Chapter 8
Regulation of Corpus Luteum Function in the Domestic Dog (*Canis familiaris*) and Comparative Aspects of Luteal Function in the Domestic Cat (*Felis catus*)

Mariusz Pawel Kowalewski

Abstract The domestic dog (*Canis familiaris*) and the cat (*Felis catus*), although sharing the same goal of ensuring maximal fertility, have developed different reproductive strategies. Significant differences can be found in the mechanisms regulating luteal function. In the dog, the lack of an acute luteolytic mechanism in the absence of pregnancy results in prolonged regression of the corpus luteum (CL), extended luteal progesterone secretion, and CL lifespan, features that are similar in pregnant and nonpregnant bitches until the acute prepartum luteolysis. This observation emphasizes the differences between pregnant and nonpregnant dogs in mechanisms regulating the termination of CL function and further highlights the interspecies differences. In the domestic cat, successful mating results in pregnancy and a luteal lifespan that extends until parturition, and after a nonfertile mating ovulation is followed by pseudo-pregnancy. However, differing from the dog, the duration of pseudo-pregnancy is approximately half the gestation length observed during pregnancy. The persistence of luteal function in pregnant queens over the duration of pseudo-pregnancy is, most probably, caused by the supportive role of placental steroidogenesis, which is lacking in the dog. Interestingly, in both species luteal function, at least in the absence of pregnancy, is independent of a uterine luteolysin, as it remains unaffected by hysterectomy. Consequently, in both species the luteal regression/luteolysis during pseudo-pregnancy appears to be a passive degenerative process in the absence of a luteolytic principle of uterine origin; however, the inherent luteal lifespan is much shorter in the feline than in the canine species, facilitating and hastening reproduction in cats.

Keywords Domestic dog (*Canis familiaris*) • Domestic cat (*Felis catus*) • Corpus luteum function • Pregnancy • Pseudo-pregnancy • Luteal regression • Luteolysis

M.P. Kowalewski (✉)
Vetsuisse Faculty, Institute of Veterinary Anatomy, University of Zurich,
Winterthurerstrasse 260, CH-8057 Zurich, Switzerland
e-mail: kowalewskipl@yahoo.de; kowalewski@vetanat.uzh.ch

R. Meidan (ed.), *The Life Cycle of the Corpus Luteum*,
DOI 10.1007/978-3-319-43238-0_8

8.1 Introduction

Among the domestic animal species, in dogs ovarian function appears to have only minimally albeit successfully evolved, representing a basic model of mammalian reproduction. Thus, dogs are classified as "aseasonal monoestrous," that is, ovulating only once per breeding season; they are polytocous and spontaneous ovulators. The periods of sexual activity are separated from each other by obligatory periods of sexual inactivity, referred to as "anestrus," which can last as long as 36 weeks [1, 2]. Contrasting with this is the "seasonally polyestrous" reproductive pattern of domestic cats, providing them with increased opportunities for facilitating fecundity and production of offspring. Cats are polytocous, predominantly induced ovulators repeating estrus until mating (or ovulation). Consequently, several regulatory mechanisms governing reproductive function in both species are species specific.

Indisputably, both species represent the most important pets, while also serving as laboratory animals. Dogs are accepted as one of the best models for studying multifactorial human diseases [3], and investigations on cats serve for better understanding of reproductive function in endangered felids, for example, lynxes [4–6]. Consequently, following increased scientific interest, knowledge concerning canine reproductive function in particular has greatly expanded during the past few years. This expansion relates mostly to the mechanisms regulating maintenance of CL function by luteotropic mechanisms. The physiological processes associated with luteal regression or luteolysis in dogs are still not fully understood. Even less is known about CL regulation in domestic cats, with most knowledge derived from clinical and endocrinological observations; little attention has been paid so far to the underlying molecular regulatory mechanisms.

Based on the available reports and reviews, including those from our own laboratory [2, 6–16], this chapter presents our current understanding of the endocrine and molecular mechanisms regulating CL function in pregnant and nonpregnant dogs, with some comparative data from studies with cats. Although some of the data generated still remain to be published, they are discussed here to provide new insights and ideas for prospective research directions. A historical perspective is provided by presenting the earliest observations published by Bischoff [17] in his pioneering work, dated 1845, regarding canine reproduction. Finally, interesting but largely neglected comparative aspects of feline and canine luteal functions are presented.

8.2 Shining a Spotlight: Species-Specific Peculiarities of Canine Luteal Function

Taking into account the indispensable role of progesterone (P4) during the establishment and maintenance of mammalian gestation, one of the most interesting peculiarities of canine reproduction is the lack of placental steroidogenesis [18, 19]. This characteristic is unique among domestic animal species, as even in those species

with gestation dependent on luteal P4, such as pigs or goats, the placenta is capable of producing steroids [20–22]. Consequently, the dog is virtually the only domestic animal species devoid of placental steroids, which further underlines the central role of the CL in regulating canine fertility. Moreover, the lack of an extra- or intraluteal luteolytic principle leads to a somewhat reverse relationship between the duration of the luteal lifespan during pregnancy, determining the length of gestation, and the extended luteal phase in nonpregnant cyclic bitches, referred to as pseudo-pregnancy, which frequently exceeds the length of normal gestation [18, 23, 24]. Therefore, as discussed later, different mechanisms must have evolved to regulate the gradual luteal regression observed in nonpregnant dogs and the acute prepartum luteolysis. Importantly, however, the similar progesterone profiles observed in these situations, that is, in pregnant and nonpregnant dogs, preclude P4 as a reliable marker for pregnancy determination in this species. Consequently, relaxin of fetal placental origin is the only marker of canine gestation identified so far [25]. There are no other markers available in dogs allowing for early pregnancy detection, i.e., preimplantation. Compared with other species, in which ovarian cyclicity is maintained by periodic uterine PGF2α production, the dog exhibits a more primitive form of CL control where there is no relationship between the uterus and control of the CL. This difference is further expressed in the lack of an embryo-derived anti-luteolytic signal; such a relationship is a more evolutionarily advanced system. Thus, in the dog there is no classical maternal recognition of pregnancy, and both during pregnancy and pseudo-pregnancy the canine genital tract is exposed to a high P4 milieu of luteal origin [26]. Concerning luteotropic support, one of the most interesting species-specific peculiarities is the fact that, although especially during the second half of the luteal phase PRL and LH act as luteotropic factors, luteal regression/luteolysis take place despite their increased availability (discussed later). Finally, regarding the CL as being the only source of circulating steroids during canine pregnancy, there is no pregnancy- and/or parturition-specific increase in estrogens [18, 23, 27].

8.3 Periovulatory Endocrine Events

The hormonal changes characterizing the periovulatory endocrine milieu in the dog are shown in Fig. 8.1. Thus, pro-estrus is the phase when the bitch is under the influence of increasing estradiol (E2) levels secreted from ovarian follicles. E2 concentrations increase continuously from levels of about 5–15 pg/ml at the beginning of pro-estrus to average levels of 70 pg/ml (40–120 pg/ml) at the peak, 1 or 2 days before the onset of estrus. Estrogens alone are, however, not responsible for the breeding activity, which is normally associated with decreasing E2 levels occurring concomitantly with rising P4 concentrations [28]. The latter, being designated as follicular luteinization, sets in before the first significant LH increase (LH surge), which indicates the final maturation of ovarian follicles. During this time, circulating P4 rises slowly from basal levels of 0.2–0.4 ng/ml to 0.6–1.0 ng/ml [29]. E2 starts to decrease progressively toward its intermediate estrous values of 10–20 pg/ml [2].

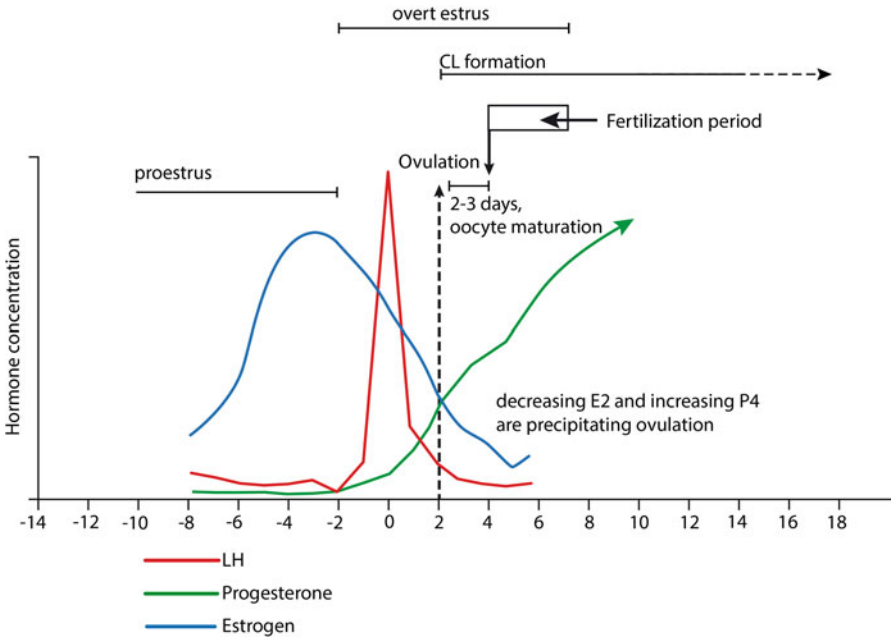

Fig. 8.1 Schematic representation of the most important endocrine patterns before and after ovulation, leading to establishment of the canine CL. A detailed explanation is provided in the text. Ovulation takes place accompanied by relatively high circulating progesterone (P4) levels, >5 ng/ml, and is precipitated by decreasing estradiol (E2) and increasing P4. The structural formation of CL begins before cessation of clinical estrus signs (overt estrus). (Modified from [26])

The LH surge is the result of increasing P4 and decreasing E2 levels, providing a strong positive feedback on the hypothalamus and hypophysis, also leading to enhanced follicle-stimulating hormone (FSH) production. Thus, hormonally, estrus that lasts on average 9 days is characterized by declining estrogen and rising P4 concentrations, strongly stimulating LH secretion and precipitating ovulation. The LH surge takes place 0.5–3 days (average, 1 or 2 days) after the E2 peak. It has been defined more accurately by Concannon [2] as the first detectable rise >200 % of preceding mean concentrations of LH and >50 % of its peak concentrations (i.e., the first significant LH increase). This abrupt surge of gonadotropins at the end of proestrus results in a 1- to 3-day elevation of LH (average, 2 days, usually peaking in the first 12–18 h) and a 1- to 4-day elevation of FSH, leading to ovulation at 48–60 h (2–3 days) after the LH surge [2, 24, 26]. It is noteworthy, and unique among the domestic animal species, that canine oocytes are ovulated at the stage of primary oocytes and that their maturation and completion of the first meiotic division are delayed, taking place in the oviducts 2–3 days after ovulation (i.e., 4–5 days after the LH surge) [24].

