[Frontiers in Bioscience 12, 297-307, January 1, 2007]

Molecular control of ovulation and luteinization in the primate follicle

Richard L. Stouffer [1], Fuhua Xu [1], and Diane M. Duffy [2]

[1]Division of Reproductive Sciences, Oregon National Primate Research Center/Oregon Health and Science University, 505 NW 185th Avenue, Beaverton, Oregon 97006; Department of Physiological Sciences, Eastern Virginia Medical School, 700 Olney Road, Lewis Hall, Norfolk, VA 23501

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Prostaglandin Synthesis and Action
4. Progesterone Synthesis and Action
5. Synthesis and Action of Angiogenic Factors
6. Perspective
7. Acknowledgments
8. References

1. ABSTRACT

In recent years, significant progress was made, particularly through the use of the macaque monkey, in identifying three types of local factors that are induced by the midcycle LH surge and play a critical role in ovulation and/or luteinization of the primate follicle. The ovulatory gonadotropin surge increases prostaglandin (PTG, typically abbreviated PG) levels in follicles prior to rupture; although considerable attention has focused on LH stimulation of the "inducible" form of PG G/H synthase (PTGS2), other aspects of PG synthesis (notably a phospholipase A_2, cPLA2, and a PGE synthase, PTGES) and metabolism (15-hydroxy PG dehydrogenase, HPGD) also appear LH-regulated and may control the timing of the PG rise in the ovulatory follicle. Local (intrafollicular) ablation and replacement of PGs suggests that PGE2 is essential for release of the oocyte; but not necessarily for follicle rupture, and not for luteinization. Novel PGE-regulated genes are being identified in macaque granulosa cells, including adipose differentiation-related protein (ADFP). Similar types of studies indicate that the rise in progesterone (P) synthesis, as well as the induction of the genomic P receptor in granulosa cells, is essential for both ovulation and luteinization of the primate follicle. Limited data suggest that P action controls cell cycle activity (via cyclin B1 and cyclin-dependent kinase inhibitor p27), cholesterol uptake and utilization (e.g., low density lipoprotein or LDL receptor), proteases and their inhibitors (matrix metalloproteinase or MMP1; tissue inhibitor of MMP or TIMP1) and cell health in the granulosa cell layer. Finally, members of two classes of angiogenic factors, originally proposed as important for embryonic and pathologic (tumorigenic) vasculogenesis, appear induced in the granulosa layer of the preovulatory follicle, i.e., vascular endothelial growth factor (VEGF) and angiopoietin (ANGPT). Local injection of antagonists to VEGF (soluble VEGF receptor) and ANGPT (the natural antagonist ANGPT2) into the preovulatory follicle suppressed ovulation and luteinization in monkeys, possibly by disrupting the structure-function of existing vessels or preventing angiogenesis in the avascular granulosa layer. Further studies using high-throughput genomic and proteomic analysis, particularly on specific cell types (e.g., granulosa, theca and microvascular cells) and distinct follicular regions (apex, base and cumulus-oocyte complex) of the dominant follicle in natural menstrual cycles, are needed. Such information is essential to advance our understanding of the cascade of events leading to ovulation and luteinization of the primate follicle, to unravel the causes of ovary-based infertility and to consider novel ovary-selective approaches to contraception.

2. INTRODUCTION

As summarized recently by Espey and colleagues (1), the ovulatory process encompasses those events in the granulosa and theca cells of the mature follicle that are initiated by the midcycle gonadotropin (luteinizing hormone, LH) surge and lead to follicle rupture and release of a fertilizable oocyte (2, 3). This "cascade" of events includes direct actions of LH, as well as indirect effects mediated by LH-induced local factors (4), that ultimately alter every cell type in (e.g., granulosa cells, theca cells, cumulus-oocyte complex (5, 6, 7)) or recruited to (e.g., immune cells (8)) the follicle. Superimposed on the ovulatory process in many species, particularly those in which LH is a primary luteotropic hormone, is the luteinization process that converts the follicle wall into the progesterone-secreting endocrine gland called the corpus luteum (9). This process begins early after the onset of the gonadotropin surge and extends beyond ovulation for a number of days until luteal development during the ovarian cycle is complete. There is some compartmental distinction; ovulation requires changes in the granulosa-cumulus-oocyte complex (COC) to allow its release from the follicle wall (10) and in the follicle apex to allow rupture and extrusion of the antral fluid and COC, whereas luteinization involves remarkable changes in the steroidogenic and vascular characteristics of the remaining follicle wall. Nevertheless, to date, investigators have routinely used whole ovaries, or follicles, or at best aspirates of one cell type (granulosa cells) to study processes in the ovulatory, luteinizing follicle.

In the past few years, modern molecular techniques permitting high-throughput genomic analysis, including differential display-reverse transcriptase-polymerase chain reaction, suppression subtractive hybridization and DNA microarrays, were used to identify gonadotropin-regulated genes and gene expression during follicular development (11). Most of the data on the temporal pattern of expression of ovulation/luteinization-related genes were obtained from rodent models (1, 12); approximately 70 such genes were identified, which can be divided into subsets associated with immediate-early gene response, steroidogenesis, angiogenesis, inflammatory response, etc. Progress in understanding the molecular and cellular events associated with follicle ovulation and luteinization in other species has been slower, in part because of logistic problems (e.g., the ovulatory process is much longer in primates and domestic animals) and the difficulty of getting adequate tissue at precise intervals after the onset of the gonadotropin surge for analysis. Nevertheless, significant advances are occurring, particularly in primate species, through the use of a nonhuman primate model, the Old World macaque monkey. This chapter will focus primarily on recent information collected on the vital roles of three types of LH-induced local factors in ovulation and/or luteinization in the primate follicle.

3. PROSTAGLANDIN SYNTHESIS AND ACTION

A critical role for prostaglandins (PTGs, or commonly PGs) in follicle function was first proposed in the 1970s (for review, see (13)), when it was determined that the nonsteroid anti-inflammatory drugs (NSAIDs), aspirin and indomethacin, inhibited ovulation in several nonprimate species independent of any actions on pituitary LH secretion. Since these drugs irreversibly inactivate the enzyme converting arachidonic acid into PGH2, the precursor for several bioactive PGs, it was hypothesized that intra-ovarian/follicular PGs were essential for follicle rupture and/or oocyte release from the follicle. Subsequent evidence that the midcycle gonadotropin surge (or an exogenous gonadotropin bolus in stimulated cycles) markedly increases the concentrations of PGE2 and PGF2alpha in follicles prior to ovulation was consistent with this hypothesis. However, the mechanisms whereby the gonadotropin surge increases intrafollicular PG levels, the individual PG(s) promoting ovulation, and the specific PG actions causing oocyte release remain under investigation. Recent studies, particularly in rodents and domestic animals, indicate that the gonadotropin surge stimulates the expression of an "inducible" form of PG G/H synthase, termed PTGS2, and that mutant mice deficient in *Ptgs2*$^{(-/-)}$, but not the constitutive *Ptgs1*$^{(-/-)}$, isoform display ovulatory defects (14). Moreover, since increased PTGS2 expression and elevated PG levels were consistently measured approximately 10 h before follicle rupture in several species, where the interval from onset of the gonadotropin surge to ovulation varied from 14 (rodents) to 48 h (horses), Sirois (15) proposed that PTGS2-mediated PG expression was the determining factor in the timing of ovulation in mammals. Limited data, again from null-mutant mice, suggest that PGE is an essential factor for ovulation, acting through the PGE receptor subtype PTGER2, or EP2 (16). However, results varied depending on the age and genetic characteristics of mice. The release of the oocyte from the follicle requires both changes in the granulosa cells surrounding the oocyte (i.e., cumulus expansion) leading to release of the COC from the follicle wall, plus the lysis of the wall at the follicle apex to expel the antral fluid and COC. There is some evidence that PGs play a role in both processes, e.g., by activating proteases (17) but PG actions in the follicle remain poorly defined.

