Follicle growth, ovulation, and luteal formation in primates and rodents: A comparative perspective

Charles L Chaffin[1] and Catherine A VandeVoort[2]

[1]Department of OB/GYN & Reproductive Sciences, University of Maryland Baltimore, Baltimore, MD, USA; [2]California National Primate Research Center and Department of Obstetrics and Gynecology, University of California, Davis, CA, USA
Correponding author: Charles Chaffin. Email: cchaffin@fpi.umaryland.edu

Abstract

Ovarian function has a great deal of functional overlap between species; antral follicles grow in response to FSH, ovulation involves proteolysis, and the steroidogenic pathway is largely the same. However, embedded in these similarities are important differences that reflect the evolutionary and natural history of species and may focus future research into these critical areas. This review compares ovarian function of rats and mice with primates, focusing on estradiol and follicle growth, steroidogenesis and rupture during the periovulatory interval, and the formation of a functional corpus luteum, drawing the conclusion that careful comparison of species yields more functional information about both than studying them in isolation.

Keywords: Ovary, rodent, primate, granulosa, ovulation, corpus luteum

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Introduction

The use of animal models as a means to understand human biology has at its core the tacit acceptance that all animal life possesses inherent relatedness based on shared ancestry. However, as part of this understanding there is also an *a priori* recognition that the utility of animals as models for humans diminishes as phylogenetic distance increases. As a generalization, non-human primates (NHP) make better biological models for humans than do rodents, and rodents better than fish, although this clearly excludes technological applications such as genetic mouse models that are not currently feasible in taxa like primates, as well as cost and ethical issues associated with the use of NHP. In evolutionary terms, the lineages leading to the Orders of Rodentia and Primates diverged from each other approximately 90 million years ago (mya), while rats and mice diverged from each other in the area of 25 mya, and the apes from the balance of the Old World cercopithicines around 15 mya.[1,2] Ovarian function is an aspect of mammalian physiology in which species divergence is especially apparent; on even the most superficial level, for example, rats and mice have a 4–5-day reproductive cycle resulting in multiple ovulations compared to the 28-day cycle of most old-world primates with a single ovulated oocyte. It can, therefore, be reasonably assumed that phylogenetic distance based on molecular systematics is parallel to physiology such that the reproductive systems of macaques and humans are far more similar than either is to rodents. There are caveats to this, however. The first is that systematics are exceedingly complicated to determine and thus the phylogenetic relationships between taxa and dates of divergence are not exact. Second is the likelihood that ovarian systems are under very high selective pressures to preserve species specificity, so that the rate of evolution may be much faster than other physiological systems. This by no means obviates the need for multiple models; in fact, as will be suggested below, a comparative approach may be critical to understanding some key concepts in ovarian physiology. A recent review covering the neuroendocrine control of the reproductive cycle in primates and rodents underscores the importance of placing common laboratory models in a comparative framework in order to better understand human physiology.[3] The current review will present differences in ovarian physiology between rodents and primates in order to illustrate the value of a comparative approach and the dangers in ignoring important differences between species, and will conclude with some new aspects in the primate corpus luteum.

Follicle growth

Different roles for estradiol in follicle development

Germ cell development occurs through several rounds of mitosis with incomplete cytokinesis, leading to the

formation of interconnected oocytes in a germ cell nest, breakdown of which is a necessary step in primordial follicle development. Primordial follicle formation occurs *in utero* in primates and during the first seven days after birth in mice, strongly suggesting different mechanisms regulate nest breakdown. In baboons, blocking estradiol synthesis with an aromatase inhibitor during the second half of gestation attenuates nest breakdown indicating that estradiol promotes primordial follicle assembly, while estradiol inhibits nest breakdown in mice.[4,5] In both primates and mice, estradiol works partly through the TGFβ family; in neonatal mice, for example, exogenous activin increases the number of primordial follicles, while estradiol suppresses activin.[6–9] Similarly, estradiol depletion in baboons during fetal development increases the expression of inhibin-α in pre-granulosa cells from germ cell nests, presumably decreasing local activin action that is important for nest breakdown.[4] While activin promotes nest breakdown in both taxa, estradiol suppresses activin in mice and augments the activin signal in primates. The adaptive value of the divergent roles for estradiol between rodents and primates is not clear, although maximum concentrations of estradiol during gestation in mice are around 60 pg/mL[10] compared to >500 pg/mL in rhesus macaques and baboons.[11,12] While out of the scope of this mini-review, it is important to bear in mind potential differences in estradiol affinity, receptor density in target tissues, and possible membrane effects between species as mitigating factors to differences in circulating hormone concentration. The theme of mice and rats as a 'low estradiol' model relative to primates will be further discussed in the context of follicle selection.