The time period from the LH surge to ovulation is characterized by rapid proliferation of follicular theca cells and emergence of a vascular network supplying them. Consequently, luteinizing follicular cells are capable of producing amounts of

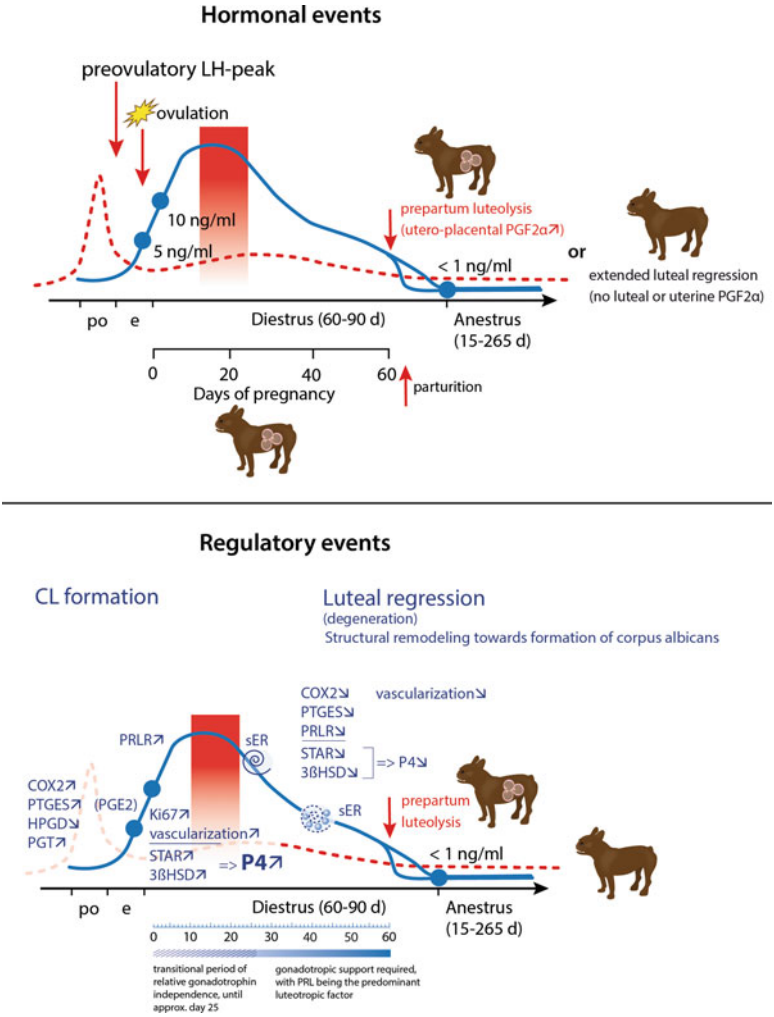

Fig. 8.2 Diagrammatic representation of the most important hormonal mechanisms regulating luteal function during pregnancy and pseudo-pregnancy in dogs. A detailed explanation is provided in the text. *COX2* cyclooxygenase 2 (PTGS2), *PTGES* PGE2-synthase, *Ki67* proliferation marker, *PRLR* prolactin receptor, *LH* luteinizing hormone, *STAR* steroidogenic acute regulatory protein, *3βHSD (HSD3B2)* 3β-hydroxysteroid-dehydrogenase, *PGT* prostaglandin transporter, *HPGD* 15-prostaglandin dehydrogenase, *sER* smooth endoplasmic reticulum, an organelle in which microsomal enzymes such as 3βHSD, CYP19arom, 17αHSD, or inducible PTGES isoform are being synthesized (ongoing degenerative processes characterized by whorl-like structures are indicated). (Modified from [8])

P4 considerably exceeding basal values, reaching levels of about 5 ng/ml at the time of ovulation (Figs. 8.1 and 8.2) [24]. The morphological changes associated with this phenomenon were described for the first time by Bischoff [17], who found

luteal-like structures and strong proliferation and folding areas in canine preovulatory follicles. Recently, these changes have been associated with high local concentrations of prostaglandin (PG) E2 and PGF2α in the newly forming CL [30], indicating the involvement of both PGs in the process of ovulation, similar to events described in other species. The structural formation of CL continues immediately following ovulation and before cessation of the clinical estrus, defined as male acceptance (overt estrus). Thus, already at this time, both functionally and morphologically, ovarian structures enter the stage of luteal dominance, commonly referred to as diestrus. Endocrinologically, estrus ends when plasma E2 concentrations decrease below 15 pg/ml, which is associated with cytological and clinical or behavioral signs of progesterone domination [28].

8.4 Luteal Steroidogenic Activity During Pregnant and Nonpregnant Cycles

The biology of canine CL has been extensively studied and thoroughly discussed recently, covering broad aspects of luteal physiology including growth and maintenance, as well as divergent patterns of slow regression and luteolysis in pregnant and pseudo-pregnant bitches [2, 7–11, 31]. A cumulative schematic representation of the most important regulatory mechanisms is shown in Fig. 8.2.

Corpora lutea develop within the ruptured follicular cavities, which release cumulus oocyte complexes within 12–96 h [32]. In dogs, the number of ovulating follicles varies and, at least to some extent, depends upon the breed and animal size, with smaller breeds ovulating fewer oocytes (2 to 10) and larger breeds ovulating more, 5–15 oocytes [28]. Those follicles not mature enough to ovulate undergo atresia. The ovulated follicles reorganize and luteinize quickly, resulting in a strongly increasing steroidogenic capacity during the early luteal phase, manifested in rapidly rising peripheral P4 levels that reach their highest levels usually within 15–30 days after ovulation [23]. At that time, the average circulating P4 concentrations range between 30 and 35 ng/ml (although sometimes displaying values of 80–90 ng/ml or higher) [2, 24]. Afterward, the steroidogenic capacity of the CL starts to decrease gradually, indicating the turning point in its functional lifespan when the slowly ongoing and considerably extended luteal regression sets in. Because canine CL function remains unaffected by hysterectomy [23, 33], this reveals an inherent lifespan, which in nonpregnant dogs is independent of any acute (luteolytic) regulatory mechanism. It can even last as long as 1–3 months until the peripheral P4 levels reach the baseline limit of <1 ng/ml, indicating, per definition, the onset of sexual quiescence, i.e., anestrus.

During canine pregnancy, the length of the luteal lifespan determines the duration of gestation. It ends rapidly at about 60 days after ovulation, when the so far slow luteal regression is interrupted by a precipitous P4 decline shortly before parturition, referred to as the prepartum luteolysis. This, in dogs, as in most other mammals, is a prerequisite of parturition. Importantly, in contrast to pseudo-preg-

nancy, the concomitant surge of PGF2α in maternal plasma indicates its key role during luteolysis and/or parturition [34].

Although the mean P4 concentrations tend to be numerically higher at pregnancy, mostly due to strong individual variations, they only rarely differ statistically between pregnant and pseudo-pregnant dogs [35]. Sometimes, however, differences can be seen, especially after days 25–30 of gestation, that is, following implantation (which in the dog takes place around day 17–18 of pregnancy) and placentation. It has been hypothesized that the elevated prolactin (PRL) concentrations measured during the same phase of pregnancy might be responsible for this increase [2, 29]. In this context, it is noteworthy that, because of high individual variations in the strongly elevated PRL levels observed in overtly pseudo-pregnant bitches (*lactatio falsa*), similar to P4, PRL cannot be used as a reliable endocrine marker for pregnancy determination in dogs.

In addition, individual E2 levels fluctuate strongly during most of the diestrus period (Fig. 8.3). Following the preovulatory peak, E2 tapers progressively downward over 9–12 days to basal values of 8–9 pg/ml when cytological diestrus is definitely established [27]. This "shift" from estrus to diestrus is characterized by a change in the vaginal cytology picture from 80–100 % of superficial cells to 80–100 % of parabasal and intermediate cells observed at diestrus [28]. Coinciding with luteal formation and increasing P4, from approximately day 10 E2 again increases significantly and stays elevated in both pregnant and nonpregnant dogs, with average levels ranging between 15 and 40 pg/ml depending on the breed, but never reaching the preovulatory levels [2, 23, 27] (Fig. 8.3).

