

Review

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Mutant mouse models and their contribution to our knowledge of corpus luteum development, function and regression

Luiz E Henkes¹, John S Davis² and Bo R Rueda*¹

Address: ¹Vincent Center for Reproductive Biology, Department of Obstetrics and Gynecology, Massachusetts General Hospital, Boston, Massachusetts 02114, USA and ²Olson Center for Women's Health, Department of Obstetrics and Gynecology, University of Nebraska Medical Center, Omaha, Nebraska 68198; VA Medical Center, Omaha, Nebraska 68105, USA

Email: Luiz E Henkes - lhenkes@partners.org; John S Davis - jsdavis@unmc.edu; Bo R Rueda* - brueda@partners.org

* Corresponding author

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Abstract

The corpus luteum is a unique organ, which is transitory in nature. The development, maintenance and regression of the corpus luteum are regulated by endocrine, paracrine and autocrine signaling events. Defining the specific mediators of luteal development, maintenance and regression has been difficult and often perplexing due to the complexity that stems from the variety of cell types that make up the luteal tissue. Moreover, some regulators may serve dual functions as a luteotropic and luteolytic agent depending on the temporal and spatial environment in which they are expressed. As a result, some confusion is present in the interpretation of *in vitro* and *in vivo* studies. More recently investigators have utilized mutant mouse models to define the functional significance of specific gene products. The goal of this mini-review is to identify and discuss mutant mouse models that have luteal anomalies, which may provide some clues as to the significance of specific regulators of corpus luteum function.

Introduction

The corpus luteum is an important byproduct of the ovulating follicle. It is a transitory, hormonally regulated organ that consists of a heterogeneous cell population. Unlike the follicle, the different cell types are not segregated into distinct compartments, but are highly integrated. The steroidogenic cells synthesize progesterone for the establishment and maintenance of pregnancy. Other cell types include the endothelial cells and immune cells, typically thought to play supportive roles. There is evidence to suggest that the endothelial cells and the immune cells play an active role in luteal formation, maintenance and regression [1]. The vascular endothelial cells make up a large portion of the corpus luteum, whereas the immune cells vary in number dependent upon the stage of the luteal phase or pregnancy [1-3].

Studies designed to elucidate the contributions of one or more of the luteal cell types are often difficult to interpret. More often than not these studies are based on *in vitro* cell culture models. Primary cultures of dispersed luteal tissue or enriched populations of specific cell types provide some opportunity for investigators to delineate potential signaling pathways, which may be engaged in response to a specific stimulus. Outcomes derived from *in vitro* studies are important but are often subject to criticism. For example, *in vitro* studies tend to favor one cell type or another. Moreover, the cell-cell interactions that are present in a multidimensional environment *in vivo* are removed when experiments are performed in a two dimensional field *in vitro* (eg, tissue culture dish). What effect this has on an outcome is not always fully appreciated and cannot be directly extrapolated to the *in vivo* model. For example prostaglandin F_{2α} (PGF_{2α}) is primarily considered a

luteolytic agent *in vivo*, yet it has no direct lytic effect on endothelial cells or steroidogenic cells *in vitro* [1,4,5]. This raises a number of questions. Is the response observed *in vitro* an artifact of the static culture systems most often employed? Alternatively, are *in vitro* cultures lacking a luteolytic agent found *in vivo*, or is cell-to-cell communication critical for the production of a luteolytic factor present only in the *in vivo* environment?

Alternatives to the current *in vitro* and *in vivo* strategies are necessary to fully understand the functional significance of putative mediators of luteal development and regression. The development of various mutant mouse models has provided an invaluable knowledgebase for defining or possibly redefining the function and/or significance of many gene products. The mutant mouse models, whether they are hypermorphs, hypomorphs, conditional knockouts or true knockouts, provide a unique opportunity to define function of the genes or their products. However, these models have inherent caveats and have provided us with a new list of disclaimers to help interpret the unexplainable findings. One such issue is redundancy. Often times there are built in safeguards within a cell type or alternatively there is system overlap to protect or compensate for the loss of a particular protein. Therefore when a protein is deficient, a gross phenotype is not always readily evident. Alternatively, the significance of a particular protein to corpus luteum function may be underestimated when a loss of the protein results in embryonic lethality. Of course this makes it very difficult to determine its function or significance in events that control the cyclic nature of the mature female.