Duffy and colleagues recently used female macaque monkeys (18, 19) to determine if the midcycle gonadotropin surge stimulates PTGS2 expression and PG production in the ovulatory follicle in primates. In controlled ovarian stimulation cycles, PTGS2 mRNA levels and immunostaining were low-to-nondetectable in granulosa cells of large antral follicles prior to administration of the ovulatory hCG bolus, rose remarkably by 12 post-hCG injection, and remained elevated through 36 h post-hCG just prior to expected ovulation (36-40 h). PGE2 and PGF2alpha concentrations in follicular fluid were low through 24 h post-hCG injection, but increased 100-fold by 36 h. Moreover, concomitant treatment with a PTGS2 inhibitor, celecoxib, beginning at hCG administration markedly suppressed PGE2 levels in follicular fluid specifically at 36 h (19). These findings are consistent with the hypothesis that the midcycle LH surge promotes ovulation by stimulating *PTGS2* gene expression and PG (E2 and F2alpha) production in the follicle. However, there may be some unique aspects in primates: (a) whereas PTGS2 expression in rodents and domestic animals appears limited to the granulosa cell layer of the

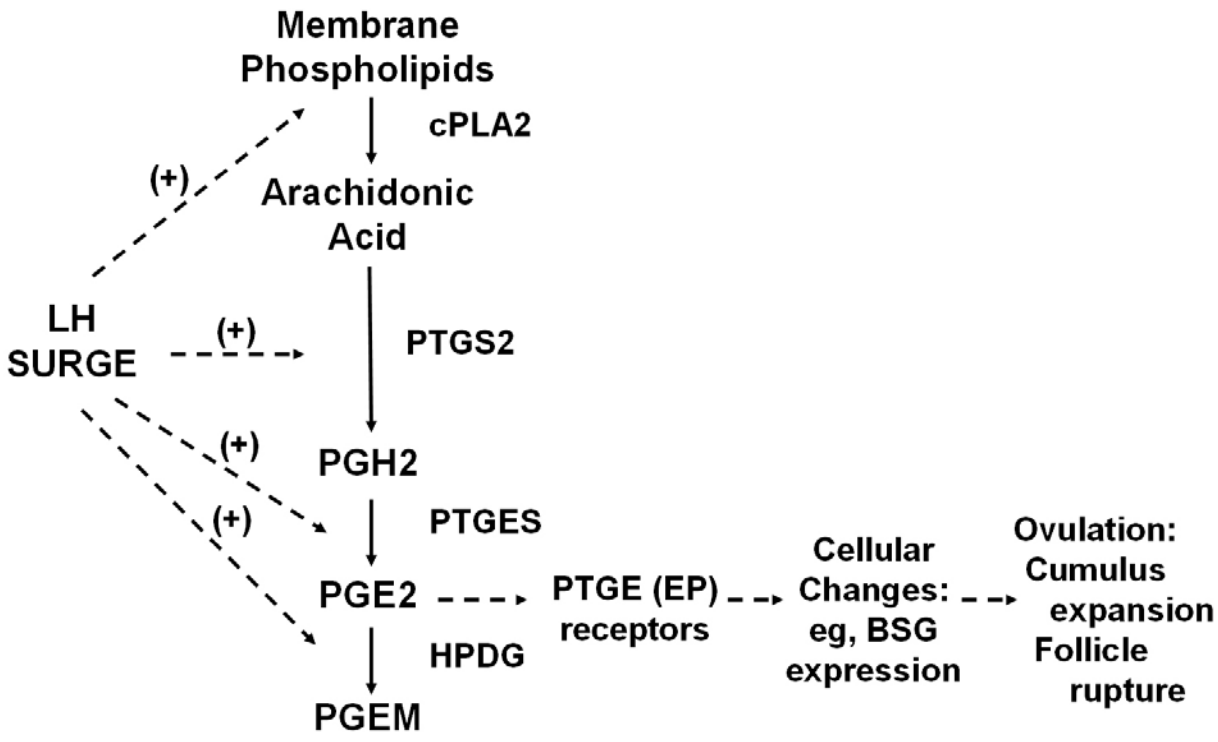

Figure 1. Proposed schematic of PGE2 synthesis, metabolism and action in the primate ovulatory follicle, and apparent sites of regulation by the midcycle LH surge. Solid lines indicate enzymatic steps in synthesis and degradation. Dotted lines indicate apparent actions of LH or PGE2 leading to cellular events culminating in cumulus expansion, rupture of the follicle wall, and release of the cumulus-oocyte-complex from the mature follicle. See text for definitions of abbreviations and the timing of events after onset of exposure to the LH surge.

follicle, PTGS2 expression and activity was detected in both the theca and granulosa cells of macaque and human follicles, and (b) the delay between PTGS2 expression (12 h post-hCG) and elevated PG levels (36 h post-hCG) suggests that the former event may not be the only factor controlling PG synthesis and PG-mediated ovulatory events in primates.

Additional studies by Duffy and colleagues suggest that the gonadotropin surge stimulates other steps (Figure 1), from the mobilization of arachidonic acid precursor to the suppression of PG metabolism, that would enhance PG, notably PGE2, levels in the primate ovulatory follicle. It is believed that members of the phospholipase A_2 (PLA2) enzyme family promote PG synthesis by the release of arachidonate precursor from cell membrane phospholipids (13). Two of the three members capable of arachidonic acid release, cytosolic (c) PLA2 (PLA2G4A) and soluble (s) PLA2v, but not sPLA2IIA, were detectable in macaque granulosa cells (20). Only one form, cPLA2, displayed 8-fold elevated mRNA expression within 24 hrs after administration of an ovulatory hCG bolus, followed by a marked increase in PLA2 activity in granulosa cell lysates by 36 h post-hCG injection. Notably, a cPLA2-selective inhibitor, arachidonyl trifluoromethyl ketone (ATFK), markedly suppressed PGE_2 production by primate granulosa cells following hCG exposure *in vivo* and acute (4 h) incubation *in vitro*. These data suggest that the gonadotropin surge in primates, and perhaps in rodents (21, 22), promotes arachidonic acid mobilization in the ovulatory follicle by selective induction of cPLA2. There are other active eicosanoids synthesized from arachidonic acid besides PGE2, including other PGs and thromboxanes (TXs) produced by the *PTGS* pathway, plus the unstable hydroperoxyeicosatetraenoic acids (HPETEs), their stable breakdown products hydroxyeicosatetraenoic acids (HETEs) and leukotrienes (LTs) produced by the lipoxygenase pathway (23, 24, 25). It is possible, if not likely, that some of these eicosanoids also influence ovulation and luteinization. For example, there are reports that a lipoxygenase inhibitor (26) and LTB4 receptor antagonist (27) inhibits ovulation in rodents, and that HETEs influence progesterone production by human luteal cells (28). However, detailed studies are needed to further evaluate the role of eicosanoids, other than PGE2, in the ovulatory, luteinizing follicle in primates.

The gonadotropin surge may also regulate the pattern and levels of follicular PGE2 by: (a) stimulating expression of the enzyme directly catalyzing PGE synthesis, and (b) controlling the degradation of PGE to inactive metabolites (Figure 1). Duffy *et al* (29) reported that following the hCG bolus in gonadotropin-stimulated cycles, macaque granulosa cells gradually increased mRNA levels for one of the three forms of *PTGE* synthase (*PTGES*, but not *PTGES2* or *PTGES3*), with *PTGES*

protein levels peaking just prior to ovulation. The latter coincided with peak PGE2 levels in follicular fluid 36 h post-hCG administration. However, PGE2 production was also evident in theca cells from monkey and human periovulatory follicles (18, 30), so a role for this compartment in PG-related events cannot be ruled out. It is well known that PGE2 can be metabolized to biologically inactive 15-keto PGE2 derivatives by 15-hydroxy PG dehydrogenase (HPGD) and then further degraded by other enzymatic and nonenzymatic methods (13). The lung is the major site of HPGD activity (accounting for the short half-life of PGs in circulation), but this enzyme is expressed in other tissues and may play a role in controlling local PG levels and action, e.g., in the placenta. Duffy and colleagues (31) recently reported that HPGD expression and activity was present in the macaque preovulatory follicle in a pattern consistent with modulating PGE2 levels leading up to ovulation. Although low prior to exposure to the ovulatory hCG bolus, macaque granulosa cells transiently expressed *HPGD* mRNA at 12 h, but not 24-36 h post-hCG administration. HPGD protein was also evident at 0-24 h, but low-to-nondetectable prior to ovulation at 36 h. Likewise, levels of PGE metabolite (PGEM) were low at 0 h, but appreciable thereafter and peaking at 24 h prior to the marked increase in bioactive PGE2 at 36 h. Thus, it is possible that gonadotropin-regulated HPGD plays a role in controlling the timing of the PGE2 rise, and hence the ovulatory interval, in the primate follicle.