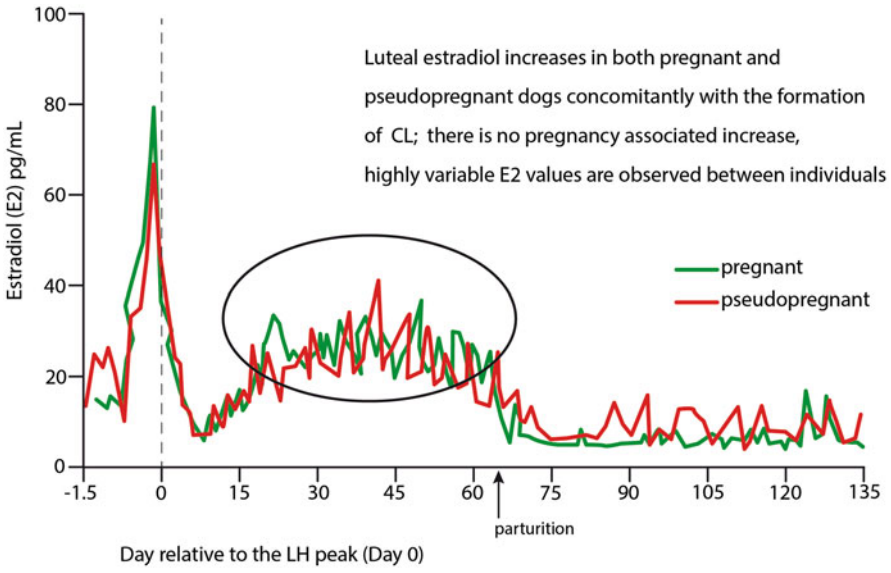

Fig. 8.3 Diagrammatic representation of estradiol (E2) profiles in pregnant and pseudo-pregnant bitches. (Modified after [27])

As mentioned before, there is no pregnancy-associated increase in E2; its profiles, at least in part, parallel those of P4. Beginning on day 60 of the extended luteal lifespan, E2 starts to decline under both conditions. Importantly, and in contrast to other domestic animal species, in pregnant dogs a prepartum drop in E2 is observed during prepartum luteolysis [18]. No hint of placental aromatase activity (CYP19arom) was found [18], and neither could aromatase expression be identified in canine placenta [19]. Its abundant expression was, however, confirmed in the canine CL [11, 19]. This finding, together with the prepartum E2 decline, further indicates its luteal origin. Both P4 and E2 seem to exert paracrine and autocrine effects on canine luteal structures as expression of their respective receptors (PGR, ERα/ESR1, ERβ/ESR2) was found throughout the luteal phase in both steroidogenic and nonsteroidogenic cells [10, 11]. Furthermore, the luteotropic effects of P4 on canine CL arise from the diminishing effects of anti-gestagens on its functionality. Thus, treatment with a PGR blocker unequivocally results in a preterm luteolysis (or abortion) [36, 37]. It is noteworthy that, in addition to the lack of a prepartum increase in estrogens seen in other species, in the dog the parturition-associated increase of cortisol in maternal plasma is not mandatory for normal parturition and can be observed only irregularly [18, 38]. This increase, when present, was attributed by Hoffmann and coworkers to maternal stress [18]. On the other hand, however, some effects of locally produced cortisol cannot be excluded, and it is plausible that the circulating levels observed in maternal blood do not fully reflect its concentrations at the feto-placental level. Accordingly, the placental expression of glucocorticoid receptor is elevated in the dog during normal prepartum luteolysis, but not in response to anti-gestagen treatment when applied to mid-pregnant dogs [39]. This finding suggests that cortisol may be involved in the local withdrawal of P4 at the time of physiological parturition, thereby resembling endocrine mechanisms found in humans [40]. In this context, it needs to be emphasized that, in addition to the divergent profiles of P4 in pregnant and pseudo-pregnant dogs, also the E2 and cortisol secretion patterns indicate the presence of different, species-specific endocrine regulatory mechanisms associated with the cessation of CL function and initiation of parturition.

8.5 Luteal Development: Morphological Aspects and Functional Implications

As in other species, the canine CL originates in ruptured ovarian follicles. The histological analysis of ovarian structures on the day of ovulation (determined by P4>5 ng/ml) reveals the presence of both freshly ovulated and preovulatory follicles, characterized by the aforementioned strong folding of theca interna layers [17], separated from the follicular cavity by the basement membrane [30]. Concomitantly, the shape of luteinizing theca cells changes from elongated to rounded. Following ovulation, in addition to further luteinization of follicular wall structures, the luteinizing granulosa cells can be clearly observed, still at least partly separated from the theca cells by remnants of the basement membrane [30].

As presented in Fig. 8.2, the abruptly increasing luteal P4 secretion is supported by strong proliferative and vasculogenic activities, as indicated by enhanced expression of the Ki67 proliferation marker and increased staining for the endothelial cell marker, endoglin [11, 41]. The vasculogenic and angiogenic activities are reflected in increased expression of vascular endothelial growth factor-A (VEGFA) and its two receptors (VEGFR1/Flk1 and VEGFR2//KDR/Flk1) in steroidogenic and non-steroidogenic cellular components, as reported for the nonpregnant canine CL [42, 43]. This increase seems, at least to some extent, to be driven by hypoxia, which may be concluded from the clearly detectable presence of the hypoxia-inducible factor-1α (HIF1α) [43]. Similarly, during pregnancy expression of the VEGF system is upregulated in steroidogenic and vascular components of the CL and increases with luteal formation, being strongest at the postimplantation stage of pregnancy (days 18–25 of embryonic life) [44]. This stage is also the time when the P4 demand increases to support the establishment of canine gestation through P4-dependent uterine secretory activity. The increased metabolic needs of the CL are reflected in the increased expression of the facilitative glucose transporter GLUT1 (SLC2A1), responsible for glucose uptake [43]. The vascular activity facilitates increased blood flow, as indicated by elevated expression of endothelin receptor B (ETB) in early canine CL of both pregnant and pseudo-pregnant dogs. Of the two endothelin (ET) receptors (ETB and ETA), ETA is responsible for vasoconstriction, whereas occupation of ETB receptors results in nitric oxide-mediated vasodilation [45]. Interestingly, within the canine CL ETB is localized both in lutein cells and vascular endothelium. Along with ETB, increased expression of one of its ligands, ET2, was noted in forming canine CL and was localized predominantly in endothelial cells, thereby implying a functional interplay between these two compartments [46]. The enhanced provision of ETs is signaled by the concomitantly increased presence of their activating enzyme, endothelin-converting enzyme 1 (ECE1), at both locations [46].

Functionally, the dynamically rising P4 output depends on the increased expression of steroidogenic acute regulatory protein (STAR) and 3-β-hydroxysteroid-dehydrogenase (3βHSD, HSDB2) [31, 36, 47, 48], which are key factors regulating steroidogenesis. Their expression throughout the luteal phase closely matches the peripheral P4 concentrations during pregnancy and in nonpregnant cycles. Functional aspects concerning the canine STAR promoter are not yet fully established. Its proximal fragment, homologous with the murine counterpart bearing transcriptional activity comparable to the full-size promoter, has been cloned and characterized [31]. It reveals several putative binding sites for transcription factors, such as C/EBP, SF1, GATA, SREBP, CRE-1, and CRE-3 (transcriptionally active half-sites of CREB found in the murine counterpart): all these are known as positive regulators of STAR expression. Additionally, binding sites for one of the strongest inhibitors of STAR expression, DAX1, were identified [31]. As expected, the cloned fragment of canine STAR promoter proved to be responsive to one of the most potent canine luteotropic factors, namely PGE2 [31].

Morphologically, only one type of steroidogenic cell can be found in mature canine CL, so unlike in other species no distinction between small and large lutein cells is possible. The process of their differentiation from both types of progenitor cells remains to be elucidated. In early CL, on day 5 after ovulation, steroidogenic

cells are irregularly shaped and are 5–10 μm in size. Their cytoplasm includes many small lipid droplets, indicating high rates of metabolic and steroidogenic activity [8, 49]. Although still in the hemorrhagic state, characterized by extravasated erythrocytes, the capillary bed is already well developed. As the lutein cells continue to grow, their size increases, reaching 20 μm at day 15; they become mature at around day 25 of the luteal lifespan with diameters approximately 30–40 μm. At that time, the luteal tissue appears dense, steroidogenic cells are polyhedral, and the vascular bed is fully established, providing virtually every lutein cell with a direct vascular supply, as indicated by the high density of endoglin staining [8, 41]. The number of small lipid droplets decreases; however, their activity remains high as indicated by the increased numbers of mitochondria and smooth endoplasmic reticulum. Proliferation and vascularization rates slow down in mature canine CL. Once the highest steroidogenic activity is over, the slowly ongoing luteal regression is characterized by signs of luteal degeneration. This is first reflected in structural changes of the endoplasmic reticulum, which by day 30 starts to exhibit "whorl-like" structures, and from day 45 on includes large lipid droplets as a further sign of degeneration [8, 10, 49]. The ER also loses its proximity to the nucleus and moves toward the periphery of lutein cells. The number of mitochondria decreases, and the cytoplasm of the lutein cells becomes filled with large vacuoles, another sign of cellular degeneration. The intercellular distances between lutein cells increase along with elevated numbers of matrix and connective tissue components [8]. The density of the vascular bed and the expression of vasculogenic and vasoactive factors decrease together with diminishing steroidogenic activity, as indicated by reduced STAR and 3βHSD expression. Around day 60–65, luteal degeneration is already strongly advanced with large, irregularly shaped lutein cells and an increased incidence of pyknotic nuclei [8]. At the subcellular level, the number of mitochondria is strongly diminished, and degenerative vacuoles fill virtually the entire cytoplasm. Already at this stage the matrix and connective tissue components may indicate the slowly ongoing transition toward corpus albicans formation.

Interestingly, in nonpregnant bitches, all the aforedescribed changes take place in the absence of strong apoptotic events, which can be observed only sporadically [10, 49], indicating that the slow luteal regression is a passive, preprogrammed, degenerative process; this is opposite to the situation in pregnant bitches, where the prepartum luteolysis and accompanying PGF2α increase are associated with massive apoptotic activity within the CL, which can be observed microscopically and is evidenced by the strong expression of active caspase-3 [8]. Interestingly, at least at the mRNA level, the vascular epithelial growth factor (VEGF) system and, therefore, vasculogenic activity remain unaffected during normal and (within the first 24 h of the anti-gestagen treatment) induced luteolysis, compared with its expression at mid-gestation [8]. At the protein level, however, the expression of VEGFA and its VEGFR1 receptor decreases, indicating possible divergence between the mechanisms regulating their mRNA expression and the turnover rates of the respective proteins [44] during cessation of canine luteal function. Concomitantly, the functionality of blood vessels reacts strongly to the luteolytic insult in both situations (i.e., during normal and induced luteolysis), reflected in increased endothelial expression of the vasoconstrictive ETA [46], which remains unaffected during late

luteal regression in pseudo-pregnant bitches. It has therefore been concluded that also where vascular activity is concerned, the extended luteal regression remains primarily a passive process [46].

Among other regulatory components that affect canine CL structurally and functionally are immune system-derived factors. Although under-investigated, CD4- and CD8-positive cells, as well as MHC II-positive cells, cumulatively representing predominantly lymphocytes and macrophages, are present in the CL throughout the luteal phase. Although cells bearing all three differentiation markers can be identified in early CL, renewed infiltration of CD8- and MHC II-positive immune cells could be found in regressing CL (at days 45 and 65 for CD8, and 65 and 75 for MHC II) [10, 41]. By applying qualitative PCR, the expression of IL-8, IL-10, IL-12, TNF-α, and TGF-β, but not of IL-1β, IL-2, and IL-4, could be confirmed [50]. Clearly, further investigations are needed to determine the role of immune system components in canine luteal function.

