Endocrinology of pregnancy in the dog: A review

K. Verstegen-Onclin*, J. Verstegen

Large Animal Clinical Sciences, Small Animal Reproduction Center, College of Veterinary Medicine, University of Florida, PO Box 100136, 2015 SW 16th Avenue, Gainesville, FL 32610-0136, United States

Abstract

Pregnancy regulation in the dog is not yet fully elucidated. Since plasma progesterone concentrations are similar in pregnant versus non-pregnant animals, it is a poor reflection on CL function and progesterone metabolism. Increased progesterone secretion by the CL in pregnant animals follows implantation and relaxin secretion by the feto-placental units. Progesterone is absolutely required to maintain pregnancy and no placental sources of progesterone have been identified. Pregnancy can be artificially maintained by progesterone administration. Prolactin secretion appears to be increased in response to the increase in relaxin production and occurs independent of estrogen production by the CL. The respective roles of LH, FSH and prolactin are still unclear, with considerable conflicting evidence among studies. However, it appears that prolactin is absolutely required, whereas LH is either permissive or facilitates CL function during pregnancy. Pre-implantation events are still poorly defined in the bitch, and no embryonic factors have been isolated or purified, preventing early pregnancy diagnosis. Parturition occurs following luteolysis, which results from the release of prostaglandin $F_2\alpha$, which begins 36 h prepartum in a process similar to that observed in other species. The role of estrogens at the time of parturition remains undefined.

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1. Introduction

Veterinary practitioners and specialist veterinarians providing reproductive services for small animals should be aware of the aspects of reproduction that are unique, or nearly unique, to the dog. These reproductive features, in some instances represent challenges not encountered with other species, and in other cases represent unique opportunities that can be clinically useful in the reproductive management of individual animals [1–4]. Endocrinology of pregnancy is among the most specific aspects, which deserves to be further studied and characterized [2,3,5–9].

2. Overview of canine reproduction

Many features of canine pregnancy are unique relative to other domestic animals. They include a delay in oocyte maturation until after ovulation; a delay in implantation of the embryo; a preovulatory rise in serum progesterone associated with the preovulatory luteinization of follicles; an elevation in serum progesterone for intervals as long as, or longer than, the 2 months of normal gestation; a serum progesterone concentration apparently similar in pregnant versus non-pregnant animals (although progesterone metabolism is significantly increased in pregnant animals); and the absence of pregnancy-specific proteins and...
exclusive placental production of relaxin [5,10–13]. There is not only a delay in oocyte maturation until 2–3 d after ovulation, but more uniquely, the canine oocyte may remain viable for an additional 3–5 d, with fertilization occurring as late as 6–8 d after ovulation, or up to 10–11 d after the preovulatory LH surge [2,3]. Thus, hormonal balance is particularly critical for the maintenance of embryo viability.

Other unique features include the occurrence of clinical pseudopregnancy (PSP) in a substantial proportion of ovarian cycles in which bitches are not bred, a high incidence of pyometra associated with the luteal phase of the cycle, and the occurrence of acromegaly and insulin resistance in association with otherwise normal luteal phases in some older intact animals [12,14,15]. Dogs may also be unique in their sensitivity to progesterone, which can elicit nearly maximum mammary as well as uterine hypertrophy and hyperplasia in both the pregnant and nonpregnant state [14,15].

A better understanding of endocrine factors and their modes of action in regulating pregnancy in the bitch will not only allow for better and safer management of pregnancy, but will also be useful for differentiation of pregnancy, pseudopregnancy and uterine diseases, including cystic endometrial hyperplasia (CEH) and the mucometra/pyometra complex [1–5].

3. Comparison of pregnancy versus non-pregnant cycles

Outwardly, there does not appear to be much difference between pregnancy and the non-pregnant cycle of the bitch [2,3,5]. The luteal phases for both last approximately 2 months, with pregnancy having a shorter duration of plasma progesterone elevation and ending with a more abrupt decline of progesterone than the non-pregnant cycle. Both pregnant and non-pregnant bitches may or may not show any physical changes or behavioral signs characteristic of pregnancy. However, even if they appear generally similar, there are hidden clinical features and scientific data that clearly demonstrate many significant differences between pregnant and non-pregnant dogs.