Some phenotypes are more controversial than others. The 'fertility' of female mice is subject to a number of biases. Some investigators will claim that if a female delivers one live offspring she is fertile. Others would argue that because the average mouse litter size is 7–8 pups, a mouse that delivers fewer than 7–8 pups has a fertility problem. For practical purposes herein a reduction in litter size will be described as sub-fertile. It is not so clear how to classify mutant mouse models that display erratic estrous cycles, because they may still be able to become pregnant and deliver a normal size litter. Although mutant mice may never reach the reproductive potential of their wild type siblings, they do deliver pups and under a strict definition of fertility they could be classified as fertile. In reality though the reproductive potential of a number of female mutant mice is sub-optimal suggesting that they are truly less fertile than their wild type counter parts.

Mutant mice models are often generated to investigate non-reproductive problems. Therefore, investigators who are not directly engaged in a reproductive study or investigators not familiar with the reproductive field may miss

or dismiss a phenotype pertinent to reproduction. Reproductive anomalies are not limited to reduced fecundity or irregular estrous cycles, but include anovulation, hypothalamic or pituitary defects, implantation defects, sub-optimal hormone concentrations, and/or parturition defects. This article provides a brief review of mouse models that have defects affecting the development, function and regression of the corpus luteum.

Mutant mice models with preovulatory/luteal development and/or luteal maintenance defects/anomalies

It is important to recognize that in some mutant mouse models ovarian follicles fail to ovulate (Table 1); yet, the steroidogenic cells may undergo luteinization spontaneously or in response to exogenous gonadotropins resulting in a luteinized unruptured follicle. A number of anovular phenotypes have been reported: gonadotropin receptors: LH receptor, FSH receptor [6]; gonadotropins: FSH β subunit [7], glycoprotein hormone α subunit [8]; steroid hormone receptors: ER α [9], ER α /ER β [10], PR [11]; cell cycle regulatory proteins: cyclin D2 [12], p27(kip) [13]; enzymes for steroidogenesis and prostaglandin synthesis: aromatase [14], and COX-2 [15]. The ability of steroidogenic cells to undergo luteinization naturally would suggest that at least some signaling between the pituitary-hypothalamic-ovarian axis is intact. Alternatively, if luteinization does not occur, but is initiated only with exogenous gonadotropins it can be predicted that one or more signaling pathways have been interrupted. For example, preovulatory follicles of cyclin-D2 $^{-/-}$ females undergo arrest and do not ovulate, however their granulosa cells undergo luteinization [16]. Similarly, inactivation of the type 4 cAMP specific phosphodiesterase (PDE4D) gene results in infertile female mice. PDE4D is critical for feedback regulation of cAMP levels and PDE4D females have a high incidence of entrapped oocytes within the follicles and yet the steroidogenic cells undergo luteinization [17]. Another example includes the nuclear corepressor Nrip1 (a.k.a. RIP140) $^{-/-}$ mouse which is infertile [18]. The infertility stems from a failure of the follicles to undergo maturation. As in the previous examples the inability to ovulate is independent of the ability to undergo luteinization [18]. Connexin 37 $^{-/-}$ female mice also fall into this category. Connexin 37 is normally present in gap junctions between oocyte and granulosa cells of the follicle and is critical for signaling [19]. Connexin 37 $^{-/-}$ female mice lack mature preovulatory follicles and fail to ovulate. Despite the lack of ovulation, luteal-like structures within the ovary displayed all the characteristics of functional corpora lutea including high intracellular lipid content, mitochondria with tubular cristae, abundant smooth endoplasmic reticulum and numerous capillaries [19]. Moreover, oocyte development arrested in connexin 37 $^{-/-}$ female mice before meiotic competence

was achieved. Thus, cell-cell signaling through intercellular channels critically regulates the highly coordinated set of cellular interactions required for successful oocyte development and ovulation [19]. In contrast, it is not necessarily obligatory for cell-cell signaling through intercellular channels to induce luteinization.

There are also mutant mouse models, which provide indirect evidence that luteinization can occur in the absence of ovulation (Table 1). For example, female mice lacking the gene for endothelial nitric oxide synthase (eNOS^{-/-}) have irregular estrous cycles and fewer pups per litter [20]. In response to gonadotropin stimulation the eNOS^{-/-} females have a significant reduction in ovulatory efficiency compared with wild type female, however there was no significant difference in plasma progesterone concentrations [21]. It appears that the luteinization process is not interrupted although ovulation rate is compromised. This difference may be due in part to unrecognized luteinized follicles.