Evidence from both monkeys and women support the concept that locally generated PGs, and particularly PGE2, are critical for ovulation but not luteinization of the primate follicle. Systemic administration of NSAIDs, as general PTGS1 inhibitors, around the time of the midcycle gonadotropin surge caused the development of luteinized unruptured follicles (32, 33). More recent evidence that oral administration of specific PTGS2 inhibitors to women can disrupt timely ovulation, led to recommendations that these drugs should be avoided during fertility efforts (34). Novel experiments injecting a general PTGS1 inhibitor (indomethacin (35)) directly into the preovulatory follicle in monkeys confirmed that local PG synthesis is required for ovulation, but not luteal development; serial sectioning of ovaries identified trapped oocytes within the luteinizing walls of follicles, half of which appeared to have ruptured. However, co-injection of PGE2 with indomethacin eliminated any evidence of trapped oocytes or unruptured follicles. Nevertheless, the specificity of PGE action was not examined, e.g., by injecting other PGs such as PGF2alpha. Whether intrafollicular conversion of PGE2 to other bioactive PGs, such as PGF2alpha, plays a role in ovulatory events has not been evaluated.

The mechanism(s) of action of PGs, and notably PGE2, in controlling ovulatory events in the primate follicle has received little attention. The presence of PG receptor subtypes is poorly defined in the primate ovary, although it is reported that human granulosa cells from ovulatory, luteinizing follicles express PTGER1 (EP1), PTGER2 (EP2) and PTGER4 (EP4) receptors for PGE2 (36, 37). Recently, Seachord and colleagues (19) used microarray analysis with the Affymetrix (Santa Clara, CA) Human Gene FL array to compare mRNA expression by nonluteinized granulosa cells from macaque antral follicles after 40 h culture in the presence of an ovulatory dose of hCG alone or hCG plus PGE2 at a level found in ovulatory follicles (1 microgram/ml). Twenty mRNAs were identified that differed by more than 2-fold expression between groups, including *BSG* (a matrix metalloproteinase inducer, +16.7-fold), *EGR1* (a transcription factor previously identified in ovulatory follicles of rodents (1), +2.4-fold), and *ADFP* (+2.4-fold). Adipose differentiation-related protein or ADFP is a lipid droplet protein involved in the intracellular transport of long-chain fatty acids, including arachidonic acid. Subsequent *in vivo* protocols confirmed that *ADFP* mRNA and ADFP protein levels increased in macaque granulosa cells of preovulatory follicles after administration of the hCG bolus, peaking at 36 h post-injection, and that co-administration of the PTGS2 inhibitor, celecoxib, markedly reduced *ADFP* mRNA levels. These studies identify ADFP as a novel LH- and PG-regulated protein in granulosa cells of the primate ovulatory follicle that may enhance PG synthesis, steroidogenesis and/or other lipid-associated processes. These studies also portend discovery of other PG-regulated processes in primate ovulatory follicles using broad scale genomic and proteomic approaches.

4. PROGESTERONE SYNTHESIS AND ACTION

Although proposed years earlier by Rothchild (38), direct evidence for local actions of progesterone (P) in the ovulatory, luteinizing follicle followed the discovery in the late 1980s that the midcycle LH surge not only promoted the development of the steroidogenic pathway catalyzing P synthesis, it also induced the expression of genomic P receptor (PGR or PR) in the luteinizing granulosa cells of monkeys and rodents (for review, see (39)). Over the past decade, this phenomenon was observed in all mammalian species studied to date, from mice to domestic animals to primates. There appear to be species-differences in PR expression in other follicular compartments, since PR is detectable in the theca layer (and surrounding stroma) in primates, but not in rats. However, studies have focused primarily on the granulosa cell as a P target because of the prevalent use of the rodent model and the ease of collecting granulosa cells from ovaries by follicle aspiration. The duration of *Pgr* mRNA and PR protein expression in the ovulatory follicle and developing corpus luteum varies markedly between species. PR is only transiently expressed in the preovulatory follicle of rats up until the time of ovulation (approx. 12 h), and only into the early luteal phase of some species, such as rabbits. However, appreciable genomic *PGR* in the developed corpus luteum occurs in species with long, functional luteal phases during the ovarian cycle, including domestic animals and primates. Both PR-A and -B isoforms are reportedly detectable, but again there may be species differences (e.g., PR-A predominates in rat granulosa cells, whereas PR-B is greater in macaque luteal tissue) and the ratio of PR-A to -B may change temporally in the tissue. In addition, mounting evidence suggests that P can have nongenomic (i.e., "rapid") actions in target

cells, that may be mediated by membrane-associated receptors (40, 41). Taken together, these data support the concept that the ovulatory, luteinizing follicle is not only a vital source of the steroid hormone P, but also a site of PR-mediated actions of P in the ovary.

Since both P synthesis and PR expression increase rapidly in granulosa cells following the midcycle gonadotropin surge, P could play a local role in the three major events of the mature follicle: (a) reinitiation of oocyte meiosis, (b) ovulation, and (c) luteinization to form the corpus luteum. In the 1970s, investigators recognized that acute suppression of steroidogenesis prevented ovulation in rats (39). Studies over the past decade have identified P as the critical steroid for ovulation in rodents, and extended this concept to primates. Two animal models were employed: (a) the *Pgr*$^{(-/-)}$ null, mutant mouse, and (b) the female rhesus monkey, in classical steroid ablation-replacement protocols wherein steroid synthesis/action is suppressed and individual steroids added back during the preovulatory interval to demonstrate specificity of action. In both models, exogenous gonadotropins are given to promote multiple follicular development followed by a bolus of hCG to stimulate periovulatory events.

Collectively, the data support a vital intrafollicular role for P in ovulation of the rodent and primate follicle. Generation of mice deficient in both PR-A and -B (*Pgr*$^{-/-}$) yielded infertile animals that did not ovulate spontaneously or in response to exogenous gonadotropins (42). Subsequently, generation of mice deficient in only one PR isoform indicated that deletion of PR-A severely impaired follicle rupture, whereas PR-B deletion did not (43). Studies in monkeys indicated that acute administration of a 3 beta-hydroxysteroid dehydrogenase (HSD3B) inhibitor (trilostane, Sanofi Pharmaceutical, Inc.), beginning at the time of hCG injection, prevented ovulation, but concomitant treatment with a potent progestin R5020, but not an androgen, dihydrotestosterone, restored follicle rupture (44). Although P generated in response to the gonadotropin surge appears essential for ovulation, limited evidence suggests that P alone cannot replace or mimic the LH/CG action. At least at the doses tested, when the progestin R5020 was administered to monkeys at midcycle in place of the ovulatory hCG bolus, follicle rupture did not occur (45).