3.1. Progesterone metabolism

There is increased production of progesterone by the CL of pregnancy in comparison with that of the non-pregnant cycle; this is characterized by a significant increase in fecal progesterone metabolites during pregnancy. Plasma progesterone concentrations increase (see below) from the end of pro-estrus to peak approximately 20–30 d after the LH surge, and then slowly decline to basal concentrations by 60–70 d in both pregnant and non-pregnant animals. Although plasma progesterone concentrations remain comparable, evaluation of fecal metabolites of progesterone clearly differentiates pregnancy status. Pregnancy-associated progesterone produced by the CL is significantly higher, but it is rapidly metabolized in the periphery and utilized by the placenta, resulting in similar plasma concentrations of native progesterone in both groups of bitches. Only the amount of the progesterone metabolite in the feces facilitates clear differentiation of pregnant versus non-pregnant animals [10]. It is noteworthy that this difference also occurs in other species of carnivores and is used to monitor pregnancy [16,17].

3.2. Progesterone requirements for pregnancy

Continuous availability of progesterone is required for initiation and maintenance of pregnancy. In dogs, plasma progesterone concentration must be ≥2 ng/mL to maintain pregnancy. Progesterone ensures the differentiation of the endometrium and endometrial gland secretion, maintenance of endometrial integrity, and attachment of the placenta. It also suppresses uterine contractility, particularly, by preventing the uterogenic activity of estrogens, which, decrease at the end of the estrual phase, but then again increase significantly approximately 10–15 d after the LH surge. Estrogens, may play a role in sustaining CL function by stimulating and increasing progesterone secretion and receptors, as observed in rabbits and other species [2,3,6,7,18].

3.3. LH and prolactin

Tonic factors regulating steroidogenesis are of only pituitary origin. No placental or embryonic secretions have been demonstrated in this species [18–21]. Both LH and prolactin have been proposed to be luteotrophic in the dog, whereas prolactin seems to be the main and essential support for the CL [14].

3.3.1. Prolactin

Prolactin (PRL) appears to be the main pituitary hormone sustaining steroidogenesis by the CL. Prolactin concentrations rise in mid-gestation and remain elevated throughout gestation and lactation. However due to individual, diurnal, and/or stress-related variations, serum PRL concentrations are unreliable for pregnancy diagnosis [22–25]. Its removal through the
use of dopamine agonists or other mechanisms which inhibit prolactin secretion leads to functional arrest of the CL and luteolysis, which in turn results in blockage of progesterone secretion and subsequent abortion [22].

3.3.2. LH

The role of pituitary LH is less obvious and to some extent, still controversial. Some studies have shown CL inhibition following administration of GnRH antagonists or after GnRH down-regulation, whereas others have not been able to reproduce these effects [19]. Administration of GnRH antagonists at concentrations high enough to inhibit plasma LH concentrations, or passive immunization against LH through injection of large quantities of anti-LH antibodies, were unable to affect CL function and progesterone secretion [25]. It has been suggested that if LH is required to maintain CL function, its role would be more luteotropic (stimulation of progesterone production by the CL) than luteotrophic (required for CL function) [25]. In later studies, it became evident that although LH was not necessary for pregnancy maintenance, it did stimulate progesterone secretion when injected for a few days into pregnant animals at 30 or 40 d of gestation [7,19,25].

3.4. Relaxin

Circulating concentrations of placental relaxin are elevated from approximately 21–24 d after the LH surge to the end of pregnancy [13]. Serum relaxin concentrations are not detectable at any time in non-pregnant (diestrus) bitches. In pregnant bitches, it reaches peak concentrations (5 ng/mL) in late pregnancy (40–50 d of gestation). Relaxin concentrations decrease after parturition, but remain detectable for at least 30 d during lactation. Relaxin is the only specific pregnancy-associated protein identified in dog. A relaxin enzyme immunoassay is available and can be used for pregnancy diagnosis or to assess viability of fetuses, however there can be false-positive results following abortion [35].