Follicle selection: levels of estradiol as a predicator of follicle number

The endocrinology of follicle selection is based on the negative feedback of estradiol from small antral follicles on FSH production, and this is a general principle across species. FSH is essential to the growth of early antral follicles, serving a range of functions that include cell survival and gene expression (i.e. CYP19, LHCGR) in granulosa cells; although while FSH is required for granulosa cell proliferation, it requires additional endocrine factors such as activin and IGF to affect necessary changes in gene expression.[13–18] Follicle selection occurs in primates around day 4–5 after menstruation and on the morning of metestrus in the rat as a result of decreasing FSH in response to the rise in estradiol synthesis from the cohort of small antral follicles.[19–21] The molecular mechanisms leading to selection in primates involve the acquisition of some degree of FSH-independence through the ability to respond to low, tonic levels of LH.[19,22–24] Follicle selection in the bovine is associated with lower levels of insulin-like growth factor binding proteins (IGFBP) and higher concentrations of bioactive IGF.[25,26] Interestingly, IGFBP4 expression in primate dominant follicles is greater than in atretic antral follicles, although it is not clear how these data translate to IGF bioactivity.[27] Given the phylogenetic distance of cows from both rodents and primates, more research is necessary before concrete extrapolation is possible. The mechanisms of follicle selection in rodents are even less clear, probably because the wide-spread use of superovulation and the fact that these taxa normally develop multiple preovulatory follicles has de-emphasized selection as an important area of investigation. In primates, the question of why one follicle is selected versus the others remains to be adequately answered; given the prevalence of overriding follicle selection with exogenous FSH as a clinical and research tool and the effects that may have on the physiological environment of maturing oocytes and early embryos makes this a critically important topic.

Controlled ovarian stimulation (COS; 'superovulation') has become a central tool in assisted reproductive technologies as well as a primary research approach with which to study ovarian function. However, there are marked endocrine abnormalities associated with COS in both rats and primates. Szoltyz *et al*.[28] showed that superovulation of rats with equine chorionic gonadotropin (eCG) resulted in higher levels of androgens with lower progesterone, hypertrophic theca cells, and more atretic follicles in response to an ovulatory hCG bolus than was observed in spontaneous cycles after the onset of the LH surge. These findings are similar to observations made in primates after COS, in which more than 40% of follicles were scored as grossly atretic 36 h after an hCG bolus.[29] While there has been significant effort expended towards improving IVF and early embryo development *in vitro*,[30] there has been limited work towards understanding the abnormalities associated with COS. This is important because the health of individual follicles during oocyte retrieval is not apparent and thus oocytes can still be obtained from degenerating follicles, and the factors leading to atresia of periovulatory follicles during COS are not clear and may have important development consequences to the embryo.