Initial evidence suggested that P was not essential for reinitiation of oocyte meiotic maturation. But recent results from rodents (46), domestic animals (47), and monkeys (45) indicate that there may be a steroid/P-regulated pathway of oocyte maturation, perhaps reminiscent of direct progestin-induced meiosis in non-mammalian oocytes or via indirect actions on the cumulus cells surrounding the oocyte. Shimada and Terada (47) proposed that P acts via gonadotropin-induced PR in cumulus cells to reinitiate meiosis in porcine oocytes by disrupting gap junctions between cells in the COC. Borman and colleagues (45) reported that whereas P treatment in place of hCG as ovulatory stimulus did not cause follicle rupture, over 40% of the oocytes collected from unruptured follicles had resumed meiosis to reach metaphase I, matured to metaphase II in hours and fertilized *in vitro*. Although detailed studies are required, defects in oocytes and embryos following various regimens of steroid depletion and replacement support the concept that both steroid-dependent and -independent actions of the gonadotropin surge combine to achieve an optimal milieu for reinitiation of meiosis and oocyte maturation that is essential for fertilization and early embryogenesis in primates.

A role for P to promote luteinization of the ovulatory follicle may differ between species. Although initial reports (42) hinted otherwise, further studies in *Pgr*$^{(-/-)}$ null mice suggest that morphologic and molecular (e.g., CYP11A1 expression) indices of luteinization remain in the unruptured follicles (48). In contrast, morphologic and functional (serum P levels in the subsequent luteal phase) indices suggest that luteinization, as well as ovulation, is suppressed in large antral follicles of monkeys during acute steroid ablation at midcycle, but restored by progestin replacement (44). The broader role in primates may be related to the extended (38 h) periovulatory interval and major role of LH- (versus prolactin, in rodents) mediated events in luteal development and lifespan. One may hypothesize that the ovulatory function of PR-A signaling is conserved among species, and the predominant PR-B form in primates serves to promote the development and maintenance of luteal structure-function.

Investigators are now using sensitive molecular techniques (differential display, and real-time PCR) to identify genes/processes that are regulated by the ovulatory gonadotropin stimulus and local P action. Compared to rodent models (1), information on the primate ovulatory follicle has been slower to accumulate (Figure 2). Controlled ovarian stimulation cycles have been employed to evaluate the expression of cellular molecules involved in P synthesis (e.g., LDL receptor, steroidogenic enzymes) in granulosa cells of macaque antral follicles after administration of the ovulatory hCG bolus. These studies (49, 50) determined that: (a) in some cases, the dynamics of mRNA expression (e.g., increased *STAR* and *HSD3B* mRNA and decreased aromatase (*CYP19A1*) mRNA) were consistent with conversion of granulosa cells from primarily estrogen- to P-producing cells; but (b) mRNA expression (e.g., for *HSD3B* and *CYP11A1*) did not necessarily correspond to cellular steroidogenic activity; also (c) steroids/P play a role in regulating cholesterol uptake and utilization (e.g., LDL receptor and possibly *STAR* mRNA) in the luteinizing follicle, but (d) any role for P in regulating the steroidogenic enzymes leading to its synthesis is unclear - investigations with P synthesis inhibitors (51) yielded negative data, whereas use of PR antagonists (52) supports a stimulatory role for P. Additional studies are needed which analyze the various compartments (including theca) of the ovulatory follicle, and include the developing corpus luteum in the early luteal phase of the cycle (53).

Studies by Chaffin and colleagues (54) broadened the concept from rodents (55) to primates that the ovulatory gonadotropin stimulus markedly suppresses granulosa cell

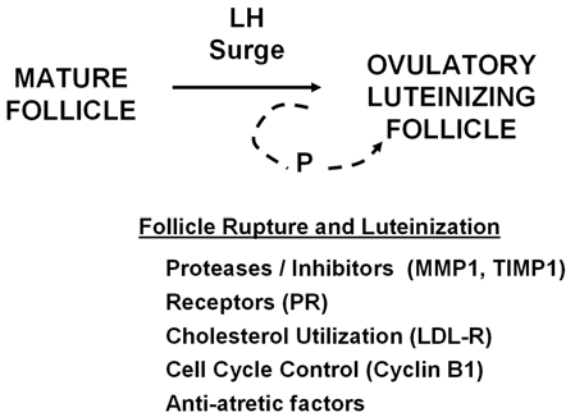

Figure 2. Schematic of the concept that the LH surge stimulates synthesis of the steroid hormone progesterone (P), which acts as a critical intrafollicular factor essential for ovulation and luteinization in primates. Some of the processes and molecules observed to date that are P-regulated in granulosa cells of the macaque ovulatory follicle are listed. For further details see the text and review by Stouffer (39).

proliferation in the ovulatory follicle. The number of cells in a proliferative cycle (as judged by Ki67 immunostaining) declined rapidly from 50% at 0 h, to 20% by 12 h post-hCG injection, and 10% by 36h post-hCG. Important differences and similarities between these species in terms of mRNA expression of key proteins (e.g., cyclins B, D, E) and inhibitors (cyclin-dependent kinase inhibitors p21 and p27) were apparent. Notably, a similar pattern of the inhibitors was observed in both species, leading to the hypothesis that the early rise (12 h) in p21 was important for the initial decline in granulosa cell mitosis and the later rise (36 h) in p27 plays a role in maintaining cell cycle arrest in luteinized cells of the developing corpus luteum. It appeared that steroids/P do not play a critical role in the early decline in granulosa cell proliferation, but can regulate mRNA levels of cell cycle components such as cyclin B1 and p27. However, more studies are needed to understand the control of cell cycle activity within specific cell types in the luteinizing follicle (56), including arrest of steroidogenic cells versus proliferation of microvascular cells.

An area that's received considerable attention is the proteases that may be important for the tissue remodeling associated with follicle rupture/oocyte release and luteinization. Chaffin and colleagues (57) provided evidence that members of the matrix metalloproteinase (MMP) family and their endogenous inhibitors (TIMPs) are dynamically expressed in granulosa cells of the primate follicle following administration of the ovulatory gonadotropin (hCG) bolus, and that expression of at least one important member (MMP1 or interstitial collagenase) and its inhibitor (TIMP1) is up-regulated by P. Moreover, expression of certain MMPs (e.g., MMP-1 (58)) and their inhibitors (e.g., TIMP-1 (58, 59) remained elevated during luteal development in the early luteal phase of the menstrual cycle. However, the complexity of the process is underscored by the realization that only five of at least 28 MMP-TIMP family members (for review, see (60)) were examined, that these enzymes/inhibitors are also regulated at the translational and post-translational level, and that their expression/activity likely varies between compartments (granulosa versus theca) and regions (apex versus base) of the ovulatory luteinizing follicle. Other classes of proteases, such as the serine proteases (e.g., plasmin/plasminogen activator) are expressed in the periovulatory follicle (61) and may be indirectly regulated by LH via P (62). Two P-regulated proteases (cathepsin L, and a disintegrin and metalloproteinase with thrombospondin motifs or ADAMTS1) were recently discovered and are suspected to contribute to ovulatory events in the rodent follicle (48). ADAMTS1 is highly expressed in the luteinizing granulosa cells of the primate ovulatory follicle, as well as in the developing corpus luteum (63), but its regulation to date is unknown. Further studies are required to elucidate the expression, regulation and role of the myriad of proteases that may serve to control the ovulatory, luteinizing follicle in primates.

5. SYNTHESIS AND ACTION OF ANGIOGENIC FACTORS

Aside from pathologic and trauma conditions, blood vessel development in healthy adults is primarily limited to the ovary and reproductive tract, i.e., tissues that undergo sequential growth and regression during the ovarian cycle. Notably, the importance of neovascularization of the luteinizing, ovulatory follicle - particularly in the previously avascular granulosa layer - for the development and subsequent function of the corpus luteum has been implied for years. Recently, considerable progress occurred following the discovery that two classes of factors with selective actions on the endothelial/peri-endothelial cells of the vasculature, i.e., the vascular endothelial growth factors (VEGF) and angiopoietins (ANGPT), are synthesized in the ovulatory, luteinizing follicle, including in the primate ovary (for review, see (64, 65)). Moreover, administration of VEGF- or ANGPT-antagonists markedly altered periovulatory events, thus supporting a critical role of these angiogenic factors in follicle health and function, including ovulation and luteal development.