The increase in relaxin is observed concomitantly, or just before the increase in prolactin. The role of relaxin is not yet clear, but it may play a major role in promoting progesterone secretion by the luteal cells of pregnant animals by directly acting at the level of the CL, or acting indirectly through increased prolactin production [26,27].

3.5. Hematologic alterations

A pregnancy-associated anemia occurs and the packed cell volume (PCV) may drop to <30%, reflecting an equivalent increase in circulating volume. The effects of litter size on the extent of anemia are unknown. There are post-implantation increases in acute phase proteins (APP) [28–32]. Mid-gestational increases in circulating concentrations of fibrinogen, C-reactive protein, and haptoglobin have also been reported, but the source has not been identified. Concurrent with this increase in APP, an increase in white blood cells (WBC) is observed, beginning at the time of implantation [33,34]. Elevations in acute phase proteins and alterations in WBC during pregnancy probably represent non-specific inflammatory-like responses to the presence of the fetal-placental unit, which is recognized as containing foreign protein [31,32,35]. These changes can be depicted from approximately Days 25 to 30 up to Days 45 to 55 of pregnancy. False positives (any inflammatory disease) are observed, as well as false negatives (too early sampling). Therefore, they are not reliable parameters to predict pregnancy, although there are commercial diagnostic assays in some countries [36,37].

3.6. LH pulse pattern

A differential pattern in LH pulsatility and secretion is observed between pregnant and non-pregnant animals. As presented earlier, the role of LH during the luteal phase of the dog is still controversial. Concannon [19] postulated that LH is a main luteotrophic factor in the bitch, whereas Okkens et al. [23] maintains that prolactin is the primary luteotrophic factor. Our previously published observations that concomitant administration of a prolactin inhibitor and LH did not sustain CL function are in agreement with previous studies on hypophysectomized ferrets or bitches, suggesting that LH is not essential for the morphological or functional integrity of the CL and that prolactin alone induces or maintains progesterone secretion. However, more recent studies using contraceptive GnRH agonist implants demonstrate that the induced estrus, when followed by mating, does not result in pregnancy, thereby raising doubts regarding previous observations. The prevention of pregnancy may be due to the GnRH agonist implant continuous down-regulation of GnRH receptors and subsequent inhibition of LH release and LH receptor synthesis at the level of the ovary, or it may be due to other mechanisms, including interference with prolactin secretion and PRL receptor expression at the level of the luteal cells, with receptors normally induced by LH [38–41]. These different hypotheses all lead to a loss of the enzymatic cascades necessary to produce
progesterone concentrations that are required for the maintenance of pregnancy. Relationships among GnRH, LH, and prolactin have been poorly studied during the early luteal phase in dogs and further investigations are warranted.

3.7. Estradiol 17β and CL function

The role of estradiol 17β in the regulation of the canine CL has not been clearly identified. That estradiol 17β increases from Days 10 to 64 of the luteal phase was reported by Steinetz et al. [27] and Onclin and Verstegen [21]. Elevated concentrations of total canine estrogens were reported by Hadley [42], whereas Concannon et al. [43] detected elevated concentrations in pregnancy, but not during the non-pregnant luteal phase. Others did not detect any significant changes [8,44]. The origin of the observed increase in estradiol concentration is unknown, but it probably originates from the CL. Indeed, in species such as rats and rabbits [45–47], estradiol is secreted by the luteal cells and plays a role in luteal regulation. In association with prolactin, estradiol is known to sustain luteal function during LH-dependant stages of pregnancy in these species and to stimulate progesterone secretion in pigs [48]. It remains possible that estradiol forms, in association with prolactin and LH, part of the luteotrophic complex in dogs as in other species [47,49].