There is an important difference in the number of ovulated oocytes per cycle, despite the overall similarities between rodents and primates in antral follicle growth and possibly selection. With the exception of marmosets (*Callithrix* spp.), all primates ovulate a single oocyte. Marmosets are recently evolved[2] and so multiple ovulations in this Genus most likely represent a derived trait. In contrast to primates, rodents ovulate several oocytes each cycle, with the number of ovulations reflecting the number of preovulatory follicles, and the number of follicles associated with circulating levels of FSH.[31] Compared to rodents, primates have very high concentrations of estradiol with correspondingly low FSH; in fact, rodents are a very 'low estradiol/high FSH' species in general (Table 1).[32–34] Here, as with estradiol above, it should be

Table 1 Comparison of estradiol and FSH peak concentrations in rats and primates

	FSH (ng/ml)	E_2 (pg/ml)
Rat	500	<50
Macaque	<160	300

From Refs. 30–32.

considered that FSH may have different affinities for the FSH receptor as well as different receptor densities on the surface of granulosa cells. In addition, changes in hormone assay reagents over time can make comparison of results difficult. It is probable that the higher FSH in rodents is the proximal cause of the increased number of selected follicles and ovulated oocytes, in essence undergoing endogenous superovulation each cycle relative to primates. In support of this hypothesis, there are several studies in which rat follicle growth is dose-dependently enhanced with increasing concentrations of PMSG or FSH,[35,36] although these data are somewhat self-evident because FSH reduces atresia in all mammalian species. An unexplored approach is to dose-dependently reduce FSH in rodents during spontaneous estrous cycles to determine if FSH can be reduced to the point where only a single dominant follicle develops. Analysis of follicle growth in heterozygote mouse models compared to wild-type and complete loss in function of FSHβ, FSHR, and CYP19A1 have either not been described or not been informative.[37–39] In rats, granulosa cell sensitivity to FSH does not change during antral follicle growth and the follicle can thus be hypothesized to respond directly to changes in circulating FSH concentrations, although receptor number and affinity were not determined.[40] Comparative differences in the mechanisms leading to lower estradiol (and thus to higher FSH) in rodents are not clear, but could involve the relative efficiencies of 17,20-lyase and aromatase or perhaps through increased peripheral clearance. An important caveat to estradiol synthesis is the fact that in rodents 17,20-lyase can utilize 17α-hydroxyprogesterone (17-OH-P) as a substrate for androstenedione, so estradiol synthesis proceeds through either the delta-4 or delta-5 pathways. This is in contrast to primates, where 17-OH-P is a poor substrate for 17,20-lyase and thus estradiol can only be produced through the delta-4 pathway.[41,42] An apparent paradox therefore exists in that rats and mice have mechanisms in place to ensure adequate estradiol synthesis while keeping estradiol at low levels (and resulting in higher FSH) relative to primates.[43]

A local role for estradiol in antral follicle growth

The local production of estradiol raises the important possibility for an autocrine/paracrine action in the growth and development of antral follicles, although while data support a role for estradiol in the growth of antral follicles in rodents, the existing data do not indicate the same function in primates. Early work on a local role for estradiol suggested a possible atretogenic action during follicle selection, where increasing estradiol synthesis could promote atresia in all but the dominant follicle,[44,45] although at the time, the lack of consensus regarding the expression of estradiol receptors (ER) in primate granulosa cells confused the situation. While the discovery of ERβ provided a potential mechanism for local estradiol action in the primate follicle,[46] the hypothesis that in primates estradiol promoted atresia directly at the level of the ovary was discounted, but not experimentally refuted, by emerging evidence that estradiol has important folliculogenic actions in mice and rats, with subsequent generation of knockout mouse models strongly supporting this hypothesis.[47] In addition, COS protocols in primates generate very high levels of estradiol even as multiple preovulatory follicles develop.[48] Thus, despite conflicting data, the vision that estradiol has a universal role in antral follicle growth across most, if not all, mammalian taxa gained support.