Although several members of the VEGF family (VEGF-A to -F), as well as isoforms of individual VEGFs (e.g., the seven forms of VEGF-A ranging from 121 to 206 amino acids), have been detected in tissues, studies on the ovary have focused on the first discovered member VEGF (or VEGF-A). It is now evident that its lower molecular weight, secreted isoforms VEGF-121 and -165, its two receptors FLT1 or VEGF-R1 and KDR or VEGF-R2, and possibly two co-receptors neuropilin-1 and -2 (which facilitate VEGF-165 binding and action), are expressed in the primate ovary (66). In addition, ANGPT1 and its TEK or Tie2 receptor, as well as the endogenous TEK antagonist ANGPT2, are expressed in the primate follicle and corpus luteum (66), but progress on this angiogenic family has been limited by the paucity of reliable reagents to detect protein product. A model originally proposed to explain

blood vessel development, maintenance and degeneration in embryonic tissues may be applicable to vascular events during growth versus atresia of follicles as well as corpus luteum development versus regression (67). It is proposed that VEGF(-A) production promotes blood vessel development by stimulating endothelial cell proliferation, migration and cell-cell interaction to form tubular vessels, whereas ANGPT1 promotes vessel maturation by recruiting peri-endothelial support cells (e.g., pericytes) and thus supporting vessel integrity and function. In contrast, ANGPT2 antagonizes ANGPT1 action thereby destabilizing vessels which, in the presence of VEGF, may promote angiogenesis from existing vessels or, in the relative absence of VEGF, can cause vessel degeneration.

It is generally believed that VEGF expression in granulosa cells of the growing antral follicle in primates is low, but becomes prevalent in the granulosa surrounding the oocyte and in the theca cells of the preovulatory follicle (64). After the ovulatory gonadotropin surge (or hCG bolus in stimulated cycles), VEGF protein levels are greatly elevated in the follicular fluid and granulosa cells prior to follicle rupture (68). Evidence that this is a direct effect of LH comes from studies where *in vitro* exposure of nonluteinized granulosa cells from macaque preovulatory follicles to surge levels of gonadotropins increased VEGF levels in the culture media 10-100-fold (68). Remarkably, hypoxic conditions, which are the primary stimulus of VEGF production and angiogenesis in many tissues, did not stimulate VEGF production by nonluteinized granulosa cells (69). Rather, hypoxia-stimulated VEGF production was not evident until just prior to ovulation (36 h post-hCG injection) or in the early luteal phase (day 2-5 post-LH surge) in luteinized granulosa cells (70) and luteal cells from primates (71). Although further studies are needed, especially on the role of other local factors (e.g., insulin-like growth factors (69)), the evidence suggests that the primary stimulus of VEGF synthesis changes as follicular granulosa cells differentiate into luteal cells, with LH losing its acute stimulatory activity and hypoxia gaining this action.

Much less is known about the synthesis and regulation of ANGPTs in the ovary, including in primates. Hazzard and colleagues (72) report that *ANGPT1* and *ANGPT2* mRNAs are detectable in preparations of granulosa cells from macaque preovulatory follicle. Moreover, administration of an ovulatory hCG bolus increases *ANGPT1* mRNA levels 30-fold between 24 and 36 h post-injection, whereas *ANGPT2* expression remained unchanged. These data are consistent with a modest role for ANGPT2 around the time of ovulation (perhaps to promote focal sites of vessel destabilization for angiogenesis), but a major action of ANGPT1 to promote pericyte recruitment and maturation of VEGF-stimulated new vessels in the luteinizing tissue of the developing corpus luteum. Although the ovulatory hCG bolus increased *ANGPT1* expression in granulosa cells, it is unclear if this is a direct effect; its late (more than 24 h post-hCG) onset may reflect the actions of other LH-induced local factors. Notably, steroid ablation during the periovulatory interval in monkeys (using trilostane as described earlier) decreased *ANGPT1* mRNA expression, which was partially restored by progestin (R5020) replacement. Since ANGPT proteins and assays (R&D Systems) recently became available, more information on these angiogenic (ANGPT1) and potentially angiolytic (ANGPT2) factors should be forthcoming. The possible angiolytic action of ANGPT2 is supported by reports of increased expression in atretic follicles and the regressing corpus luteum (73).

To examine the local actions of VEGF and ANGPTs in the ovulatory follicle in primates, Hazzard, Xu and colleagues injected either a VEGF antagonist (recombinant human soluble FLT1/Fc chimera, (74)) or the endogenous antagonist ANGPT2 (75) directly into the preovulatory follicle (day before or of the midcycle LH surge) during spontaneous menstrual cycles in rhesus macaques. Compared to vehicle-injected controls, sFLT1/Fc treatment caused a dose-dependent decline in P levels circulating during the subsequent luteal phase, but did not shorten the lifespan of the corpus luteum. Laparoscopic evaluation of the ovaries 3 days post-injection, and removal of ovaries bearing the injected follicle for serial sectioning, indicated that: (a) all vehicle-injected follicles ovulated and displayed morphologic indices of luteinization, whereas, (b) the highest doses of VEGF antagonist prevented 50% of the follicles from ovulating (n=8 of 17), as confirmed by the absence of an ovulatory canal/stigmata and presence of a trapped oocyte. These unruptured follicles also displayed few signs of luteinization. Histologic evaluation of luteal tissue 6 days after injecting the antecedent follicle detected similar numbers of endothelial (PECAM1-positive) and steroidogenic (HSD3B-positive) cells in vehicle versus sFLT1/Fc treatment groups, but the less developed microvasculature, smaller luteal cells and large areas of vacant extracellular space in the later group, indicated defects in luteal structure-function.

In contrast to VEGF antagonism, intrafollicular injection of the endogenous ANGPT antagonist ANGPT2 (but not the agonist ANGPT1) completely blocked ovulation and prevented any rise in circulating P during the next two weeks, i.e., eliminated the anticipated luteal phase (75). Laparoscopy 3 days post-injection revealed the absence of a stigmata on a darkened follicle, and serial sectioning identified an unruptured follicle whose antrum was filled with blood cells and a trapped, degenerating oocyte. Subsequent evaluation of hormone levels indicated that ANGPT2 injection was soon followed by another follicular phase, as estradiol levels rose and elicited another LH surge 11-12 days later in 3 of 5 animals, followed by a rise in serum P levels. A subsequent laparotomy on one of these animals 10 days post-injection revealed that the ANGPT2 injected follicle had disappeared, but another large follicle had developed on the contralateral (non-injected) ovary - which was presumably the source of circulating estradiol and the follicle destined to ovulate and form a functional corpus luteum a few days later.

These data are consistent with growing evidence that systemic administration of VEGF antagonists to

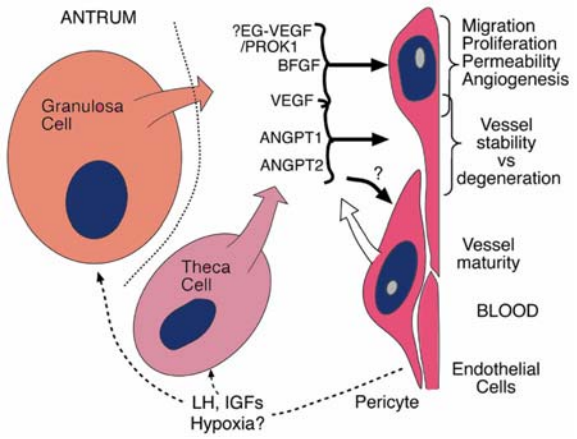

Figure 3. Conceptual diagram of the interactions between granulosa, theca, vascular endothelial and peri-endothelial (pericyte) cells in the ovulatory, luteinizing follicle for the production of angiogenic factors that promote follicle maturation, rupture and luteinization during the menstrual cycle in primates. Open arrow - synthesis; black arrows = actions; dotted arrows = regulation by hormones and local factors. See text for abbreviations and further details.

monkeys during the mid-to-late follicular phase can disrupt follicle maturation, ovulation and corpus luteum function, presumably through disruption of vessel structure:function (e.g., vascular permeability) as well as angiogenesis (65). However, the complete disruption of the ovulatory follicle by ANGPT antagonism, to the extent that follicle rupture is abolished, and the follicle degenerates rather than differentiating into the corpus luteum, supports a vital role for the ANGPT, as well as VEGF, system in controlling the maturation and differentiation of the ovulatory, luteinizing follicle in primates. Further studies on members of the VEGF and ANGPT families, as well as other factors that may have selective angiogenic activity in the ovary or steroidogenic glands (e.g., the recently discovered endocrine gland (EG)-VEGF/prokineticin 1 (PROK1 (76, 77, 78)) are needed to define their roles in normal cyclic ovarian function (Figure 3), as well as in the etiology of ovarian dysfunctions, e.g., luteal phase defects and ovarian hyperstimulation syndrome.