Luteal regulation in the diestrous bitch appears to be a complex and dynamic process in which several complementary hormones interplay, the most classical being progesterone and prolactin, with LH, estradiol and relaxin playing either a specific direct role or being fundamental components of this luteotrophic complex. However, when native progesterone alone was provided by injection and subcutaneous implants before and after ovariectomy performed before implantation, implantation occurred normally, and pregnancy was maintained to term. The increase in prolactin was not different from that of control pregnancies, despite the absence of effective systemic concentrations of estrogen, even in the face of a typical castration-response in LH and FSH [11]. Therefore, the pregnancy-associated increase in prolactin concentrations does not require an increase in or the presence of maternal estrogen [11].

The endocrine regulation of the diestrous CL in dogs, as in other species, appears to be more complicated than originally thought, and still remains to be completely elucidated. In addition to endocrine factors, which are currently being analyzed, the roles of the autocrine and/or paracrine parameters regulating the CL locally remain unknown.

3.8. Lack of placental gonadotropin

No specific gonadotrophins of placental origin have been identified and purified in the bitch [50–52] and there is no evidence to support placental progesterone secretion [20]. Furthermore, ovariectomy at any time in pregnancy results in abortion.

3.9. Insulin resistance

Pregnancy may cause resistance to insulin exacerbating diabetes in bitches with a pre-existing condition, or inducing mild insulin resistance in some normal bitches [14,15,53]. This increase in insulin resistance is associated with an increased production of growth hormone (GH), leading to subclinical or clinical hyperglycemia. The GH increase in relation to the increase in progesterone in both pregnant and non-pregnant animals appears to be secondary to mammary gland GH de novo synthesis. It has been clearly demonstrated that during the luteal phase of the non-pregnant cycle, especially in older bitches, progesterone can stimulate GH hypersecretion [14,15,54,55]. In healthy cyclic non-pregnant bitches, the pulsatile secretion pattern of GH changes during the progression of the luteal phase, with basal GH secretion being higher and pulsatile GH secretion being lower when plasma progesterone concentration is high. This may be explained by partial suppression of pituitary GH release by progesterone-induced GH production from the mammary gland [14,15,49,50]. Similarly, exogenous progesterone administration in the bitch (for estrus suppression or contraception) has been frequently reported to produce similar effects, which can result in acromegaly and diabetes mellitus.

3.10. Induction of parturition by prostaglandin synthesis

At the end of pregnancy, endogenous prostaglandins increase and are luteolytic, just as exogenous prostaglandins used to induce abortion or whelping (see above). Exogenous prostaglandin F₂α (PGF₂α), depending on the type and dose given and the age of the CL, can directly cause CL regression.

3.11. Cortisol production in late pregnancy

Cortisol concentrations rise, reaching a plateau a few days prepartum, and subsequently decrease postpartum. Corticosteroid administration in late gestation can induce abortion [2,3,7,56,57].
4. Pregnancy: reproductive hormones and clinical observations

Pregnancy in the bitch can be divided into three periods: (1) the period of fertilization and the early embryo encompass the time from fertilization to approximately Days 20 to 22 post LH surge when implantation occurs; (2) the period of the late embryo is from implantation to fetal calcification/ossification at 40 to 42 d after the LH surge, and corresponds mainly to the start of fetal development and organogenesis; and (3) the period of the fetus is from fetal calcification/ossification to parturition.

4.1. Pre-implantation events: period of fertilization and the early embryo

During the first third of gestation, pregnancy diagnosis is difficult to perform. This period is characterized by an apparent refractoriness of the CL to exogenous treatment or exogenous support. This refractoriness may be due to relative autonomy of the CL or to the existence of a complex interplay of regulatory elements, complementary or compensatory, which regulate function of the CL, thereby allowing resistance to the withdrawal of one or the other of its supports.