Collectively the mutant models with ovulation defects described above provide evidence to suggest that luteinization is independent of ovulation and that ovulation of the oocyte is not obligatory for luteinization. There are also examples of mutant mice which have an ovulation defect but there is no evidence that the follicles undergo luteinization (Table 1). One example is the Progesterone Receptor (PRKO) and Progesterone Receptor alpha (PRAKO) knockout mice. The importance of progesterone derived from the corpus luteum in the establishment of pregnancy is well accepted. However the pervasive impact of progesterone on reproduction became more evident with the development of the PRKO mice [11,22]. The PRKO model was designed by targeting both the PRA and PRB isoforms. The females develop normally, however they have multiple reproductive defects including an inability to ovulate, uterine hyperplasia, limited mammary development and an inability to exhibit sexual behavior [22]. All of these symptoms likely contribute to their reported infertility. The PRAKO mouse, generated by selective ablation of the PRA gene [23], are also infertile. Gonadotropin stimulation of PRKO and PRAKO mice results in the development of follicles, however only PRAKO mice ovulate. Pregnancy is not possible due a defect in decidualization [22,23]. Collectively, the available data indicate that progesterone is required for more than just the establishment of pregnancy; it is required for ovulation, a prerequisite for true CL formation. There are no data provided to determine whether or not the unruptured follicles become luteinized.

An additional example of an anovulatory mutant mouse would include the estrogen receptor mutant mice. There is no doubt that estrogen plays a significant physiological

role in folliculogenesis. Estrogen stimulates both granulosa cell proliferation and differentiation [24,25]. Estrogen is also responsible for the induction of follicle stimulating hormone (FSH) and luteinizing hormone (LH) receptors [24,26]. Estrogen binds both estrogen receptors; ER α [27,28] and ER β [29]. Both are expressed in granulosa cells of preantral and antral follicles [25] and have a highly conserved DNA binding domain [29]. ER α is more prevalent in stromal and theca cells while ER β is predominant in antral follicles [30]. ER α knockout mice (α ERKO) females are acyclic, infertile and display enlarged, hemorrhagic and cystic follicles with a high incidence of ovarian tumors [10]. In contrast to the ER α female mice, which are completely infertile, the ER β null mice females are subfertile. The ER β ^{-/-} mice (β ERKO) have decreased ovulation rates, fewer litters, less pups per litter and sparse corpora lutea. The double knockouts (α ERKO and β ERKO) present with a phenotype similar to ER α knockout [10].

Luteinizing hormone (LH), obviously by its name, is well recognized as a luteotropic agent and is pivotal to mammalian reproduction. LH contributes to the maintenance of gametogenesis and reproductive tract development in the female [31-34]. Receptors for LH are found predominantly in the ovary, but numerous reports over the past 15 years demonstrate expression of functional LH receptors in numerous extra-gonadal tissues [33]. Mutations in gonadotropin and gonadotropin receptor genes are very rare [35,36], however, these mutations have helped to define the physiology and pathophysiology of gonadotropin action [37]. Targeted disruption of the LHR gene causes infertility in both sexes [34,38-40]. Other phenotypes include gross underdevelopment of internal and external genitalia [38,40]. With respect to the mouse ovary, the adult LHR^{-/-} female displays small ovaries and follicular development up to the preantral stage [40]. The mutant mice had no discernable reductions in FSH receptor or progesterone receptor mRNA [38]. Furthermore, there was no apparent difference in the development of the theca layer surrounding the developing follicle. However, the theca in mutant mice displayed a marked reduction in the expression of mRNA for P450 17-hydroxylase [41] and steroidogenic acute regulatory (StAR) protein [38]. As a result steroid hormone levels were markedly reduced, which can account for the observed hypoplastic uterus and elevated gonadotropin levels [38,41]. No evidence of preovulatory follicles or corpora lutea are observed in the LHR^{-/-} mice [38-40]. Injections of PMSG, or injections of PMSG followed by hCG failed to induce ovulation and luteal formation [38,39]. Additionally, estrogen and progesterone replacement therapy could not restore the ovulatory defect and fertility [38]. It seems clear; therefore that development of antral follicles to the ovulatory stage and luteinization of the ovulated

follicle requires the actions of LH. Recent studies using the LHR^{-/-} mutant mouse model also provide evidence for possible extra-gonadal roles for the LH receptor in uterine morphogenesis [39].