8.6 Hypophyseal Hormones

Both PRL and LH are luteotropic within the canine CL. There is, however, controversy concerning the exact timing and the extent to which both factors are required for luteal maintenance in the dog [51–55].

Thus, during the first 2–4 weeks of its development, the canine CL appears to be at least in part refractory to hypophyseal influence as early hypophysectomy (on day 4 after ovulation) resulted in only temporary suppression of P4 secretion, which was attributed to postoperative stress. This was followed by a 6 to 10-day recovery phase, with the luteal lifespan, however, being shortened compared with controls [55]. In the same study, when dogs were hypophysectomized on day 18 after ovulation, the suppression of CL function was permanent. Based on these observations, it has been concluded that in the dog luteal function is autonomous during a certain period, at least regarding hypophyseal support, or in a broader sense, gonadotropic support. Taking into account the length of the recovery phase observed after early hypophysectomies, it has been postulated that the refractory phase ends on about day 24–28 after ovulation [55]. A contradiction exists, however, with results in a study by Concannon [52], in which hypophysectomies performed between days 10 and 50 of the luteal phase always resulted in premature cessation of CL function within 3–17 days, supporting the conclusion that canine CL is chronically dependent upon gonadotropic support. Further studies are needed to clarify these discrepancies.

Nevertheless, during the second half of diestrus, hypophyseal hormones are needed for maintaining canine CL function, with PRL being the predominant luteotropic factor. The latter is absolutely required from day 25 of the luteal lifespan onward, as clearly presented in functional studies utilizing the dopamine agonist, bromocriptine, for suppression of PRL secretion [53]. Interestingly PRL, but not LH, was able to reverse the negative effects of bromocriptine on P4 production [56]; this further supports the afore-presented, postulated time-dependent luteal sensitivity to

hypophyseal support. Suppression of LH function at comparable time points or later during the luteal lifespan (i.e., on days 25, 30, 31 of the luteal phase [53], or day 42 after the onset of estrus [51], or between days 30–34 and at day 40 after the LH surge [54]), resulted either in temporarily decreased P4 release, or did not affect circulating P4 levels, indicating the subordinate role of LH as a luteotropic agent compared with PRL. Its role, however, in regulating canine CL function is illustrated by the stimulatory effects evoked by LH treatment on PRL secretion and its direct positive effects on P4 production observed in some studies [52, 54]. PRL, on the other hand, did not stimulate LH secretion and neither did it directly stimulate P4 levels, indicating its role in maintenance or support of CL function and slowing down of luteal regression, rather than active stimulation of P4 secretion [56]. This finding is supported by the observation that the progressive luteal regression that takes place during the second half of diestrus cannot be prevented despite the increasing bioavailability of PRL and LH [57–61]. Especially in pregnant bitches, the levels of PRL increase continuously and significantly during the second half of gestation toward parturition, displaying maximal values of about 50 ng/ml close to term [57]. In pseudo-pregnant bitches, it remains at basal levels during most of the luteal lifespan, increasing only about two- to threefold from initial values of 2–4 ng/ml to their maximal levels of approximately 9 ng/ml at the time point corresponding to parturition [57, 58]. Interestingly, PRL can be produced by the CL, but its expression is low and does not seem to contribute significantly to the overall circulating levels (our data, unpublished). Apparently, some local autocrine or paracrine effects cannot be excluded, especially because the PRL receptor (PRLR) is continuously expressed in CL [62]. The expression of PRLR is time dependent, at both mRNA and protein levels, being abundantly present in early CL and decreasing significantly during regression toward the end of the luteal life in nonpregnant and pregnant dogs, respectively. It could be speculated that the decreased expression of PRLR during the course of luteal regression might be, at least in part, responsible for the diminishing sensitivity of the CL toward hypophyseal support, contributing thereby to its degeneration. On the other hand, however, the degenerative processes might be responsible for falling PRLR expression. Analogous to what has been described for other species, such as pigs or monkeys [63, 64], it could be hypothesized that the enhanced PRL secretion observed during pregnancy derives from an increasing secretion of relaxin from placental syncytiotrophoblast [25], simultaneously or slightly earlier (days 25–30 after the preovulatory LH surge), which stimulates, in turn, hypophyseal PRL production.

8.7 Intraluteal and Extraluteal Prostaglandins

As in other species, in the dog, prostaglandins (PGs) appear to be major players regulating canine CL function. The locally produced, that is, intra-CL, PGs are especially involved in its formation and establishing P4 production, but not in the cessation of luteal function. Consequently, the early luteal phase is associated with strongly increased cyclooxygenase 2 (COX2/PTGS2) and PGE2 synthase (PGES, PTGES) [31, 36, 65], accompanying progressively rising P4 levels. Their

expression decreases significantly in regressing CL and remains low until the end of the luteal lifespan in pregnant and nonpregnant dogs. A similar expression pattern, indicating the involvement of PGs in CL formation, was identified for the PG transporter (PGT) [31]. The expression of HPGD (15-prostaglandin dehydrogenase), the enzyme responsible for degradation of PGs, seemed to be negatively correlated with PTGES and PGT expression, possibly increasing the bioavailability of PGs in the canine CL [8]. At the cellular level, PGE2 stimulates STAR expression, and phosphorylation (i.e., activation) as evidenced by its increased protein expression and steroidogenic output from cultured canine lutein cells isolated during early diestrus [31], proving the luteotropic capacity of PGE2 in canine CL.

The PGE2-mediated regulation of STAR is cAMP/PKA dependent, and two of the PGE2 receptors (EP2/PTGER2 and EP4/PTGER4) known to act via this pathway are clearly detectable in the canine CL throughout the luteal phase [31, 66]. Interestingly, the expression of 3βHSD remains unaffected by PGE2 treatment [31]. A functional in vivo proof, and compelling evidence for the luteotropic function of PGs in canine CL, have been provided by applying the selective COX2 blocker firocoxib in nonpregnant dogs up to day 30 after ovulation [30, 67]. This treatment resulted in inhibition of the steroidogenic machinery, reflected in lowered STAR and 3βHSD expression and reduced P4 concentrations. Additionally, the expression of PTGES and PRLR was significantly suppressed. The latter, together with stimulatory effects of PGE2 on PRLR expression presented in the same study in in vitro cultured lutein cells, indicates possible indirect effects of PGE2 on local PRL availability, by regulating the expression of its receptor [30, 67]. Additional indirect effects of PGE2 in the dog CL arise from the observation that it increased ETB expression in early lutein cells in vitro, possibly contributing thereby to the increased blood flow in the forming CL [46].

It is noteworthy that, although only low or no expression of PGF2α-synthase (PGFS/AKR1C3) can be detected in canine CL throughout the luteal phase under both pregnancy and pseudo-pregnancy, the PGF2α receptor (FP, PTGFR) can be clearly detected throughout the luteal lifespan [36, 68, 69]. PGFS/AKR1C3 is the only canine-specific PGF2α-synthase identified so far and is responsible for the direct conversion of PGH2 to PGF2α [68].

The sole target of PGF2α appear to be on lutein cells, where the FP receptor is localized [69]. Its constitutive expression points toward a basic capability of the canine CL to respond to PGF2α during its entire lifespan. Indeed, PGF2α is luteolytic in the dog even as early as day 5 of the luteal lifespan [70, 71], although this requires high dosages or repeated treatments accompanied by strong side effects.

8.7.1 Prepartum Luteolysis

Although the nonpregnant canine CL apparently lacks an internal PGF2α source, allowing it to persist for a long time, the prepartum increase in circulating PGF2α seems to originate in the pregnant uterus, where the increased COX2 expression is predominantly localized in fetal trophoblast cells [37] (Fig. 8.4).