There are several empirical tests to the hypothesis that estradiol promotes antral follicle development in primates. First, there are a few reports of naturally occurring mutations to relevant genes, for example, 17,20-lyase deficiency that attenuates estradiol synthesis by reducing androgenic substrates for aromatase.[49] In one patient, small antral follicles were able to develop, while another patient was able to undergo a controlled ovarian stimulation protocol with multiple follicles developing to >10 mm.[50,51] In addition, granulosa cells isolated following COS produced normal levels of progesterone in response to hCG, suggesting that despite the lack of appreciable estradiol, granulosa cell luteinization occurred normally.[52] A second test was done experimentally through the administration of the aromatase inhibitor 1,4,6-Androstatrien-3,17-dione (ATD) to macaques during COS cycles, finding that steroid reduction did not reduce follicular growth.[53] However, because estradiol concentrations in the ATD group remained just under 20% of controls, making a definitive conclusion difficult, a broader steroid synthesis inhibitor (the HSD3B inhibitor trilostane; TRL) was tested. Similar to ATD, TRL treatment during COS cycles did not retard follicle growth, although estradiol following TRL was still 7% of controls.[54] However, it is highly unlikely that these residual concentrations were sufficient to promote the growth and development of multiple preovulatory follicles in primates, but that does remain a possibility. These studies further found that oocyte fertilization was compromised in the TRL treatment group, although this is most likely due to the loss of progesterone rather than estradiol as Borman *et al*.[55] showed that progesterone promotes the resumption of meiosis in primates. Given that there are data supporting a role for estradiol in other large mammals such as cows,[56] primates may be somewhat exclusive in the lack of estradiol-mediated follicle growth. The expression of ER in the dominant follicle of primates, particularly the granulosa layer, does argue for an autocrine/paracrine role for estradiol, although what that may be remains to be determined. However, the existing data from primates, even those stemming back to the atretogenic hypothesis, should not simply be 'shoe-horned' into the rodent model – they just do not fit well. Clearly there are differences between these models that need to be considered.

Placing estradiol and follicle growth into an evolutionary perspective

There are a great number of important and relevant consistencies between primate and rodent antral follicle growth, including the general process of selection, the central role of FSH, markers of cytodifferentiation such as LH receptor and aromatase, as well as cell cycle control. However, there are important differences, namely the regulation and local action of estradiol. By placing these differences into an

evolutionary context, the functional consequences can be better understood.

The natural history of rodents in the wild is represented by a very short reproductive lifespan, and thus the selective pressures resulting in a reproductive strategy centered on maximizing the number ovulations per cycle and the number of cycles per reproductive lifespan can be envisioned. In fact, there is a general tendency for larger mammals to ovulate a single oocyte along with longer reproductive cycles and higher proportional off-spring survival rates than poly-ovular taxa like rats and mice.[57] In addition to multiple ovulations, the lack of a functional luteal phase in a non-fertile cycle of rodents permits the immediate resumption of follicle growth, with a new round of small antral follicle growth initiated within 24 h of the onset of the LH surge,[58] and this represents another important adaptation to a short reproductive lifespan. In contrast, primates have a much longer reproductive lifespan and a far greater maternal investment into offspring, including mono-ovulation, which may permit a greater degree of fetal growth and development than occurs with multiple births.[59] In the case of primates, the rapidity of follicle growth is less relevant than ensuring the selection of a single – presumably healthy – follicle.

Finally, the ability of 17,20-lyase to metabolize 17α-hydroxyprogesterone in rodents but not primates underscores the importance of estradiol to the rodent system. In addition, blocking androgen synthesis from 17-OH-P in primates opens up an important local role for mineralocorticoids and glucocorticoids to be produced from progesterone and 17α-hydroxyprogesterone, respectively. Primate, but not rodent, granulosa cells are capable of expressing CYP21A2 (21-hydroxylase), which is necessary to produce steroids down these pathways, the function of which remains to be conclusively determined.[60]

Periovulatory events

The periovulatory interval encompasses the onset of the LH surge, or hCG in stimulated cycles, until the extrusion of the fertilizable oocyte. Follicle rupture occurs around 14–16 h after an ovulatory stimulus in rodents, but averages 38 h after the onset of ovulatory stimulus in humans, with a range of 34–46 h.[61] In primates undergoing controlled ovulation (COv, as compared to COS), in which the single dominant follicle is controlled by exogenous hormones during only the last two days of follicle maturation, half of the ovulations occurred before 36 h and the other half after, although magnitude of temporal variation around this mean was not reported.[62]