Another example of where mutant mice display an inability to develop corpora lutea includes the mice deficient in CATT/enhancer binding protein (C/EBP β) [42]. CATT/enhancer binding protein (C/EBP β) is expressed in granulosa cells of the ovary after LH stimulation, *in vitro*. Similarly, C/EBP β is expressed in granulosa and not thecal cells of antral follicles derived from hCG treated wild type females. C/EBP β is not evident in the intact corpus luteum in wild type mice suggesting a functional role for C/EBP β in the granulosa cells. This role is apparently lost or severely down regulated during the luteinization of granulosa cells. The obligatory role of C/EBP β is demonstrated in the mice deficient in this protein [42]. C/EBP β ^{-/-} female mice fail to ovulate and therefore cannot initiate or maintain a pregnancy. There were no gross abnormalities in the uterine tissue and uterine wet weights are similar between the C/EBP β ^{-/-} and wild type females. Marked differences in ovarian function were observed when females were subjected to gonadotropin-induced superovulation regime. The heterozygous females ovulated an average of 30 oocytes whereas the C/EBP β ^{-/-} females ovulated 3 to 6 oocytes. The ovaries of the C/EBP β ^{-/-} female mice had evidence of large, often hemorrhagic antral follicles which were not evident in the wild type females [42]. These observations suggest that there is a transition failure in ovulation and luteinization. To verify that the infertility was intrinsic to the C/EBP β ^{-/-} ovarian phenotype, ovaries deemed to be normal were transplanted to homozygous null females and the infertility was resolved arguing that the pituitary, hypothalamus and uterus were hormonally responsive and intact. In contrast, corpora lutea never formed when ovaries of mutant mice were transplanted into normal females. Sterneck *et al.*, [42] summarized that the morphology of superovulated ovaries of C/EBP β ^{-/-} females were indicative that these mice lacked the necessary mechanisms required to induce ovulation and support luteinization. The ovarian transplant experiments further support the significance of C/EBP β to luteal formation.

The final example of a mouse model with an ovulation/luteinization defect is the Large tumor suppressor homolog 1 mutant mouse (Lats-1^{-/-}) [43]. Lats1 is a tumor suppressor originally identified in the *Drosophila melanogaster*. Lats1^{-/-} mice display infertility, growth retardation and lack of mammary gland development. They also exhibit hyperplastic changes in the pituitary and decreased serum hormone levels (*i.e.* LH, prolactin (PRL) and growth hormone). Based on vaginal cytology Lats1^{-/-} mice do not exhibit an estrous cycle and remain in mete-

strus. The majority of the follicles are primary and secondary follicles with few follicles if any attaining antrum formation. There is no evidence of CL formation. Gonadotropins stimulate estrous cyclicity, although is reported to be prolonged [43]. It is not clear what phase of the cycle is prolonged. Ovaries from Lats-1^{-/-} females contain fewer follicles than wild type females of the same litter. The reproductive hormone defects of the Lat1 mutant mice are similar to that LH-hypogonadotropic hypogonadism and CL insufficiency in humans [43].

It is well recognized that luteinization of the steroidogenic cells of the follicle marks a significant point whereby the steroidogenic cells undergo hypertrophy and hyperplasia only to be followed by cellular differentiation and a dramatic reduction in cellular proliferation. Concurrent with this process there are significant changes in the levels and actions of specific cyclins, their corresponding cyclin dependent kinases, and cell cycle inhibitors (*i.e.* p27 and p21). The cell cycle is regulated by cyclin interaction with cyclin dependent kinases (CDKs) [44]. Progression through G1 is regulated by the Cyclin D and E dependent kinases. In the G1 phase type D cyclins bind and activate CDK4 or CDK6. Cyclin E activates CDK2 in the late G1 phase. The CDKs can be inhibited by CDK inhibitors, which are classified into two groups, Kip/Cip and Ink4 inhibitors. Kip/Cip family includes p21, p27 and p57. The Ink4 inhibitor family includes p15, p16, p18 and p19 [44]. Many of the changes observed in these regulators of cell cycle are believed to be mediated in part by hormones also implicated in follicular growth, ovulation, luteal formation/luteinization [45].