The initial part of this period is characterized by delayed oocyte maturation. Maturation is estimated to occur 60 h after the preovulatory LH surge; therefore, it can occur any time between late proestrus to mid-estrus, depending on when the behavioral shift occurs in the individual cycle. The timing of ovulation can be estimated by monitoring vulvar swelling, vaginal cytology and through the observation of changes in vaginal mucosal morphology, shape and color with vaginoscopy [58–66]. Accuracy is increased by monitoring blood progesterone concentrations every 1 to 3 d. In the dog, unlike many species, luteinization occurs before ovulation. Initially there is a minor but morphologically and endocrinologically detectable luteinization in small patches on the interior wall of individual follicles during mid- to late-proestrus (during the 2–3 d prior to the preovulatory LH surge); this increases serum progesterone concentrations from non-detectable to 0.4 to 0.8 ng/mL. Next, pre-ovulatory luteinization occurs throughout and following the LH surge. There is a rapid and generalized proliferation of luteinizing cells throughout the periphery of the preovulatory follicles; this is accompanied by a rise in serum progesterone concentrations to 1.0–3.0 ng/mL at the time of the LH surge and from 4 to 8 ng/mL at the time of ovulation in the “average” dog. This rapid rise in progesterone can be used during breeding management to estimate the occurrence of the LH surge, determine the time of ovulation and to define the optimal window for fertilization [1].

The abrupt progesterone increase at the beginning of this luteinization process is essential for induction of the LH surge. Irregularities in the LH surge and ovulation can occur as a result of abnormal early luteinization; this can result in clinical conditions such as false estrus, anovulation, partial ovulation and/or prolonged proestrus, and infertility. These conditions are relatively common in some lines or in older bitches.

The “normal” bitch ovulates primary oocytes approximately 2 d after the LH surge (Day 2) which then require, in the “average” bitch, another 2 to 3 d to reach final maturation and formation of the secondary oocyte (Days 4 to 5).

Morulae develop in the distal oviduct into blastocysts, which enter the uterus approximately Days 10 to 12 after the LH surge. The embryos will remain free within the uterine lumen, floating, and transmigrating within the uterine horns, and absorbing nutrients from “uterine milk” produced by the early developing endometrial glands, under the influence of increasing progesterone concentrations from the developing CL. During this period, the free-floating embryos are exceptionally sensitive to all treatments and to modifications of uterine milk, potentially leading to infertility [11].

Under mechanisms not yet completely understood, blastocysts evenly attach to the endometrium at approximately Days 18 to 20 after the LH surge, with final implantation (invasion of the endometrium by the fetal trophoblast) occurring approximately Days 20 to 22 after the LH surge. Uterine swellings at the sites of implantation are noted from as early as Day 14 in some bitches, but in most bitches it is ~Days 16 to 20. Histological observation of localized inflammatory reactions (edema, neutrophils and lymphocytes infiltration) with congestion and hyper-vascularization, are noted as early as Days 10 to 14 at various locations of the uterine horns and body, which are possible sites for future implantation (Verstegen, unpublished results). The observation of localized reactions seems to indicate the presence of some form of communication between the uterus and the embryos (although no embryonic signals for maternal recognition have been demonstrated in the dog).

4.2. Post-implantation events: periods of the late embryo and fetus

The endotheliochorial, zonary (circumferential), modified deciduate placenta develops at approximately
Days 20 to 22. Marginal hematomas are observed at the edges of the zonary band, and are formed from maternal blood (vascular lakes) where hemoglobin is metabolized into uteroverdin. This pigment has a black–green color, and is characteristic of the carnivore placenta. The embryo does obtain some metabolites from these areas, notably iron [62].

The prolonged duration of the luteal phase and associated elevation in serum progesterone is similar in non-pregnant and pregnant bitches. Thus, progesterone assays cannot ever be used to detect pregnancy. However, evaluation of progesterone metabolites in the feces can be used to diagnose pregnancy after Days 25 to 30 [10,37].