A number of events occur during this interval of time and these can be considered in terms of either ovulation or corpus luteum (CL) development. The former includes cumulus expansion, the resumption of meiosis, and follicle rupture, while the latter is characterized by the differentiation of somatic cells to favour progesterone synthesis over estradiol, neovascularization of the developing CL, and remodeling of the follicle wall into a corpus luteum. While overlapping (certainly the case for LH) or independent mechanisms leading to both ovulation or luteal development could be argued; a follicle can luteinize without rupturing, and a follicle can ovulate but the CL be insufficiently developed to support early pregnancy. Regardless, these processes occur in all mammalian taxa in response to an ovulatory stimulus, whether an endogenous LH surge or an exogenous hCG bolus, suggesting strong evolutionary conservation. However, the development of a longer functional luteal phase, especially during the non-fertile cycle, is highly species-specific; this includes luteotropic support (for example, LH and progesterone in primates, prolactin in rats and mice, and estradiol in rabbits) and mechanisms leading to luteolysis, which despite being well-described in rodents remain a mystery in primates.[63,64] During the non-fertile cycle, the primate CL retains functionality – primarily steroid synthesis – for around 7–12 days and does not regress until nearly two weeks after the LH surge, in direct contrast to rodents that have an 'ultra-short' luteal life-span of around 24 h.[63] Thus, during the periovulatory interval, there are (a) commonalities between taxa that are especially prevalent during the early part of the periovulatory interval and involved in differentiation of the follicle into a corpus luteum, (b) similarities in the activities that lead to a common outcome (e.g. follicle rupture) occurs in both taxa although through distinct molecular components, and (c) frank divergences involved in the maintenance of a functional CL. Examples of these three relationships between rodents and primates will be illustrated in the following paragraphs.

Early periovulatory aspects of steroidogenesis: high conservation across species

In all mammalian species examined to date, an ovulatory stimulus rapidly induces progesterone synthesis concomitantly with a reduction in estradiol.[48,65] This is driven by changes in gene expression/activity, including an increase in steroidogenic acute regulatory protein (StAR), HSD3B, and CYP11A1 with a reduction in CYP17A1 and HSD17A1.[66–68] The induction of progesterone synthesis is exceedingly rapid and occurs in primates within 15 min of an hCG bolus and in less than 2 h in rodents.[48,65] At least in primates, part of this rapidity lay in the fact that lipid droplets in the luteinizing granulosa cells contain sufficient cholesterol esters to support up to 24 h of maximal rates of progesterone synthesis in the absence of an external lipoprotein source. Thereafter, LH/hCG-induced scavenger receptor-BI (SR-BI) serves to replenish the cholesterol supply, primarily from LDL,[69,70] and human SR-BI variants with lower activity lead to impairment of LH/hCG-induced progesterone and are associated with infertility,[71,72] In contrast, HDL may be the more relevant cholesterol source for both theca and granulosa cells in rats.[73,74] The LH/hCG-induced increase in SR-BI, as well as in HSD3B and StAR, is due to the rapid (<3 h) reduction of liver X receptors (LXRα, β; NR1H2, NR1H3) following an ovulatory stimulus, which also serves to reduce reverse cholesterol transport.[75,76] The regulation of LXRs in the rodent ovary has not yet been reported, although there is clear evidence that LXR regulates similar genes involved in cholesterol homeostasis in mice,[77] so it would be highly surprising if LH/hCG

down-regulation of LXR was not an early event leading to progesterone synthesis in rodents. An interesting aspect of these studies is that cholesterol balance in the follicle appears to be regulated almost entirely by gonadotropins instead of through dogmatic homeostatic mechanisms, and this has been clearly shown in both rats and primates.[76,78] The early events in steroidogenesis are highly conserved between rodents and primates and result in the rapid increase in progesterone synthesis mediated by increased cholesterol acquisition/decreased efflux and expression of steroidogenic enzymes.