The significance of cyclins, CDK and their inhibitors becomes readily apparent in the mutant mouse models. FSH or bromo-cAMP failed to induce proliferation of granulosa cells derived from cyclin D2^{-/-} female mice [12,16]. Moreover, the cyclin D2^{-/-} female mice fail to ovulate, but undergo luteinization [12]. The p27^{-/-} mice exhibit a number of abnormalities including gigantism with multi-tissue hyperplasia, benign adenomas in the pituitary, and female infertility [46]. Of interest to this review is the fact that granulosa cells in the ovary of p27^{-/-} mice continue to proliferate beyond the LH surge, suggesting that p27 plays a critical role in establishing quiescence or differentiation of luteinizing granulosa cells. Cyclin dependent kinase 4^{-/-} mice are also infertile and females exhibit prolonged estrous cycles [47,48]. Although the CDK4^{-/-} mice develop corpora lutea, the postovulatory progesterone secretion is low and results in disruption of implantation. Progesterone supplementation will reverse the defect indicating that the infertility is the result of luteal dysfunction [47]. It is unclear whether receptors for luteotropic agents or steroidogenic enzymes are disrupted in CDK4^{-/-} mice.

Table 1: Summary of Reproductive Female Phenotypes in Mutant Mice models.

Mutant mice models with preovulatory/luteal development defects/anomalies		
MUTANT	REPRODUCTIVE PHENOTYPE	REF
^a CDK2	Infertile – Follicle arrest with granulosa luteinization; Gigantism with multi tissue hyperplasia and benign adenomas in the pituitary	[16,78]
^b CDK4	Impaired postovulatory progesterone secretion and disruption of implantation	[12,47,48]
^c p27 ^{kip}	Ovulation defect; granulosa cells continue to proliferate after luteinization	[46]
^d PDE4D	Infertile – Follicles with entrapped oocytes that undergo luteinization	[17]
^e Nrip1	Infertile – Unruptured follicles; granulosa cells undergo luteinization	[18]
^f Con-37	Infertile – Mature oocytes fail to ovulate	[19]
^g PRLR	Infertile – Fewer follicles, reduced ovulation, irregular cycles, reduced fertilization rates, defective preimplantation embryo development an lack the ability to initiate pseudopregnancy	[54]
^h PRKO	Infertile – Inability to ovulate, uterine hyperplasia, limited mammary development and inability to exhibit sexual behavior	[11,22,23]
ⁱ αERKO	αERKO females are acyclic, infertile and display enlarged, hemorrhagic cystic follicles with a high incidence of ovarian tumors	[9,10,30]
^j βERKO	βERKO females are sub fertile, have decreased ovulation rates, fewer litters, less pups and sparse corpora lutea	[10,30,79]
^k LHR	Infertile – arrested follicular development at the early antral stage	[38-40,80]
^l Lats – 1	Infertile-Growth retardation and lack of mammary development; Most follicles are primary and secondary; No evidence of corpora lutea formation	[43]
^m C/EBPβ	Infertile – Fail to initiate or maintain a pregnancy	[42]
ⁿ eNOS	Irregular estrous cycle and reduced ovulation rate	[20,21]
Mutant mice models with reduced luteal function		
MUTANT	REPRODUCTIVE PHENOTYPE	REF
^o Hyt	Infertile – Continuous diestrous	[50]
^p TIMP-1	Corpora lutea develop/sub-optimal progesterone	[51]
Mutant mice models with delayed or disrupted luteal regression		
MUTANT	REPRODUCTIVE PHENOTYPE	REF
^q FP	No regression of corpora lutea and fail to spontaneously undergo parturition	[59,60]
^r COX-1	Delayed parturition	[57,58]
^s COX-2	Reduced ovulation rates, reduced fertilization rates, implantation and decidualization defects	[15]
^t lpr	Irregular follicular development/corpora lutea undergo luteolysis at irregular intervals	[71]
^u gld	Irregular follicular development/corpora lutea undergo luteolysis at irregular intervals	[71]
^v TNFR	Increased number of ovulations, irregular estrous cycles, eventually get locked into a diestrous phase	[67]
^w Casp3	Delayed structural luteal regression, independent of decrease in progesterone	[76,77]
^x InsI3	Disrupted cycle length and increased ovarian apoptosis including follicles and corpora lutea	[64]