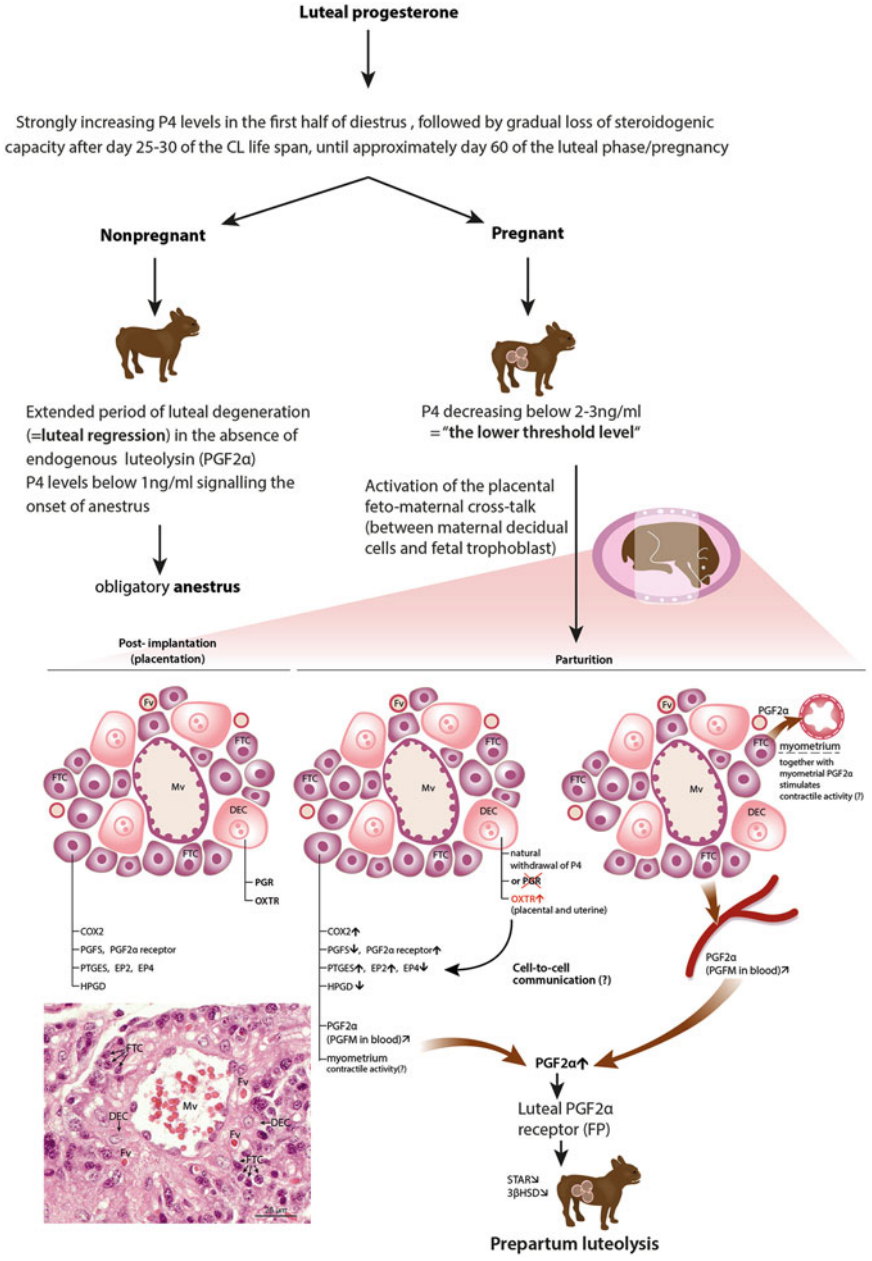

Fig. 8.4 Schematic illustration of the differential mechanisms regulating luteal function in pregnant and pseudo-pregnant dogs. A proposed model of the placental endocrine cascade involved in the prepartum output of the luteolytic PGF2α is presented. A fragment of the canine placenta endotheliochorialis is represented schematically and depicted in the micrograph. Shown are maternal decidual cells (DEC, the only cells of the canine placenta expressing progesterone receptor,

The uterine expression of COX2 is targeted mostly to the myometrium, indicating its contractile functions [72]. PGFS/AKR1C3 does not seem to be responsible for the utero-placental synthesis of PGF2α at the time of prepartum luteolysis, as its expression is downregulated at that time. Instead, the concomitantly increased expression of microsomal PTGES implies the presence of alternative pathways involved in prepartum PGF2α release in the dog, for example, those utilizing PGE2 as a substrate for PGF2α synthesis. Indeed, during prepartum luteolysis the respective biochemical capabilities of canine uterine and placental homogenates have been confirmed [37, 68, 73].

As demonstrated by applying an anti-gestagen (aglepristone) to mid-pregnant dogs, P4 signaling seems to play a major role in the underlying feto-maternal communication leading to the prepartum PGF2α output. While the P4 receptor (PGR) is localized solely in the maternal stroma-derived decidual cells, interfering with its function evokes changes in the uterine and placental PG system similar to those observed during normal prepartum luteolysis, resulting in enhanced PGF2α synthesis and, unequivocally, leading to pre-term luteolysis [37] (Fig. 8.4). The role of the placental oxytocin receptor (OXTR) as a possible signaling molecule mediating the prepartum PGF2α production arises from its colocalization with PGR in the maternal placenta and increased expression during both normal and induced parturition [74].

8.8 Perspectives

Having discussed the most important endocrine mechanisms governing luteal function, it becomes obvious that many of the regulatory aspects, especially those related to the cessation of CL function in pregnant and pseudo-pregnant bitches, remain to be further elucidated. In line with this, global transcriptomic studies involving next-generation sequencing (RNA-Seq) have been initiated aiming at identifying novel potential regulatory pathways and new candidate genes involved in the underlying cellular processes (our data, unpublished). Genes differentially expressed in CL collected from pseudo-pregnant bitches during late luteal regression (day 65 after ovulation) were compared with those expressed in CL derived from normal prepartum

Fig. 8.4 (continued) PGR, and oxytocin receptor, OXTR), and fetal trophoblast cells (FTC). Intercellular communication during the onset of parturition is presented, including cross-communication between these two cell types, resulting in strong induction of fetal placental prostaglandin (PG) synthesis and coinciding with the high prepartum PGF2α output. The expression of several regulatory factors is indicated. Blocking PGR function in the placenta materna (decidual cells) leads to similar cellular effects (at least with respect to utero-placental PG synthesis) as during normal parturition. A detailed explanation is provided in the text. *Mv* maternal blood vessel, *Fv* fetal vessels, *COX2* cyclooxygenase 2 (PTGS2), *PGFS* PGF2α-synthase (AKR1C3), *FP* PGF2α-receptor, (*PTGFR*) *PTGES* PGE2-synthase, *EP2* and *EP4* respective PGE2 receptors (PTGER2 and PTGER4), *HPGD* 15-prostaglandin dehydrogenase (deactivator of PGs). (Modified after Kowalewski [7, 8])

luteolysis. Most of the functional terms identified during late luteal regression were related to the cellular and extracellular matrix remodeling processes. On the other hand, prepartum luteolysis was dominated by expression of genes related to immune and inflammatory responses, indicating an ongoing acute process, contrasting thereby with the passive formation of the corpus albicans, and further pointing towards the luteolytic nature of circulating PGF2α. This was also indicated by the higher expression of genes related to steroid receptor activity in samples derived from late luteal regression, which were acutely suppressed during prepartum luteolysis. When compared with samples obtained from dogs in which luteolysis was induced at mid-gestation using the anti-gestagen aglepristone, the inflammatory events prevailed in those samples derived from normal prepartum luteolysis. Among the most important overrepresented functional terms resulting from the anti-gestagen-mediated withdrawal of P4 function were events related to the inhibition of transcriptional activity, negative regulation of gene expression, and negative regulation of cell proliferation. In both luteolytic groups, genes related to lipogenesis and steroid synthesis were affected. It thus seems that, even though similar at the functional level, that is, resulting in diminished steroidogenic output due to apoptotic events preventing STAR production and function, luteolysis evoked by PGR blockage is more strongly related to the deprivation of luteotropic P4 effects than to the PGF2α-related inflammatory reaction observed in natural parturition.

8.9 Feline Luteal Function: Species-Specific Peculiarities and Comparative Aspects

The most important endocrine events characterizing the reproductive cycle of the domestic cat are presented in Fig. 8.5.

8.9.1 Periovulatory Events

Domestic cats are typically seasonally polyestrous, especially when kept in temperate zones. Variations, however, can occur between latitudes, depending on the length of photoperiods to which the females are exposed, resulting in year-round cycles observed under equatorial or near-equatorial photoperiods. Thus, long-day photoperiods are stimulatory for estrus, and melatonin-suppressing effects on estrogen synthesis, post-coital LH release and, thereby, cyclicity were described [75]. In temperate climate zones, besides the late autumn and winter anestrus, and in the absence of mating or pseudo-pregnancy, cats are polyestrous. The breeding season usually starts in January to February and continues until September [28]. An average interestrous interval, with low (below 20 pg/ml) or basal E2 concentrations, usually lasts 8 days [28] but can be as long as 2–4 weeks [76]. During estrus, average E2 concentrations range approximately between 20 and 80 pg/ml [77]. The LH release starts

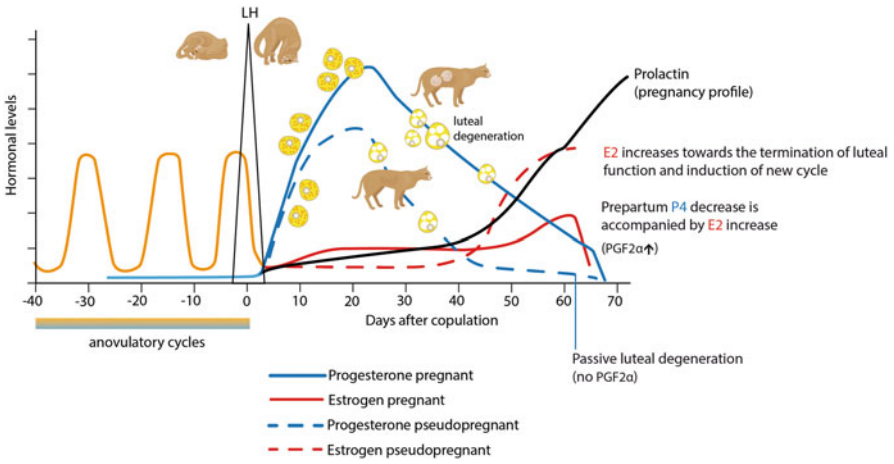

Fig. 8.5 Diagrammatic representation of the reproductive cycle in the domestic cat. Dynamic hormonal changes characteristic of pregnancy and pseudo-pregnancy are depicted. The degenerative processes associated with luteal regression are indicated: type I vacuolation (small vacuoles positive for lipid staining) is associated with increased steroidogenic output and is observed during formation and maintenance of the CL; type II vacuolation displaying large degenerative vacuoles negative for lipid staining can be observed during luteal regression in both pregnant and pseudo-pregnant queens [13]

within minutes following coitus, peaks at 2–4 h, and returns to baseline within 16 h or less [78, 79]. Ovulation begins about 24 h from the initial increase of LH and continues until approximately 32 h from the initial copulation; only one coitus can be enough to induce prolonged LH release and can result in ovulation [78]. However, more frequently, multiple copulations are needed to achieve the higher LH levels required for induction of the ovulation process, with about 50 % of queens ovulating after a single copulation [79]. The number of matings does not influence the number of ovulated follicles and thereby the subsequent number of CL.

Although traditionally considered as induced ovulators, spontaneous ovulations can be observed in queens even at frequencies of 35 % to approximately 60 % when they are kept in proximity to each other, and in pheromonal but not physical contact with males [80, 81].

8.9.2 Postovulatory Endocrine Patterns During Pregnancy and Pseudo-Pregnancy, and Sources of Circulating Hormones

Following ovulation, P4 starts to increase within 1–2 days [82] reflecting the formation of the functional CL. In cats that ovulated but did not conceive, CL of pseudo-pregnancy are formed, while in pregnant queens *corpora lutea graviditatis* develop.

