, 424). Furthermore, the endothelial cells of the bovine corpus luteum have been identified as a putative source of mRNA expression and MCP-1 secretion (416, 425). Although it is well established that treatment with PGF2 alpha elevates MCP-1 mRNA in vivo, the large luteal cells possessing the PGF2 alpha receptor were not the source of MCP-1 mRNA. Recent experiments by Caviccio *et al* (75, 427) demonstrate that PGF2 alpha treatment *in vitro* was incapable of elevating MCP-1 in mixed luteal cells or luteal-derived endothelial cells. Furthermore, these investigators demonstrated that the cytokines TNF alpha and IFN gamma stimulated MCP-1 mRNA expression and protein secretion in purified populations of endothelial cells (75). These results suggest that PGF2 alpha may contribute to the activation of a variety of cytokine signaling cascades in various cell types in the corpus luteum.

6. SUMMARY

To date there is a wealth of knowledge implicating a number of factors in the inhibition of steroidogenesis and/or the involution of the luteal structure. To what extent each of these factors play a role is questionable. Clearly, not all the factors identified are directly involved in loss of function and the structural involution of the corpus luteum. Attempts to delineate their importance have often yielded conflicting results. A majority of the contradictions can be attributed to differences in species, models (*in vitro* vs. *in vivo*), concentration and/or route of administration of a particular ligand or overall technique. Despite the differences in methodology, progress has been made and will continue to be made. The present review was designed to look beyond the cause and effect relationships and to discuss the mechanisms by which these events occur and to identify where information was lacking in order to provide some insight as to potential pathways that may be involved.

7. ACKNOWLEDGEMENTS

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