List of acronyms or abbreviations: ^aCyclin dependent kinase 2, ^bCyclin dependent kinase 4, ^cp27(kip), ^dType 4 cAMP-specific phosphodiesterase, ^eNuclear receptor co-repressor Nrip1 (a.k.a. R1P140), ^fConnexin-37, ^gProlactin receptor, ^hProgesterone receptor, ⁱαEstrogen receptor, ^jβEstrogen receptor, ^kLuteinizing hormone receptor, ^lLarge tumor suppressor homolog 1, ^mCCAAT/enhancer-binding protein β, ⁿEndothelial nitric oxide synthase, ^oHypothyroid, ^pTissue inhibitor of metalloproteinase-1, ^qProstaglandin F2α receptor, ^rCyclooxygenase-1, ^sCyclooxygenase-2, ^tlymphoproliferation, ^ugeneralized lymphoproliferative disease, ^vTumor necrosis factor receptor, ^wCaspase-3, ^xInsulin-like factor 3

Not all mutants are the product of human intervention. Hypothyroid (*hyt*) mice are autosomal recessive for hypothyroidism [49]. The *hyt* females display continuous diestrous contributing to their infertility [50]. Stimulation of immature female *hyt* and wild type mice with exogenous gonadotropins will induce follicle development at the same rate. However in gonadotropin stimulated mature female *hyt* mice, the number of oocytes ovulated were less than their wild type counterparts and pregnancy is never achieved. Mature *hyt* females have significantly fewer corpora lutea > 500 microns in diameter and significantly lower progesterone. Thyroxine treatment before mating reverses the insufficiencies; the mice have well-developed corpora lutea and progesterone levels are increased [50].

Tissue inhibitor of metalloproteinase-1 (TIMP-1) has been implicated as a potential regulator of steroidogenesis [51]. This has been recently validated by evaluation of the luteal phenotype of TIMP-1 mutant mice [51]. To validate TIMP-1 functional significance to steroidogenesis in the corpora lutea, wild type and TIMP^{-/-} mice were treated with eCG, followed by hCG to induce ovulation. Progesterone increased post hCG treatment in both genotypes, however, the progesterone concentrations in TIMP^{-/-} were less than that observed in wild type mice. The lack of progesterone was not attributed to insufficient luteal formation since a similar number of oocytes were harvested from both wild type and TIMP^{-/-} mice suggesting a similar number of corpora lutea were formed. Although the mean mass of the corpora lutea in the two genotypes was not

reported, the weights of the ovaries from TIMP^{-/-} mice were significantly larger than their wild type counterparts following gonadotropin stimulation [51]. These data provide evidence to support a significant role of TIMP-1 in steroidogenesis.

Mutant mice models with luteal regression defects/anomalies

The prolactin receptor knockout mice provide some interesting insights into luteal function and regression. Prolactin (PRL) is a pituitary hormone recognized for its luteotropic and luteolytic actions [52,53]. More specifically, prolactin regulates corpora lutea formation, steroidogenesis, gonadotropin receptors and luteal demise in rodents [52]. Therefore it is not unexpected that prolactin receptor null mice (PRLR^{-/-}) have multiple reproductive anomalies. PRLR^{-/-} mice have fewer follicles, reduced ovulation, irregular cycles, reduced fertilization rates, defective preimplantation embryo development and lack the ability to initiate pseudopregnancy. The length of the estrous cycle does not appear to differ between the PRLR^{-/-} females and their wild type counterparts [54]. Moreover the number of oocytes ovulated normally or in response to gonadotropin stimulus were the same between the two phenotypes [54]. These data suggest that PRL deficiency does not affect the ovarian responsiveness to gonadotropins. Corpora lutea form but display an elevated level of apoptosis. Moreover, there is little evidence of PECAM/CD31, an indirect index for vascularization. These data suggest that the corpora lutea of PRLR^{-/-} mice have reduced vascularization [54]. Collectively, the reproductive anomalies observed in the PRLR^{-/-} mice have been attributed to impaired luteal function resulting in insufficient levels of progesterone to support implantation.