The duration of the initial increase in P4 production is similar in pregnant and pseudo-pregnant cats until approximately days 10–12 of gestation, when implantation takes place. Afterward, in pregnant animals P4 concentrations increase dynamically, reaching peak values of approximately 30–40 ng/ml at day 21. Thereafter, a gradual decrease begins, with circulating P4 concentrations falling to approximately 13 ng/ml at day 50, and decreasing further toward parturition (days 63–65). Baseline P4 concentrations are not prerequisite for the onset of parturition [77]. These baseline levels, that is, <1 ng/ml, are observed immediately after parturition [83]. A similar initial P4 secretion pattern is observed during pseudo-pregnancy with peak levels, however, lower than during pregnancy, reaching concentrations of about 20–30 ng/ml on day 21 [82, 83]. This is followed by a gradual decline of luteal activity with P4 dropping to <1 ng/ml by days 36–46 post coitum [82, 84]. Thus, the luteal phase in pseudo-pregnant cats lasts about half of its length in pregnant queens [5]. Following pseudo-pregnancy, ovarian activity recommences within 7–10 days [84].

The basic steroidogenic capacity of CL seems to reflect the circulating P4 profiles [16]. The expression of STAR increases toward mid-gestation (3–4 weeks), but not toward the mid-luteal phase during pseudo-pregnancy (days 10–15 of the pseudo-pregnant luteal lifespan); 3βHSD is highest in mid-pregnancy and mid-pseudo-pregnancy, with higher relative amounts of the respective mRNA observed in pregnant animals [16].

Factors at least partly originating from the pregnant uterus and placenta were suggested to be responsible for the differences in the CL lifespan and its P4 output during pregnancy versus pseudo-pregnancy [12]. Thus, contrasting with its canine counterpart, the feline placenta is capable of producing both P4 and E2 [16, 85]. Expression of the respective steroidogenic factors and enzymes (STAR, 3βHSD, and aromatase) has been confirmed [16, 85]. Interestingly, STAR and 3βHSD are localized only in the maternal part of the placenta, namely in decidual cells [16]. The placental P4 and E2 levels and secretion patterns do not, however, mirror their circulating levels [16, 85]. Especially for P4, an inverse relationship between the placental and circulating levels was obvious in the study by Braun and coworkers [85]. It seems, therefore, that in cats as in dogs, the peripheral P4 during pregnancy is predominantly of luteal origin; this is supported by the fact that ovariectomies result in a strong decrease in plasma P4 [86, 87]. However, locally, that is, intraplacentally produced P4 seems to have a supplemental role in supporting pregnancy with mostly local effects. It appears to be sufficient to protect pregnancy in some queens, but not in all, depending on the stage of gestation. Thus, 100 % of cats aborted when ovariectomies were performed on day 35 of gestation, 80 % aborted following ovariectomy on day 40, 40 % aborted after surgery on day 45, and 60 % of queens aborted when ovaries were removed on day 50 of gestation [86].

As in dogs, the feto-placental unit is the main source of circulating relaxin in the cat, although it is also locally produced in the feline CL [88, 89]. It is not detectable during the estrous cycle or pseudo-pregnancy [89]. Relaxin becomes detectable at about day 20–25 of gestation, then increases rapidly, reaches a plateau between days 30–35, staying elevated until 10–15 days before parturition when it starts to decrease gradually toward term, and is undetectable 24 h after delivery [89]. Analogous to

the dog, the mRNA and protein have been found solely in fetal trophoblast cells as the cellular source of relaxin [90].

There is a pregnancy-specific increase in PRL: it is elevated during the last one third of pregnancy, beginning to rise from baseline values of around 7 ng/ml during the 6th week of gestation, and displaying strongly elevated levels from the 7th week, with values of 31 ng/ml on average, and reaching maximal values of around 43 ng/ml for the last 3 days of gestation [91]. During pseudo-pregnancy, PRL fluctuates on a daily basis, but remains generally at its basal levels of around 7 ng/ml [91]. PRL is needed not only for initiation of mammary gland growth and lactogenesis, but also acts as a luteotropic factor important for the maintenance of feline pregnancy; interfering with its secretion, for example, by applying bromocriptine during its pregnancy-related elevated secretion, leads to abortion [92, 93]. In contrast to PRL, LH fluctuates throughout the luteal phase, however, no pregnancy- or pseudo-pregnancy-related increase is observed; instead, it remains low [77, 94].

As already indicated, in the domestic cat, similar to the dog, E2 seems to be also primarily of luteal origin. It is high around the time of mating and decreases afterward in both pregnant and pseudo-pregnant queens. The level of E2 remains low during the first 35–40 days of pseudo-pregnancy; average values of 13–24 pg/ml can be detected. Thereafter, toward the termination of luteal function, it becomes more variable [83, 94]. A similar secretion pattern, with somewhat higher values, is observed during pregnancy; in the second half of gestation E2 concentrations start to vary, commencing with decreasing P4 concentrations, and increase toward parturition [77, 83].

PGF2α is luteolytic as early as days 21–25 of the luteal phase in pseudo-pregnant cats, leading to significant depression of circulating P4 [82]. When applied at days 11–15 of pseudo-pregnancy, PGF2α resulted in only temporary suppression of P4 secretion [82]. In pregnant cats, 100 % aborted when treated with PGF2α from day 33 of gestation [93]. As in dogs, parturition is associated with a prepartum luteolytic mechanism. A significant increase in fecal and serum PGF2α metabolite (PGFM) is observed during the last trimester of pregnancy [95, 96], beginning at about day 41 and reaching a peak about 3 days before parturition [96]. Similarly, elevated PGF2α concentrations can be detected in the feline placenta, mirroring the serum profile and indicating its luteolytic function [95]. This placental signal is missing in pseudo-pregnant queens, with fecal PGFM remaining at basal levels [96]. Moreover, it needs to be emphasized that in cats, as in dogs, ovarian cyclicity is maintained following hysterectomy, precluding the existence of a uterine luteolysin in the absence of pregnancy [97].

8.9.3 Morphological and Functional Implications

As in other species, in cats the residual cells of ovulated ovarian follicles give rise to CL formation. Similar to other species, but in contrast to the dog, the feline CL is composed of large and small lutein cells, both populations possessing steroidogenic activity.

Morphologically, the developmental stages of CL resemble those described for the dog. In this context, an interesting feature of cat CL is the presence of two types of vacuole that, analogous to canine CL, have been identified in CL of both pregnant and pseudo-pregnant queens [13]. The first type (type 1), characterized by small lipid droplets, stains positively with Sudan II and thus reveals the lipid nature of their content, and was associated with the high steroidogenic capacity of the cells. The second type of vacuole (type 2) are larger and scattered throughout the cytoplasm, remaining negative to lipid staining, and are associated with the process of cell degeneration [13]. In pregnant queens, this type of vacuolation replaced the first type by day 38 of gestation, concomitant with greatly decreased P4 production. Strong signs of luteal degeneration were observed by day 48 of pregnancy, with deformed lutein cells containing small, condensed nuclei and increased numbers of non-steroidogenic cells. A similar shift in morphological features of the CL was found in pseudo-pregnant cats during luteal regression.

8.10 Conclusions

Despite obvious differences between dogs and cats concerning their reproductive patterns and the underlying endocrine regulatory mechanisms, at least when it comes to the process of luteal regression, there is a similarity between the two species. In both species, the CL has an inherent lifespan that is not modulated by any luteolysin of uterine origin, dissimilar to most other domesticated animals. Because of this, in both dogs and cats, the luteal phase is greatly prolonged, resulting in physiological pseudo-pregnancy. In contrast, in pregnant animals of both species, there is an active prepartum luteolysis that causes gestation to end and allows parturition to ensue. As this mechanism is absent in nonpregnant females, the length of the luteal lifespan during canine and female pseudo-pregnancy seems to be regulated by aging processes, causing the CL to degenerate and structurally remodel toward *corpus albicans* formation. The inherent luteal lifespan of the CL in nonpregnant cats seems to be, however, much shorter than in the dog. The reason for the persistence of P4 and maintenance of pregnancy over the time span of pseudo-pregnancy may lie in factors of placental origin, including placental steroidogenesis. Finally, also *per analogiam* with the canine species, intraluteally produced PGs appear to be more involved in formation of the feline CL, with increased activity of PGE2, than in the luteolytic action of PGF2α during its termination [98].

Finally, the control of CL function in dogs and cats appears to represent a more primitive mechanism than in other domesticated animals, in which luteotropic and/or luteolytic agents have evolved to play a role in its longevity or demise, respectively.

Acknowledgments The author thanks Jeanne Peter from the Department of the Scientific Communication of the Vetsuisse Faculty, Zurich, University of Zurich for her help with preparing the schematic figures, and Professors Barry Bavister and Bernd Hoffmann for careful editing of the manuscript, critical corrections, and discussion.