6. PERSPECTIVE

As summarized in Figures 1-3, considerable progress is occurring in our understanding of the vital roles of locally-synthesized arachidonic acid products, steroids and angiogenic factors in mediating LH-induced ovulation and luteinization of the primate follicle. However, research to date typically focused on one member of each family, when it is likely, if not very probable, that other members have important roles in the ovulatory, luteinizing follicle. In addition, little is known regarding the mechanisms whereby these local factors control follicle rupture or luteinization. Until recently, investigators necessarily focused on a single process (e.g., tissue remodeling) and a limited number of gene products (e.g., MMPs and TIMPs). With the advent of high-throughput genomic and proteomic techniques, rapid advances are possible by analyzing gene and protein expression in the ovary on a broad scale (79). However, researchers will need to rigorously validate that their results apply to the ovulatory, luteinizing follicle in the natural cycle, particularly if artificial, gonadotropin-stimulated cycles are the model of choice for initial tissue analyses. Information is also needed on gene and protein expression in specific cell types (e.g., granulosa, theca, microvascular, immune cells) and follicular regions (e.g., apex, base, COC) that have different activities in the ovulatory, luteinizing follicle. Such studies will provide an essential database for investigations on the role(s) of novel, under-appreciated gene products in follicle rupture, COC maturation, and luteal development. This information should also provide a basis for better understanding the causes and treatment of ovary-based infertility and for developing novel ovary-based contraceptives.

7. ACKNOWLEDGMENTS

A special thanks to Ms. Carol Gibbins, Mr. Joel Ito and Dr. Ted Molskness for their assistance in preparing this manuscript. The support and dedication of the animal care staff at ONPRC and EVMS, the surgical unit of the Division of Animal Resources, ONPRC, and the technical expertise of members of the Imaging and Morphology (IM), Molecular and Cell Biology (MCB) and Endocrine Services (ES) Core Laboratories at ONPRC is gratefully acknowledged. Research reported from the authors' laboratories was supported by the National Institutes of Health, as part of the NICHD-sponsored Specialized Cooperative Centers Program in Reproduction Research (U54 HD18185; R.L.S., F.X.), an NCRR-sponsored Primate Center Grant (RR00163), and as individual grants HD38972 (to D.M.D.), HD20869 (to R.L.S.), plus the Rockefeller Foundation (RF96020, to R.L.S.) under the World Health Organization Initiative on Implantation Research, and the Andrew W. Mellon Foundation (D.M.D.)

8. REFERENCES

1. Espey L. L., A. S. Bellinger, & J. A. Healy: Ovulation: An inflammatory cascade of gene expression. In: The Ovary. Eds: Leung PCK, Adashi EY, Elsevier Academic Press, San Diego, 145-165 (2004)
2. Richards J. S.: Ovulation: New factors that prepare the oocyte for fertilization. *Mol Cell Endocrinol* 234, 75-79 (2005)
3. Tsafriri A., X. Cao, H. Ashkenazi, S. Motola, M. Popliker, & S. H. Pomerantz: Resumption of oocyte meiosis in mammals: on models, meiosis activating sterols, steroids and EGF-like factors. *Mol Cell Endocrinol* 234, 37-45 (2005)
4. Richards J. S., D. L. Russell, S. Ochsner, M. Hsieh, K. H. Doyle, A. E. Falender, Y. K. Lo, & S. C. Sharma: Novel signaling pathways that control ovarian follicular development, ovulation, and luteinization. *Recent Prog Horm Res* 57, 195-220 (2002)
5. Richards J. S., I. Hernandez-Gonzalez, I. Gonzalez-Robayna, E. Teuling, Y. Lo, D. Boerboom, A. E. Falender, K. H. Doyle, R. G. LeBaron, V. Thompson, & J. D. Sandy: Regulated expression of ADAMTS family members in