Cyclooxygenase (COX) catalyzes the conversion of arachidonic acid into prostaglandin H₂ (PGH₂) a substrate required for the generation of other prostaglandins including PGF₂α. Prostaglandin F₂α is especially important in the process of luteolysis [1-3,55]. The distribution and varied levels of COX expression in different tissues suggest that the biological actions of cyclooxygenase may be tissue specific [56]. COX activity is considered a rate-limiting step and disruption of COX activity and subsequent diminished prostaglandin levels was hypothesized to have a significant negative effect on reproductive function. COX-1^{-/-} female mice have multiple defects [57,58]. Of importance herein, the COX-1^{-/-} mice have a delayed parturition. In a normal pregnant wild type mouse there is an increase in uterine PGF₂α production on day 19 associated with luteolysis and parturition. This increase is not evident in the COX-1^{-/-} pregnant females [57,58]. Administration of PGF₂α will reverse the parturition defect. These data support an obligatory role for COX-1 in parturition and hence luteal regression. The reproductive

defects displayed by COX-1 deficient mice are similar to that displayed by FP^{-/-} mice [59,60]. The COX-2^{-/-} females have evidence of disrupted ovulation, reduced fertilization rates, implantation and decidualization defects [15,58]. Simultaneous inhibition of COX-1 and COX-2 resulted in more severe effects than either isoform alone [61].

Prostaglandin F₂α has long been implicated as a primary luteolytic agent, however the development of the PGF₂α receptor mutant mice (FP^{-/-}) provides additional insight into the overall significance of PGF₂α to the regression of the corpora lutea. Sugimoto and colleagues [59] demonstrated that homozygous females cycled normally and achieved pregnancy. Interestingly, FP^{-/-} pregnant females failed to undergo spontaneous parturition similar to that observed in the COX mutant females [58]. There was no decline in progesterone levels and no morphological evidence of regression. Parturition could only be induced by an ovariectomy on day 19; likely the result of a fall in progesterone levels. It is interesting that the effect on the corpus luteum is limited to the corpora lutea of pregnancy. There is no evidence that the lack of PGF₂α signaling had any effect on the corpora lutea of the estrous cycle or in the corpora lutea formed in response to pseudopregnancy.

The insulin-like factor 3 (Insl3), a member of the insulin-like hormone family or relaxin family [62] is also important for gonadal function. In the female, low amounts of RLF (Insl3) are produced in both the uterus and ovary, particularly in the theca cells of small antral follicle, where expression of the hormone is correlated with the selection of the follicles to become preovulatory [63]. In knockout mice, there is an altered female phenotype, with disturbed cycle length and increased ovarian apoptosis, particularly in follicles and corpora lutea [64]. This was demonstrated following the collection of ovaries from 40-day-old- and 6-month-old wild type and Insl3^{-/-} mice littermates, which were serially sectioned and assessed. It was determined that the number of zonae pellucidae is higher in Insl3^{-/-} ovaries of both ages than in ovaries of wild-type sisters. Wild type mice of both ages possess threefold more corpora lutea than their Insl3^{-/-} littermates. In general, wild-type corpora lutea appear healthy, show GS I-positive endothelial cells and no apoptotic cells whereas corpora lutea from mutants are rich in regressing GS I luteal cells, and an increased number of apoptotic cells. It was concluded that follicular atresia and luteolysis are accelerated in ovaries of Insl3^{-/-} mice probably because of increased apoptosis. The Insl3 function may provide survival signals to rescue endocrine cells from the apoptotic pathway.

The overall impact of some reproductive phenotypes exhibited in mutant mice is not so clear. For example if a

mouse has irregular estrous cycles what exactly does that mean? Is the irregular estrous cycle attributed to only disrupted follicle development, delayed luteal development or disruption of luteal regression or can it be a combination of all three. Examples of mutant mice models with irregular estrous cycles other than those discussed above would include the tumor necrosis factor receptor mutants (TNFR1^{-/-}), generalized lymphoproliferative disease (*gld*) mutants and lymphoproliferation (*lpr*) mutant mice (Table 1). Evidence for the involvement of TNF α in ovarian function is provided in recent reviews [1,65,66] which was further supported by Roby *et al* [67] who described the reproductive anomalies associated with the TNFR1^{-/-} female mice. Prepubertal TNFR1^{-/-} mice stimulated with gonadotropins ovulate more ova compared to their wild type controls. This increase in number of ovulations by TNFR1^{-/-} mice was associated with higher serum levels of progesterone. The increased ovulatory response was lost when the mutant females matured. At an early age the TNFR1^{-/-} female mice have the same length of estrous cycle as their wild type counterparts. However the TNFR1^{-/-} females spent more time in diestrous than did the control mice. By 6 months of age only 40% percent of the females remained cyclic and those that did not cycle appeared to be 'locked' into a diestrous phase. Also of interest was that an increased number of TNFR1^{-/-} females failed to deliver and pups suggesting that there was a higher incidence of infertility [67]. This study implicates TNF α as a critical regulator of luteal regression. These results are supported indirectly by an earlier study in which anti-thymocyte antiserum was injected in rats to inhibit immune function [68]. Similar to the TNFR1^{-/-} mice these rats failed to progress past the diestrous phase. Although this study does not directly implicate TNF α , it does provide additional support that the immune system plays an integral role in the physical regression of the corpora lutea.