Follicle rupture: conserved physiologic outcome with diverging molecular mechanisms

All follicles must rupture to release the oocyte for fertilization, and this involves the proteolytic degradation of the follicle wall.[79–81] However, the specific proteolytic genes vary across species. There are between 626 and 641 distinct proteases in the rat and mouse genome compared to 561 in human,[82] and to date, differences and similarities in the LH/hCG-induced expression of specific proteases have been reported. There are LH/hCG-induced genes in common between rats and primates, such as MMP1 and MMP10,[81,83–85] those that are expressed in one taxa but not the other (e.g. PRSS35 in mouse but not primate,[86] and those that increase in both rodents and primates but with different temporal patterns. An excellent example of this last group is a disintegrin and metalloproteinase with thrombospondin-like repeats-1 (ADAMTS1). In mice, ADAMTS1 expression increases between 6 and 12 h after an ovulatory bolus to immature animals undergoing superovulation protocols and declines by 16 h, and genetic knockout models implicate this gene product in follicle rupture, possibly through cleavage of versican in the extracellular matrix of the cumulus–oocyte complex to permit cumulus expansion and ovulation.[87–89] In contrast, ADAMTS1 expression does not increase until after follicle rupture in macaques, peaking in the early luteal phase,[81,90] implicating a role in early luteal development or function rather than follicle rupture or cumulus expansion. The hypothesized functional divergence of ADAMTS1 between rodents and primates could be through, for example, changes in the promoter structure or signaling pathways, or through secondary factors such as changes in protein or mRNA stability.

The regulatory control of ADAMTS1 appears to be different in primates. Doyle *et al.*[88] showed in rats that the progesterone receptor (PR) regulates ADAMTS1 during the periovulatory interval; importantly, the expression of ADAMTS1 parallels that of PR in rats and mice.[91,92] However, primate granulosa cells also express PR rapidly after an ovulatory stimulus although ADAMTS1 does not increase in a coordinate manner, nor does progesterone regulate ADAMTS1 during the luteal phase.[90,93] Interestingly, equine granulosa cells also express ADAMTS1 mRNA after hCG, peaking at 12 h and then declining, a pattern temporally coordinate with PR. However, there is a second increase in ADAMTS1 in the early CL while no secondary rise in PR mRNA occurs,[94] suggesting that progesterone regulates ADAMTS1 in rat and horse prior to ovulation, but that something else regulates it in the CL of horse and primate. Despite the fact that both rodents and primates have some form of membrane progestin receptor,[95–97] it is unlikely that ADAMTS1 is regulated through a non-classical/membrane PR since steroid ablation during the early luteal phase does not alter ADAMTS1 mRNA expression in macaques.[81] There are hypothesized to be at least two points of divergence in the regulation of the ovarian ADAMTS1 gene between species. The first is regulation by progesterone/PR, and given that an autocrine/paracrine role for progesterone during the periovulatory interval is widespread among mammals,[98–100] the most plausible explanation is that primates have diverged away from progesterone regulation of the ADAMTS1 gene. The second is progestin-independent expression of ADAMTS1 in the early CL of horse and primate; something occurs in these species that is not present in rodents. Whether it is the unmasking of an enhancer or suppression of a repressor remains to be seen. A recent example of how this could occur is the epigenetic regulation of the inhibin α gene during luteinization of rat granulosa cells.[101]

Maintenance of a functional CL: divergence between rodent and primate

One difference in luteal development and function between rodents and primates is the expression of inhibin A (INH-A) in the corpus luteum,[102] a main function of which is to suppress FSH secretion. In rodents, the inhibin-α gene is repressed by the LH surge and this permits the secondary rise in FSH, the key factor in promoting the next round of antral follicle growth,[20,103] while in primates, continued circulation of INH helps keep FSH at lower levels throughout the luteal phase. LH/hCG-induced repressors of inhibin α in the rodent are known (e.g. C/EBP β[104]) although the repression is transient, and so Meldi *et al.*[101] recently showed in rat luteal cells that sustained suppression of inhibin α occurs through DNMT3a-mediated methylation of the cAMP response element and abrogation of CREB binding. While the regulation of DNMT3a (or other methyl/acetyltransferases) in the luteinizing primate follicle is not currently known, it is probable that expression remains low during the early luteal phase. While it is unlikely that the evolutionary shift from ultra-short CL lifespan in rodents to the functional luteal phase of primates is a simple, single gene process, the stories ofADAMTS1 and inhibin α regulation indicate how such divergence could be manifest. Importantly, they underscore that the development of a functional CL is an important and relevant point of divergence between the rodent and primate models of ovarian function.[63]