follicles and cumulus oocyte complexes: evidence for specific and redundant patterns during ovulation. *Biol Reprod* 72, 1241-1255 (2005)
6. Yacobi K., A. Wojtowicz, A. Tsafriri, & A. Gross: Gonadotropins enhance caspase-3 and -7 activity and apoptosis in the theca-interstitial cells of rat preovulatory follicles in culture. *Endocinology* 145, 1943-1951 (2004)
7. Shimada M., M. Nishibori, Y. Yamashita, J. Ito, M. Takahide, & J. S. Richards: Down-regulated expression of A disintegrin and metalloproteinase with thrombospondin-like repeats-1 by progesterone receptor antagonist is associated with impaired expansion of porcine cumulus-oocyte complexes. *Endocrinology* 145, 4603-4614 (2004)
8. Brannstrom M. & A. Enskog: Leukocyte networks and ovulation. *J Reprod Immunol* 57, 47-60 (2002)
9. Murphy B. D.: Luteinization. In: The Ovary. Eds: Leung PCK, Adashi EY, Elsevier Academic Press, San Diego, 185-199 (2004)
10. Salustri A., C. Fulop, A. Camaioni, & V. C. Hascall: Oocyte-granulosa cell interactions. In: The Ovary. Eds: Leung PCK, Adashi EY, Elsevier Academic Press, San Diego, 131-143 (2004)
11. Hennebold J. D.: Characterization of the ovarian transcriptome through the use of differential analysis of gene expression methodologies. *Hum Reprod Update* 10, 227-239 (2004)
12. Richards J. S., D. L. Russell, S. Ochsner, & L. L. Espey: Ovulation: New dimensions and new regulators of the inflammatory-like response. *Annu Rev Physiol* 64, 69-92 (2002)
13. Sirois J., D. Boerboom, & K. Sayasith: Prostaglandin biosynthesis and action in the ovary. In: The Ovary. Eds: Leung PCK, Adashi EY, Elsevier Academic Press, San Diego, 233-247 (2004)
14. Matsumoto H., W. Ma, W. Smalley, J. Trzaskos, R. M. Breyer, & S. K. Dey: Diversification of cyclooxygenase-2-derived prostaglandins in ovulation and implantation. *Biol Reprod* 64, 1557-1565 (2001)
15. Sirois J. & M. Dore: The late induction of prostaglandin G/H synthase-2 in equine preovulatory follicles supports its role as a determinant of the ovulatory process. *Endocrinology* 138, 4427-4434 (1997)
16. Kobayashi T. & S. Narumiya: Function of prostanoid receptors: studies on knockout mice. *Prostaglandins Other Lipid Mediat* 68-69, 557-573 (2002)
17. Duffy D. M. & R. L. Stouffer: Prostaglandins modulate LH-stimulated periovulatory processes in primate granulosa cells in vitro. *Endocrine* 22, 249-256 (2003)
18. Duffy D. M. & R. L. Stouffer: The ovulatory gonadotropin surge stimulates cyclooxygenase expression and prostaglandin production by the monkey follicle. *Mol Hum Reprod* 7, 731-739 (2001)
19. Seachord C. L., C. A. VandeVoort, & D. M. Duffy: Adipose differentiation-related protein: a gonadotropin- and prostaglandin-regulated protein in primate periovulatory follicles. *Biol Reprod* 72, 1305-1314 (2005)
20. Duffy D. M., C. L. Seachord, & B. L. Dozier: An ovulatory gonadotropin stimulus increases cytosolic phospholipase A2 (cPLA2) expression and activity in granulosa cells of primate periovulatory follicles. *J Clin Endocrinol Metab* doi:10.1210/jc.2005-0980, (2005)
21. Kurusu S., S. Motegi, M. Kawaminami, & I. Hashimoto: Expression and cellular distribution of cytosolic phospholipase A2 in the rat ovary. *Prostaglandins Leukot Essent Fatty Acids* 58, 399-404 (1998)
22. Kurusu S., M. Iwao, M. Kawaminami, & I. Hashimoto: Involvement of cytosolic phospholipase A2 in the ovulatory process in gonadotropin-primed immature rats. *Prostaglandins Leukot Essent Fatty Acids* 58, 405-411 (1998)
23. Homaidan F. R., I. Chakroun, H. A. Haidar, & M. E. El-Sabban: Protein regulators of eicosanoid synthesis: role in inflammation. *Curr Protein Pept Sci* 3, 467-484 (2002)
24. Roman R. J.: P-450 metabolites of arachidonic acid in the control of cardiovascular function. *Physiol Rev* 82, 131-185 (2002)
25. Kroetz D. L. & D. C. Zeldin: Cytochrome P450 pathways of arachidonic acid metabolism. *Curr Opin Lipidol* 13, 273-283 (2002)
26. Mikuni M., M. Yoshida, P. Hellberg, C. A. Peterson, S. S. Edwin, M. Brännström, & C. M. Peterson: The lipoxygenase inhibitor, nordihydroguaiaretic acid, inhibits ovulation and reduces leukotriene and prostaglandin levels in the rat ovary. *Biol Reprod* 58, 1211-1216 (1998)
27. Matousek M., K. Mitsube, M. Mikuni, & M. Brannstrom: Inhibition of ovulation in the rat by a leukotriene B_4 receptor antagonist. *Mol Hum Reprod* 7, 35-42 (2001)
28. Ichikawa F., Y. Yoshimura, & T. Oda: The effects of lipoxygenase products on progesterone and prostaglandin production by human corpora lutea. *J Clin Endocrinol Metab* 70, 849-(1990)
29. Duffy D. M., C. L. Seachord, & B. L. Dozier: Microsomal prostaglandin E synthase-1 (mPGES-1) is the primary form of PGES expressed by the primate periovulatory follicle. *Hum Reprod* 20, 1485-1492 (2005)
30. Patwardhan V. V. & A. Lanthier: Prostaglandins PGE and PGF in human ovarian follicles: endogenous contents and in vitro formation by theca and granulosa cells. *Acta Endocrinol (Copenh)* 97, 543-550 (1981)
31. Duffy D. M., B. L. Dozier, & C. L. Seachord: Prostaglandin dehydrogenase and prostaglandin levels in periovulatory follicles: implications for control of primate ovulation by prostaglandin E2. *J Clin Endocrinol Metab* 90, 1021-1027 (2005)
32. Wallach E. E., A. de la Cruz, J. Hunt, K. H. Wright, & V. C. Stevens: The effect of indomethacin on hMG-hCG induced ovulation in the rhesus monkey. *Prostaglandins* 9, 645-658 (1975)
33. Killick S. & M. Elstein: Pharmacologic production of luteinized unruptured follicles by prostaglandin synthetase inhibitors. *Fertil Steril* 47, 773-777 (1987)
34. Norman R. J.: Reproductive consequences of COX-2 inhibition. *The Lancet* 358, 1287-1288 (2001)
35. Duffy D. M. & R. L. Stouffer: Follicular administration of a cyclooxygenase inhibitor can prevent oocyte release without alteration of normal luteal function in rhesus monkeys. *Hum Reprod* 17, 2825-2831 (2002)
36. Harris T. E., P. E. Squires, A. E. Michael, A. L. Bernal, & D. R. Abayasekara: Human granulosa-lutein cells express functional EP1 and EP2 prostaglandin receptors. *Biochem Biophys Res Commun* 285, 1089-1094 (2001)

37. Narko K., K. Saukkonen, I. Ketola, R. Butzow, M. Heikinheimo, & A. Ristimaki: Regulated expression of prostaglandin E_2 receptors EP2 and EP4 in human ovarian granulosa-luteal cells. *J Clin Endocrinol Metab* 86, 1765-1768 (2001)
38. Rothchild I.: The regulation of the mammalian corpus luteum. *Rec Prog Horm Res* 37, 183-298 (1981)
39. Stouffer R. L.: Progesterone as a mediator of gonadotropin action in the primate corpus luteum: beyond steroidogenesis. *Hum Reprod Update* 9, 99-117 (2003)
40. Peluso J. J. & A. Pappalardo: Progesterone mediates its anti-mitogenic and anti-apoptotic actions in rat granulosa cells through a progesterone-binding protein with gamma aminobutyric $acid_A$ receptor-like features. *Biol Reprod* 58, 1131-1137 (1998)
41. Peluso J. J., A. Pappalardo, G. Fernandez, & C. A. Wu: Involvement of an unnamed protein, RDA288, in the mechanism through which progesterone mediates its antiapoptotic action in spontaneously immortalized granulosa cells. *Endocrinology* 145, 3014-3022 (2004)
42. Lydon J. P., F. J. DeMayo, C. R. Funk, S. K. Mani, A. R. Hughes, C. A. Montgomery, Jr., G. Shyamala, O. M. Conneely, & B. W. O'Malley: Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev* 9, 2266-2278 (1995)
43. Mulac-Jericevic B. & O. M. Conneely: Reproductive tissue selective actions of progesterone receptors. *Reproduction* 128, 139-146 (2004)
44. Hibbert M. L., R. L. Stouffer, D. P. Wolf, & M. F. Zelinski-Wooten: Midcycle administration of a progesterone synthesis inhibitor prevents ovulation in primates. *Proc Natl Acad Sci USA* 93, 1897-1901 (1996)
45. Borman S. M., C. L. Chaffin, K. M. Schwinof, R. L. Stouffer, & M. B. Zelinski-Wooten: Progesterone promotes oocyte maturation, but not ovulation, in primate follicles without a gonadotropin surge. *Biol Reprod* 71, 366-373 (2004)
46. Hammes S. R.: Steroids and oocyte maturation - a new look at an old story. *Mol Endocrinol* 18, 769-775 (2004)
47. Shimada M. & T. Terada: FSH and LH induce progesterone production and progesterone receptor synthesis in cumulus cells: a requirement for meiotic resumption in porcine oocytes. *Mol Hum Reprod* 8, 612-618 (2002)
48. Robker R. L., D. L. Russell, L. L. Espey, J. P. Lydon, B. W. O'Malley, & J. S. Richards: Progesterone-regulated genes in the ovulation process: ADAMTS-1 and cathepsin L proteases. *Proc Natl Acad Sci* 97, 4689-4694 (2000)
49. Chaffin C. L., D. L. Hess, & R. L. Stouffer: Dynamics of periovulatory steroidogenesis in the rhesus monkey follicle after ovarian stimulation. *Hum Reprod* 14, 642-649 (1999)
50. Chaffin C. L., G. A. Dissen, & R. L. Stouffer: Hormonal regulation of steroidogenic enzyme expression in granulosa cells during the periovulatory interval in monkeys. *Mol Hum Reprod* 6, 11-18 (2000)
51. Espey J. J., R. F. Adams, & N. Tanaka: Effects of epostane on ovarian levels of progesterone, 17β-estradiol, prostaglandin E_2 and prostaglandin $F_{2\alpha}$ during ovulation in the gonadotropin-primed immature rat. *Endocrinology* 127, 259-263 (1990)
52. Tanaka N., J. Iwamasa, K. Matsuura, & H. Okamura: Effects of progesterone and anti-progesterone RU486 on ovarian 3β-hydroxysteroid dehydrogenase activity during ovulation in the gonadotrophin-primed immature rat. *J Reprod Fertil* 97, 167-172 (1993)
53. Lui K., Q. Feng, & H. J. Gao: Expression and regulation of plasminogen activators (PA), plasminogen activator inhibitor type-1 (PAI-1) and steroidogenic acute regulatory protein (StAR) in rhesus monkey corpus luteum. *Endocrinology* 144, 3611-3617 (2003)
54. Chaffin C. L., K. M. Schwinof, & R. L. Stouffer: Gonadotropin and steroid control of granulosa cell proliferation during the periovulatory interval in monkeys. *Biol Reprod* 65, 755-762 (2001)
55. Robker R. L. & J. S. Richards: Hormone control of the cell cycle in ovarian cells: proliferation versus differentiation. *Biol Reprod* 59, 476-482 (1996)
56. Chaffin C. L., R. S. Brogan, R. L. Stouffer, & C. A. VandeVoort: Dynamics of Myc/Max/Mad expression during luteinization of primate granulosa cells in vitro: association with periovulatory proliferation. *Endocinology* 144, 1249-1256 (2003)
57. Chaffin C. L. & R. L. Stouffer: Expression of matrix metalloproteinases and their tissue inhibitor messenger ribonucleic acids in macaque periovulatory granulosa cells: time course and steroid regulation. *Biol Reprod* 61, 14-21 (1999)
58. Young K. A., J. D. Hennebold, & R. L. Stouffer: Dynamic expression of mRNAs and proteins for matrix metalloproteinases and their tissue inhibitors in the primate corpus luteum during the menstrual cycle. *Mol Hum Reprod* 8, 833-840 (2002)
59. Chen X. L., H. J. Gao, F. Gao, P. Wei, Z. Y. Hu, & Y. X. Liu: Temporal and spatial expression of MMP-2, -9, 14 and their inhibitors TIMP-1, -2, -3 in the corpus luteum of the cycling rhesus monkey. *Front Biosci* in press, (2005)
60. Curry T. E., Jr. & K. G. Osteen: The matrix metalloproteinase system: changes, regulation, and impact throughout the ovarian and uterine reproductive cycle. *Endocrine Rev* 24, 428-465 (2003)
61. Liu Y.-X., K. Liu, Q. Feng, Z.-Y. Hu, H.-Z. Liu, G.-Q. Fu, Y.-C. Li, R.-J. Zou, & T. Ny: Tissue-type plasminogen activator and its inhibitor plasminogen activator type 1 are coordinately expressed during ovulation in the rhesus monkey. *Endocrinology* 145, 1767-1775 (2004)
62. Tanaka N., L. L. Espey, S. Stacy, & H. Okamura: Epostane and indomethacin actions on ovarian kallikrein and plasminogen activator activites during ovulation in the gonadotropin-primed immature rat. *Biol Reprod* 46, 665-670 (1992)
63. Young K. A., B. Bumlinson, & R. L. Stouffer: ADAMTS-1/METH-1 and TIMP-3 expression in the primate corpus luteum: divergent patterns and stage-dependent regulation during the natural menstrual cycle. *Mol Hum Reprod* 10, 559-565 (2004)
64. Hazzard T. M. & R. L. Stouffer: Angiogenesis in ovarian follicular and luteal development. In: Clinical Obstetrics & Gynaecology. Angiogenesis in the Female Reproductive Tract. Eds: Arulkumaran S, Bailliere Tindall, London, 883-900 (2000)
65. Fraser H. M. & C. Wulff: Angiogenesis in the corpus luteum. *Reprod Biol Endocrinol* 1, 88-(2003)
66. Xu F., T. M. Hazzard, A. Evans, S. Charnock-Jones, S. Smith, & R. L. Stouffer: Intraovarian actions of anti-