References

1. Okkens AC, Kooistra HS. Anoestrus in the dog: a fascinating story. Reprod Domest Anim. 2006;41(4):291–6.
2. Concannon PW. Reproductive cycles of the domestic bitch. Anim Reprod Sci. 2011;124(3-4):200–10.
3. Starkey MP, Scase TJ, Mellersh CS, Murphy S. Dogs really are man's best friend: canine genomics has applications in veterinary and human medicine! Brief Funct Genomic Proteomic. 2005;4(2):112–28.
4. Braun BC, Vargas A, Jewgenow K. The molecular detection of relaxin and its receptor RXFP1 in reproductive tissue of *Felis catus* and *Lynx pardinus* during pregnancy. Reproduction. 2012;143(3):399–410.
5. Brown JL, Wasser SK, Wildt DE, Graham LH. Comparative aspects of steroid hormone metabolism and ovarian activity in felids, measured noninvasively in feces. Biol Reprod. 1994;51(4):776–86.
6. Jewgenow K, Amelkina O, Painer J, Goritz F, Dehnhard M. Life cycle of feline Corpora lutea: histological and intraluteal hormone analysis. Reprod Domestic Anim. 2012;47 suppl 6:25–9.
7. Kowalewski MP. Endocrine and molecular control of luteal and placental function in dogs: a review. Reprod Domestic Anim. 2012;47 suppl 6:19–24.
8. Kowalewski MP. Luteal regression vs. prepartum luteolysis: regulatory mechanisms governing canine corpus luteum function. Reprod Biol. 2014;14(2):89–102.
9. Concannon PW. Research challenges in endocrine aspects of canine ovarian cycles. Reprod Domest Anim. 2012;47 suppl 6:6–12.
10. Hoffmann B, Busges F, Engel E, Kowalewski MP, Papa P. Regulation of corpus luteum-function in the bitch. Reprod Domest Anim. 2004;39(4):232–40.
11. Papa PC, Hoffmann B. The corpus luteum of the dog: source and target of steroid hormones? Reprod Domest Anim. 2011;46(4):750–6.
12. Jewgenow K, Painer J, Amelkina O, Dehnhard M, Goeritz F. Lynx reproduction: long-lasting life cycle of corpora lutea in a feline species. Reprod Biol. 2014;14(2):83–8.
13. Amelkina O, Braun BC, Dehnhard M, Jewgenow K. The corpus luteum of the domestic cat: histologic classification and intraluteal hormone profile. Theriogenology. 2015;83(4):711–20.
14. Zschockelt L, Amelkina O, Koster S, Painer J, Okuyama MW, Serra R, et al. Comparative analysis of intraluteal steroidogenic enzymes emphasises the functionality of fresh and persistent corpora lutea during pro-and metoestrus in the lynx. J Steroid Biochem Mol Biol. 2015;154:75–84.
15. Zschockelt L, Amelkina O, Siemieniuch MJ, Koster S, Jewgenow K, Braun BC. Corpora lutea of pregnant and pseudopregnant domestic cats reveal similar steroidogenic capacities during the luteal life span. J Steroid Biochem Mol Biol 2014;144(pt B):373–81.
16. Siemieniuch MJ, Jursza E, Szostek AZ, Skarzynski DJ, Boos A, Kowalewski MP. Steroidogenic capacity of the placenta as a supplemental source of progesterone during pregnancy in domestic cats. Reprod Biol Endocrinol. 2012;10:89.
17. Bischoff TLW. Entwicklungsgeschichte des Hunde-Eies. (Eng.: The development of the canine oocyte.). Braunschweig, Druck und Verlag von Friedrich Veweg und Sohn. 1845.
18. Hoffmann B, Hoveler R, Nohr B, Hasan SH. Investigations on hormonal changes around parturition in the dog and the occurrence of pregnancy-specific non conjugated oestrogens. Exp Clin Endocrinol. 1994;102(3):185–9.
19. Nishiyama T, Tsumagari S, Ito M, Kimura J, Watanabe G, Taya K, et al. Immunohistochemical study of steroidogenic enzymes in the ovary and placenta during pregnancy in the dog. Anat Histol Embryol. 1999;28(2):125–9.
20. Tarraf CG, Knight JW. Effect of uterine space and fetal sex on conceptus development and in vitro release of progesterone and estrone from regions of the porcine placenta throughout gestation. Domestic Anim Endocrinol. 1995;12(1):63–71.

21. Weng Q, Medan MS, Ren L, Watanabe G, Tsubota T, Taya K. Immunolocalization of steroidogenic enzymes in the corpus luteum and placenta of the Japanese Shiba goat. J Reprod Dev. 2005;51(2):247–52.
22. Sheldrick EL, Ricketts AP, Flint AP. Placental production of progesterone in ovariectomized goats treated with a synthetic progestagen to maintain pregnancy. J Reprod Fertil. 1980;60(2):339–48.
23. Hoffmann B, Hoveler R, Hasan SH, Failing K. Ovarian and pituitary function in dogs after hysterectomy. J Reprod Fertil. 1992;96(2):837–45.
24. Concannon PW, McCann JP, Temple M. Biology and endocrinology of ovulation, pregnancy and parturition in the dog. J Reprod Fertil Suppl. 1989;39:3–25.
25. Klonisch T, Hombach-Klonisch S, Froehlich C, Kauffold J, Steger K, Steinetz BG, et al. Canine preprorelaxin: nucleic acid sequence and localization within the canine placenta. Biol Reprod. 1999;60(3):551–7.
26. Kowalewski MP, Gram A, Kautz E, Graubner FR. The dog: nonconformist, not only in maternal recognition signaling. Adv Anat Embryol Cell Biol. 2015;216:215–37.
27. Onclin K, Murphy B, Verstegen JP. Comparisons of estradiol, LH and FSH patterns in pregnant and nonpregnant beagle bitches. Theriogenology. 2002;57(8):1957–72.
28. Feldman EC, Nelson RW. Ovarian cycle and vaginal cytology. In: Canine and feline endocrinology and reproduction, 3rd edn. St. Louis: Saunders; 2004. p. 752–74.
29. Concannon PW. Endocrinologic control of normal canine ovarian function. Reprod Domestic Anim. 2009;44 Suppl 2:3–15.
30. Kowalewski MP, Ihle S, Siemieniuch MJ, Gram A, Boos A, Zdunczyk S, et al. Formation of the early canine CL and the role of prostaglandin E2 (PGE2) in regulation of its function: an in vivo approach. Theriogenology. 2015;83(6):1038–47.
31. Kowalewski MP, Fox B, Gram A, Boos A, Reichler I. Prostaglandin E2 functions as a luteotrophic factor in the dog. Reproduction. 2013;145(3):213–26.
32. Jeffcoate I (1998) Physiology and endocrinology of the bitch. In: Simpson G, editor. Manual of small animal reproduction and neonatology. London: British Small Animal Association; 1998. p. 1.
33. Okkens AC, Dieleman SJ, Bevers MM, Willemse AH. Evidence for the non-involvement of the uterus in the lifespan of the corpus luteum in the cyclic dog. Vet Q. 1985;7(3):169–73.
34. Nohr B, Hoffmann B, Steinetz BE. Investigation of the endocrine control of parturition in the dog by application of an antigestagen. J Reprod Fertil Suppl. 1993;47:542–3.
35. Steinetz BG, Goldsmith LT, Harvey HJ, Lust G. Serum relaxin and progesterone concentrations in pregnant, pseudopregnant, and ovariectomized, progestin-treated pregnant bitches: detection of relaxin as a marker of pregnancy. Am J Vet Res. 1989;50(1):68–71.
36. Kowalewski MP, Beceriklisoy HB, Aslan S, Agaoglu AR, Hoffmann B. Time related changes in luteal prostaglandin synthesis and steroidogenic capacity during pregnancy, normal and antiprogestin induced luteolysis in the bitch. Anim Reprod Sci. 2009;116(1-2):129–38.
37. Kowalewski MP, Beceriklisoy HB, Pfarrer C, Aslan S, Kindahl H, Kucukaslan I, et al. Canine placenta: a source of prepartal prostaglandins during normal and antiprogestin-induced parturition. Reproduction. 2010;139(3):655–64.
38. Concannon PW, Butler WR, Hansel W, Knight PJ, Hamilton JM. Parturition and lactation in the bitch: serum progesterone, cortisol and prolactin. Biol Reprod. 1978;19(5):1113–8.
39. Gram A, Trachsel A, Boos A, Kowalewski MP. Reproduction. 2016 Oct;152(4):303-11. doi: 10.1530/REP-16-0213.
40. Karalis K, Goodwin G, Majzoub JA. Cortisol blockade of progesterone: a possible molecular mechanism involved in the initiation of human labor. Nat Med. 1996;2(5):556–60.
41. Hoffmann B, Busges F, Baumgartner W. Immunohistochemical detection of CD4-, CD8- and MHC II-expressing immune cells and endoglin in the canine corpus luteum at different stages of dioestrus. Reprod Domestic Anim. 2004;39(6):391–5.
42. Mariani TC, do Prado C, Silva LG, Paarmann FA, Lima MC, Carvalho I. Immunohistochemical localization of VEGF and its receptors in the corpus luteum of the bitch during diestrus and anestrus. Theriogenology. 2006;66(6-7):1715–20.

43. Papa PC, Sousa LM, Silva RS, de Fatima LA, da Fonseca VU, do Amarala VC. Glucose transporter 1 expression accompanies hypoxia sensing in the cyclic canine corpus luteum. Reproduction. 2014;147(1):81–9.
44. Gram A, Hoffmann B, Boos A, Kowalewski MP. Expression and localization of vascular endothelial growth factor A (VEGFA) and its two receptors (VEGFR1/FLT1 and VEGFR2/FLK1/KDR) in the canine corpus luteum and utero-placental compartments during pregnancy and at normal and induced parturition. Gen Comp Endocrinol. 2015;223:54–65.
45. Yanagisawa M, Masaki T. Endothelin, a novel endothelium-derived peptide. Pharmacological activities, regulation and possible roles in cardiovascular control. Biochem Pharmacol. 1989;38(12):1877–83.
46. Gram A, Latter S, Boos A, Hoffmann B, Kowalewski MP. Expression and functional implications of luteal endothelins in pregnant and non-pregnant dogs. Reproduction. 2015;150(5):405–15.
47. Kowalewski MP, Hoffmann B. Molecular cloning and expression of StAR protein in the canine corpus luteum during dioestrus. Exp Clin Endocrinol Diabetes. 2008;116(3):158–61.
48. Kowalewski MP, Mason JI, Howie AF, Morley SD, Schuler G, Hoffmann B. Characterization of the canine 3beta-hydroxysteroid dehydrogenase and its expression in the corpus luteum during diestrus. J Steroid Biochem Mol Biol. 2006;101(4-5):254–62.
49. Sonnack M. Investigations on the formation, regression and functionality of the corpus luteum in the non pregnant bitch: morphological and biochemical aspects (in German). Germany: Justus-Liebig-University Giessen; 2009.
50. Engel E, Klein R, Baumgartner W, Hoffmann B. Investigations on the expression of cytokines in the canine corpus luteum in relation to dioestrus. Anim Reprod Sci. 2005;87(1-2):163–76.
51. Concannon PW, Weinstein P, Whaley S, Frank D. Suppression of luteal function in dogs by luteinizing hormone antiserum and by bromocriptine. J Reprod Fertil. 1987;81(1):175–80.
52. Concannon P. Effects of hypophysectomy and of LH administration on luteal phase plasma progesterone levels in the beagle bitch. J Reprod Fertil. 1980;58(2):407–10.
53. Okkens AC, Bevers MM, Dieleman SJ, Willemse AH. Evidence for prolactin as the main luteotrophic factor in the cyclic dog. Vet Q. 1990;12(4):193–201.
54. Onclin K, Verstegen JP, Concannon PW. Time-related changes in canine luteal regulation: in vivo effects of LH on progesterone and prolactin during pregnancy. J Reprod Fertil. 2000;118(2):417–24.
55. Okkens AC, Dieleman SJ, Bevers MM, Lubberink AA, Willemse AH. Influence of hypophysectomy on the lifespan of the corpus luteum in the cyclic dog. J Reprod Fertil. 1986;77(1):187–92.
56. Onclin K, Verstegen JP. In vivo investigation of luteal function in dogs: effects of cabergoline, a dopamine agonist, and prolactin on progesterone secretion during mid-pregnancy and -diestrus. Domestic Anim Endocrinol. 1997;14(1):25–38.
57. De Coster R, Beckers JF, Beerens D, De Mey J. A homologous radioimmunoassay for canine prolactin: plasma levels during the reproductive cycle. Acta Endocrinol (Copenh). 1983;103(4):473–8.
58. Graf KJ. Serum oestrogen, progesterone and prolactin concentrations in cyclic, pregnant and lactating beagle dogs. J Reprod Fertil. 1978;52(1):9–14.
59. Onclin K, Verstegen JP. Secretion patterns of plasma prolactin and progesterone in pregnant compared with nonpregnant dioestrous beagle bitches. J Reprod Fertil Suppl. 1997;51:203–8.
60. Hoffmann B, Schneider S. Secretion and release of luteinizing hormone during the luteal phase of the oestrous cycle in the dog. J Reprod Fertil Suppl. 1993;47:85–91.
61. Olson PN, Bowen RA, Behrendt MD, Olson JD, Nett TM. Concentrations of progesterone and luteinizing hormone in the serum of diestrous bitches before and after hysterectomy. Am J Vet Res. 1984;45(1):149–53.
62. Kowalewski MP, Michel E, Gram A, Boos A, Guscetti F, Hoffmann B, et al. Luteal and placental function in the bitch: spatio-temporal changes in prolactin receptor (PRLr) expression at dioestrus, pregnancy and normal and induced parturition. Reprod Biol Endocrinol. 2011;9:109.