The primate corpus luteum: some new ideas on luteolysis

In rodents, functional luteolysis is defined by the drop in progesterone and is necessary for parturition. This appears driven by both uterine and local synthesis of

prostaglandin F2a, which does not suppress progesterone synthesis, but rather promotes its metabolism to 20α-dihydroprogesterone through the induction of 20α-hydroxysteroid dehydrogenase. Luteolysis in the rat has been comprehensively reviewed by Stocco *et al*.[64] In contrast, luteolysis of the primate CL remains an enigmatic event, with the mechanisms leading to functional regression still unclear. However, two recent reports have offered new and important insight into this event. In the first, Dickinson *et al*.[105] report that LH receptor splicing changes throughout the luteal phase of primates, favoring a truncated variant called LHRd lacking the transmembrane and C-terminal portions late in the luteal phase. The truncated LHRd form is unable to transduce a signal from LH and also further prevents full-length LHR from responding to LH. Casarini *et al*.[106] showed recently that LH and hCG elicit different signaling effects through the LH receptor. Importantly, hCG in culture prevents the shift from LHRa to LHRd, although the comparison of LH with hCG was not performed,[105] which leads to the intriguing hypothesis that hCG rescues the CL from luteolysis by preventing the expression of LHRd while pituitary-derived LH is unable to do so. Studies like these help resolve the long-standing paradox that primate CL regression is not associated with a reduction in total LH receptor expression, and while pulse frequency declines toward the end of the luteal phase, that the reduction does not seem to promote luteolysis.[107–109]

The second report tests the hypothesis that functional luteolysis (i.e. the decline in progesterone) is associated with a shift in the balance of cholesterol towards reduced uptake/increase reverse transport through the increased expression of the liver X receptors (LXR).[110,111] The expression of luteal LXRα (NR1H3) increases during the middle and late phase, preceding the decline in progesterone synthesis associated with luteal regression, and there are increases in ABCA1 and ApoE and declining SR-BI and LDLR expression. Luteolysis is associated with the appearance of extra-cellular cholesterol, supporting an important role for reverse transport. The primate corpus luteum produces several endogenous LXR ligands, including 27-hydroxycholesterol that activates LXRα to increase ABCA1, IDOL (an E2 ubiquitin ligase that targets LDLR protein,[112]) LXRα, and cholesterol efflux, and decrease LDLR protein in luteal cells.[111] While these data implicate LXRα in the induction of functional luteal regression in the primate, its regulation is not entirely clear. However, LXRα mRNA is strongly induced in response to an activator of the peroxisome proliferator activated receptor (PPAR)-γ in luteinizing primate granulosa cells[76] and this could an important factor in the CL as well. An additional hypothesis stems from the fact that cAMP/PKA phosphorylates and inactivates LXRα, thus as the LHR isoforms shift in the late luteal phase to favor the inactive LHRd, this could promote the activity of newly expressed LXRα. Figure 1 depicts a hypothesized model linking these studies together. Finally, these demonstrate the central role LXRα plays in both the induction and repression of progesterone in the CL, and shows how a single gene product can serve multiple functions.