The homozygous *gld* mice have a non-functional Fas ligand (FasL) and *lpr* mice have reduced expression of FAS (receptor) [69,70]. The corpora lutea of these mice undergo luteolysis but at irregular intervals. Moreover they have irregular follicle development. The *lpr* mice have increased numbers of secondary follicles [71]. Therefore it is not clear as to where the defect lies. Regardless, these studies do provide evidence to suggest that FAS mediated events are critical to the cyclicity of the female mouse. FasL or FAS activating antibodies can induce luteal cell death in the human, mouse, rat, and cow [71-75] and induce luteal regression in wild type mice [71,76]. FAS-mediated cell death results in the activation of caspase-3, a primary effector caspase [76]. More interestingly, the onset of FAS mediated cell death is attenuated in caspase-3^{-/-} mice when compared to wild type mice [76].

Carambula *et al.*, [77] predicted that corpora lutea derived from caspase-3^{-/-} mice would exhibit a delayed onset of apoptosis during luteal regression when compared with corpora lutea derived from wild type mice. Upon examination of ovaries of wild type mice stimulated with gonadotropins only residual luteal tissue at day 6 post-ovulation, ovaries collected from caspase-3^{-/-} mice retained many corpora lutea at day 6 post-ovulation that were similar in size to those observed in the early luteal phase of wild type mice. Notably, there was no dramatic increase in apoptosis in corpora lutea of caspase-3^{-/-} mice at any time point examined post-ovulation, indicating that luteal involution had been delayed. On the contrary, the levels of progesterone declined regardless of genotype. These data provide evidence that caspase-3 is functionally required for apoptosis to proceed normally during luteal regression. Moreover, these data suggest caspase-3 is not a direct mediator of the decrease in steroidogenesis associated with luteolysis [77]. Using this same model it was demonstrated that caspase-3 was downstream of PGF2 α and FAS mediated luteal regression [76]. Treatment with PGF2 α or Jo2 post-ovulation induced caspase-3 activation and increased the number of apoptotic cells when compared to IgG treated controls. In contrast, corpora lutea in ovaries collected from caspase-3^{-/-} mice, whether treated with PGF2 α , Jo2 or control IgG, showed little evidence of active caspase-3 or apoptosis. Corpora lutea of wild type mice treated with Jo2 had increased the caspase-8 activity, an activator of caspase-3 that is coupled to the FAS death receptor. Treatment of wild type mice with PGF2 α or Jo2 resulted in a increase in caspase-8 activity in the corpora lutea [76]. Based on these data it is suggested that luteolysis, at least in part, can be mediated by increasing the bioactivity or bioavailability of cytokines, such as FasL and that multiple endocrine factors can activate caspase-3-driven apoptosis during luteolysis [76].

Conclusions

A number of examples of mutant mice, which display a luteal phenotype, have been provided (Table 1). Of these there are those that fail to ovulate but undergo luteinization, granulosa cells that continue to proliferate after luteinization and those that never luteinize. Some mutant mice develop corpora lutea but the level of progesterone synthesized is not adequate to allow implantation or maintain pregnancy. There are also mutant mice that display irregular estrous cycles, possibly due to a delay in ovulation, luteinization or regression. It may be the culmination of all three of these processes. Lastly, there are mice which fail to undergo regression associated with pregnancy or alternatively, there are mice that have delayed structural regression irregardless of a decline in progesterone. Collectively, these findings provide us with predictable and not so predictable results. Some findings add to the complexity and may contradict the more tradi-

tional views. Regardless, a better understanding of the significance of specific proteins and/or their receptors in corpora lutea development, function and regression can be gained from information obtained from mutant mice.

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