63. Bethea CL, Cronin MJ, Haluska GJ, Novy MJ. The effect of relaxin infusion on prolactin and growth hormone secretion in monkeys. J Clin Endocrinol Metab. 1989;69(5):956–62.
64. Li Y, Huang C, Klindt J, Anderson LL. Stimulation of prolactin secretion in the pig: central effects of relaxin and the antiprogesterone RU 486. Endocrinology. 1993;133(3):1205–12.
65. Kowalewski MP, Schuler G, Taubert A, Engel E, Hoffmann B. Expression of cyclooxygenase 1 and 2 in the canine corpus luteum during diestrus. Theriogenology. 2006;66(6-7):1423–30.
66. Kowalewski MP, Mutembei HM, Hoffmann B. Canine prostaglandin E2 synthase (PGES) and its receptors (EP2 and EP4): expression in the corpus luteum during dioestrus. Anim Reprod Sci. 2008;109(1-4):319–29.
67. Janowski T, Fingerhut J, Kowalewski MP, Zdunczyk S, Domoslawska A, Jurczak A, et al. In vivo investigations on luteotropic activity of prostaglandins during early diestrus in nonpregnant bitches. Theriogenology. 2014;82(6):915–20.
68. Gram A, Buchler U, Boos A, Hoffmann B, Kowalewski MP. Biosynthesis and degradation of canine placental prostaglandins: prepartum changes in expression and function of prostaglandin F2alpha-synthase (PGFS, AKR1C3) and 15-hydroxyprostaglandin dehydrogenase (HPGD). Biol Reprod. 2013;89(1):2.
69. Kowalewski MP, Mutembei HM, Hoffmann B. Canine prostaglandin F2alpha receptor (FP) and prostaglandin F2alpha synthase (PGFS): molecular cloning and expression in the corpus luteum. Anim Reprod Sci. 2008;107(1-2):161–75.
70. Romagnoli SE, Camillo F, Novellini S, Johnston SD, Cela M. Luteolytic effects of prostaglandin F2alpha on day 8 to 19 corpora lutea in the bitch. Theriogenology. 1996;45(2):397–403.
71. Romagnoli SE, Cela M, Camillo F. Use of prostaglandin F2 alpha for early pregnancy termination in the mismated bitch. Vet Clin N Am Small Anim Pract. 1991;21(3):487–99.
72. Kowalewski MP, Kautz E, Hogger E, Hoffmann B, Boos A. Interplacental uterine expression of genes involved in prostaglandin synthesis during canine pregnancy and at induced prepartum luteolysis/abortion. Reprod Biol Endocrinol. 2014;12:46.
73. Gram A, Fox B, Buchler U, Boos A, Hoffmann B, Kowalewski MP. Canine placental prostaglandin E2 synthase: expression, localization, and biological functions in providing substrates for prepartum PGF2alpha synthesis. Biol Reprod. 2014;91(6):154.
74. Gram A, Boos A, Kowalewski MP. Uterine and placental expression of canine oxytocin receptor during pregnancy and normal and induced parturition. Reprod Domestic Anim. 2014;49 suppl 2:41–9.
75. Leyva H, Madley T, Stabenfeldt GH. Effect of melatonin on photoperiod responses, ovarian secretion of oestrogen, and coital responses in the domestic cat. J Reprod Fertil Suppl. 1989;39:135–42.
76. Concannon PW, Castracane VD, Temple M, Montanez A. Endocrine control of ovarian function in dogs and other carnivores. Anim Reprod. 2009; 6(1):172–93.
77. Schmidt PM, Chakraborty PK, Wildt DE. Ovarian activity, circulating hormones and sexual behavior in the cat. II. Relationships during pregnancy, parturition, lactation and the postpartum estrus. Biol Reprod. 1983;28(3):657–71.
78. Shille VM, Munro C, Farmer SW, Papkoff H, Stabenfeldt GH. Ovarian and endocrine responses in the cat after coitus. J Reprod Fertil. 1983;69(1):29–39.
79. Concannon P, Hodgson B, Lein D. Reflex LH release in estrous cats following single and multiple copulations. Biol Reprod. 1980;23(1):111–7.
80. Lawler DF, Johnston SD, Hegstad RL, Keltner DG, Owens SF. Ovulation without cervical stimulation in domestic cats. J Reprod Fertil Suppl. 1993;47:57–61.
81. Gudermuth DF, Newton L, Daels P, Concannon P. Incidence of spontaneous ovulation in young, group-housed cats based on serum and faecal concentrations of progesterone. J Reprod Fertil Suppl. 1997;51:177–84.
82. Shille VM, Stabenfeldt GH. Luteal function in the domestic cat during pseudopregnancy and after treatment with prostaglandin F2 alpha. Biol Reprod. 1979;21(5):1217–23.
83. Verhage HG, Beamer NB, Brenner RM. Plasma levels of estradiol and progesterone in the cat during polyestrus, pregnancy and pseudopregnancy. Biol Reprod. 1976;14(5):579–85.
84. Paape SR, Shille VM, Seto H, Stabenfeldt GH. Luteal activity in the pseudopregnant cat. Biol Reprod. 1975;13(4):470–4.

85. Braun BC, Zschockelt L, Dehnhard M, Jewgenow K. Progesterone and estradiol in cat placenta: biosynthesis and tissue concentration. J Steroid Biochem Mol Biol. 2012;132(3-5): 295–302.
86. Tsutsui T, Suzuki Y, Toyonaga M, Oba H, Mizutani T, Hori T. The role of the ovary for the maintenance of pregnancy in cats. Reprod Domestic Anim. 2009;44 suppl 2:120–4.
87. Verstegen JP, Onclin K, Silva LD, Wouters-Ballman P, Delahaut P, Ectors F. Regulation of progesterone during pregnancy in the cat: studies on the roles of corpora lutea, placenta and prolactin secretion. J Reprod Fertil Suppl. 1993;47:165–73.
88. Addiego LA, Tsutsui T, Stewart DR, Stabenfeldt GH. Determination of the source of immunoreactive relaxin in the cat. Biol Reprod. 1987;37(5):1165–9.
89. Stewart DR, Stabenfeldt GH. Relaxin activity in the pregnant cat. Biol Reprod. 1985;32(4):848–54.
90. Klonisch T, Hombach-Klonisch S, Froehlich C, Kauffold J, Steger K, Huppertz B, et al. Nucleic acid sequence of feline preprorelaxin and its localization within the feline placenta. Biol Reprod. 1999;60(2):305–11.
91. Banks DR, Paape SR, Stabenfeldt GH. Prolactin in the cat: I. Pseudopregnancy, pregnancy and lactation. Biol Reprod. 1983;28(4):923–32.
92. Jochle W, Jochle M. Reproduction in a feral cat population and its control with a prolactin inhibitor, cabergoline. J Reprod Fertil Suppl. 1993;47:419–24.
93. Verstegen JP, Onclin K, Silva LD, Donnay I. Abortion induction in the cat using prostaglandin F2 alpha and a new anti-prolactinic agent, cabergoline. J Reprod Fertil Suppl. 1993;47: 411–7.
94. Wildt DE, Chan SY, Seager SW, Chakraborty PK. Ovarian activity, circulating hormones, and sexual behavior in the cat. I. Relationships during the coitus-induced luteal phase and the estrous period without mating. Biol Reprod. 1981;25(1):15–28.
95. Siemieniuch MJ, Jursza E, Szostek AZ, Zschockelt L, Boos A, Kowalewski MP. Placental origin of prostaglandin F2alpha in the domestic cat. Mediators Inflamm. 2014;2014:364787.
96. Dehnhard M, Finkenwirth C, Crosier A, Penfold L, Ringleb J, Jewgenow K. Using PGFM (13,14-dihydro-15-keto-prostaglandin F2alpha) as a non-invasive pregnancy marker for felids. Theriogenology. 2012;77(6):1088–99.
97. Miller DM. Ovarian remnant syndrome in dogs and cats: 46 cases (1988–1992). J Vet Diagn Invest. 1995;7(4):572–4.
98. Zschockelt L, Amelkina O, Siemieniuch MJ, Kowalewski MP, Dehnhard M, Jewgenow K, Braun BC. Reproduction. 2016 Aug;152(2):111-26. doi:10.1530/REP-16-0180.