angiogenic agents disrupt periovulatory events during the menstrual cycle in monkeys. *Contraception* 71, 239-248 (2005)
67. Hanahan D.: Signaling vascular morphogenesis and maintenance. *Science* 277, 48-50 (1997)
68. Christenson L. K. & R. L. Stouffer: Follicle-stimulating hormone and luteinizing hormone/chorionic gonadotropin stimulation of vascular endothelial growth factor production by macaque granulosa cells from pre- and periovulatory follicles. *J Clin Endocrinol Metab* 82, 2135-2142 (1997)
69. Martinez-Chequer J. C., R. L. Stouffer, T. M. Hazzard, P. E. Patton, & T. A. Molskness: Insulin-like growth factor (IGF) -1 and -2, but not hypoxia, synergize with gonadotropin hormone to promote vascular endothelial growth factor (VEGF) -A secretion by monkey granulosa cells from preovulatory follicles. *Biol Reprod* 68, 1112-1118 (2003)
70. Friedman C. I., D. R. Danforth, C. Herbosa-Encarnacion, L. Arbogast, B. M. Alak, & D. B. Seifer: Follicular fluid vascular endothelial growth factor concentrations are elevated in women of advanced reproductive age undergoing ovulation induction. *Fertil Steril* 68, 607-612 (1997)
71. Tesone M., R. L. Stouffer, S. M. Borman, J. D. Hennebold, & T. A. Molskness: Vascular endothelial growth factor (VEGF) production by the monkey corpus luteum during the menstrual cycle: isoform-selective mRNA expression in vivo and hypoxia regulated protein secretion in vitro. *Biol Reprod* 73, 927-934 (2005)
72. Hazzard T. M., T. A. Molskness, C. L. Chaffin, & R. L. Stouffer: Vascular endothelial growth factor (VEGF) and angiopoietin regulation by gonadotrophin and steroids in macaque granulosa cells during the peri-ovulatory interval. *Mol Hum Reprod* 5, 1115-1121 (1999)
73. Maisonpierre P. C., C. Suri, P. F. Jones, S. Bartunkova, S. J. Wiegand, C. Radziejewski, D. Compton, J. McClain, T. H. Aldrich, N. Papadopoulos, T. J. Daly, S. Davis, T. N. Sato, & G. D. Yancopoulos: Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. *Science* 277, 55-60 (1997)
74. Hazzard T. M., F. Xu, & R. L. Stouffer: Injection of soluble vascular endothelial growth factor receptor 1 into the preovulatory follicle disrupts ovulation and subsequent luteal function in rhesus monkeys. *Biol Reprod* 67, 1305-1312 (2002)
75. Xu F. & R. L. Stouffer: Local delivery of angiopoietin-2 into the preovulatory follicle terminates the menstrual cycle in rhesus monkeys. *Biol Reprod* 72, 1352-1358 (2005)
76. LeCouter J., J. Kowalski, J. Foster, P. Hass, Z. Zhang, L. Dillard-Telm, G. Frantz, L. Rangell, L. DeGuzman, G. A. Keller, F. Peale, A. Gurney, K. J. Hillan, & N. Ferrara: Identification of an angiogenic mitogen selective for endocrine gland endothelium. *Nature* 412, 877-884 (2001)
77. Fraser H. M., J. Bell, H. Wilson, P. D. Taylor, K. Morgan, R. A. Anderson, & W. C. Duncan: Localization and quantification of cyclic changes in the expression of endocrine gland vascular endothelial growth factor in the human corpus luteum. *J Clin Endocrinol Metab* in press, (2004)
78. Kisliouk T., H. Podlovni, K. Spanel-Borowski, O. Ovadia, Q.-Y. Zhou, & R. Meidan: Prokineticins (endocrine gland-derived vascular endothelial growth factor and BV8) in the bovine ovary: expression and role as mitogens and survival factors for corpus luteum-derived endothelial cells. *Endocrinology* 146, 3950-3958 (2005)
79. Xu J., R. L. Stouffer, & J. D. Hennebold: Discovery of luteinizing hormone (LH)-regulated genes in the primate corpus luteum. *Mol Hum Reprod* 11, 151-159 (2005)

Key Words: Prostaglandin, Progesterone, Vascular Endothelial Growth Factor, Angiopoietin, Rhesus Monkey, Ovary, Oocyte, Granulosa Cells, Luteinizing Hormone, Review

Send correspondence to: Richard L. Stouffer, Ph.D., Oregon National Primate Research Center, 505 NW 185th Avenue, Beaverton, OR 97006, U.S.A., Tel: 503-690-5366, Fax: 503-690-5563, E-mail: stouffri@ohsu.edu

http://www.bioscience.org/current/vol12.htm