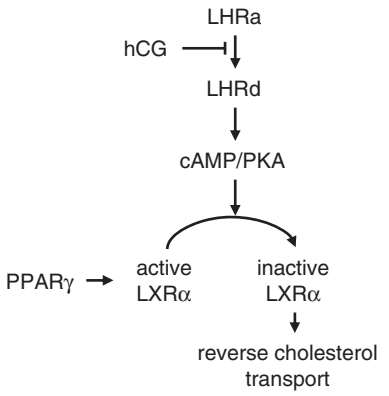

Figure 1 Hypothesized model linking PPARγ to the increase in liver X receptor α (LXRα) during luteal regression in primates, and the shift in LH receptor isoforms from functional to non-functional as a mechanism to promote the activity of newly expressed liver X receptor, leading to reverse cholesterol transport

Concluding thoughts

The modern study of reproductive physiology has been largely dominated by the use of rats and mice, partly for the pragmatic reasons of cost and ethics, continuity with other aspects of biology, and more recently, because of the ability to generate genetic models.[113] While there are merits to the study of rodents in-and-of-themselves, their use as models of human ovarian physiology has rarely been evaluated critically. There are immediate and obvious differences between rodents and primates, such as the number of ovulations and length of the cycle, but many other differences are more subtle or under-appreciated, such as the role of estradiol in follicle development and growth. Still, much of the core physiology is same and therefore represents variations on a theme, such as FSH trophic support, steroidogenesis, and proteolytic degradation of the ovulating follicle. As the lifecycle of the follicle progresses, species divergence becomes more apparent, with the development of the CL representing a point of profound species divergence. As a consequence, corpora lutea differ in length of the luteal phase, luteotropins, and mechanisms of luteal regression. Such differences make sense when viewed from an evolutionary perspective.

Rats and mice have short lifespans and high offspring mortality, and so their reproductive systems have evolved to maximize the number of offspring possible in a short period of time. By recognizing the constraints imposed by the natural history of the model, more informed choices can be made regarding which comparisons with other species are meaningful and which are not, and just as importantly, inform the interpretation of the data. As this review has summarized, there are important differences between rodents and human/primate ovarian follicle biology. By simply assuming that a particular animal model represents a faithful recapitulation of human biology without empirically determining the validity of this assumption renders the conclusions potentially invalid, and worse, self-propagating. The use of multiple animal models for studies aimed at better understanding human health therefore requires a

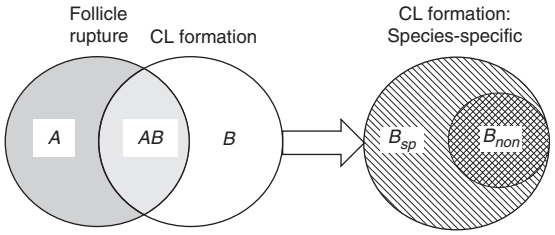

Figure 2 Depiction of gene expression during the periovulatory interval. Cluster **A** represents genes involved primarily in follicle rupture, having common functionality (but potentially different genes) between taxa. These are best represented by proteolytic enzymes. Cluster **AB** are genes having multiple, widely over-lapping functions such as signal transduction pathways. These are of limited value in discerning functional versus non-functional luteal development. Cluster **B** are genes specific to luteinization and luteal formation, for example, steroidogenic enzymes. Cluster **B** can be further divided into $\mathbf{B_{non}}$, which are genes common to all species luteal development regardless of the duration of functionality. A good example of this is the steroidogenic acute regulatory protein (StAR). The final group is $\mathbf{B_{sp}}$, which is predicted to be a set of LH/hCG-induced genes unique to species with a functional luteal phase like primates. $\mathbf{B_{sp}}$ can also represent genes expressed only in ultra-short luteal phase models like rodents, suggesting a role in inhibiting functional luteal development

comparative approach. First, to determine where similarities lay and what can be directly extrapolated and second, to determine where and why differences exist. There is a constant push-and-pull regarding the utility of specific animals as human models, and differences are almost always viewed as being non-instructive. However, species differences can be a powerful tool to subtract out those areas of overlap, with the resulting differences giving clear clues as to how the ovary works. Take, for example, corpus luteum development. By comparing gene expression in rats and primates during the late periovulatory interval and subtracting out all the expressed genes in common, those remaining in primates should be involved in the formation of a functional CL and those in rodent the reverse (Figure 2). By studying rodent models in isolation, we may be learning far more about the way the rat and mouse ovary works than we are about humans.

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