In the dog, the ovaries are essential throughout pregnancy; ovariectomy or corpora luteal excision is associated with rapid abortion. The luteal cells are dependent on “luteotropins” which are either endocrine, paracrine, or autocrine in origin. In bitches, the two main luteotropic hormones are LH and prolactin. Hypophysectomy induces immediate abortion in pregnant animals and a dramatic decrease in plasma progesterone concentration in the non-pregnant animal [22]. However, before Day 10 of pregnancy, hypophysectomy is unable to induce a functional arrest of the CL. Similarly, luteolytic drugs (dopamine agonists, GnrH antagonists or prostandolins) are unable to induce functional arrest of the CL and luteolysis during the same period. The reasons for this relative and apparent independence of the CL during this period remain unknown. This appears to be independent of LH or prolactin receptor variations, as Fernandes et al. [63] reported a constant expression of both receptors during luteal phases in non-pregnant animals.

Progesterone is absolutely required to maintain pregnancy, with concentrations ~2 ng/mL considered as the minimum. There is no required maximum, and progesterone reaches a plateau of approximately 15–90 ng/mL between Days 20 to 30 of diestrus and undergoes a continuous slow decline thereafter (the range and variation in concentrations is considerable). During the gradual decrease in plasma progesterone concentrations, the progesterone pattern appears unrelated to changes in either estradiol or prostaglandin F metabolite (PGFM) concentrations. The PGFM pattern is significantly different between diestrous and pregnant bitches. There is an apparent progressive but slow increase in PGFM in pregnant bitches from Days 30 to 60, followed by a large increase prior to parturition, and then concentrations decline immediately postpartum. However, there were no increases in PGFM during the same interval in non-pregnant bitches [64].

Low progesterone concentrations can occur during pregnancy, particularly at Days 30 to 35, and may be the result of the increased extraction of progesterone from the plasma by the gravid uterus, increased metabolism, or as a result of defective ovaries with production of insufficient progesterone concentrations (hypoluteoidism) [65,66]. Enhanced progesterone secretion by the corpora lutea during pregnancy may be impaired in some dogs. The origin of this impairment is not fully understood, but may be mediated by abnormal placental secretion of relaxin, insufficient prolactin secretion (that normally starts increasing by Days 27 to 30) or other unidentified causes (including infectious diseases such as herpes virus) [65–67]. Serum progesterone should be monitored every 1 to 2 wk in bitches with a history of pregnancy failure or luteal insufficiency.

More than 20–22 d after the LH surge, pregnancy diagnosis can be done [37]. The CL becomes sensitive to treatments or any factors interfering directly or indirectly with its function. The fetus is still exceptionally sensitive to everything, including teratogens or drugs that can impact either its development or uterine function. Abortion can easily be induced or observed during this period of pregnancy, with resorption of fetuses if death occurs early, or expulsion of fetuses when abortion occurs after ossification/calcification (Onclin, unpublished). Resorption is not generally detected clinically, but can be detected with ultrasonography. During the period of the embryo, organogenesis is at its most critical state in terms of sensitivity of the fetus to external agents, and no drugs should be administered to the bitch during this period.

During the period of the fetus, the fetuses are well-developed, ossified, and their bodyweights increase dramatically and exponentially. The CL are extremely dependent on pituitary luteotropic support; any changes in prolactin, LH or hormonal equilibrium can lead to dramatic loss of CL function and pregnancy failure.

5. Parturition

Normal parturition occurs as a result of a rapid decline in progesterone from the 4 to 10 ng/mL in the preceding days, to <2 ng/mL over a 12–24-h interval, beginning 1–2 d before whelping. The decline in progesterone occurs in response to an increase in prostaglandin to luteolytic concentrations, which in turn occurs in response to maturation of the fetal pituitary-adrenal axis and elevation in fetal glucocorticoid concentrations [4,68]. Prostaglandin F₂α [57,68–70], together with endocrine and paracrine actions of
oxytocin [71–75], are important for stimulating uterine contractility, softening of the cervix and relaxation of the birth canal. A marked elevation of plasma vasopressin concentration is also observed during the expulsion of the first few puppies, followed by a decline before the end of labor [56]; this was associated with stress and pain [76]. Monitoring and/or examining for the decline in progesterone can be an important tool in managing bitches with an apparent or presumed prolonged gestation, in evaluating bitches presented for dystocia, and in timing elective C-sections, especially in bitches in which the day of ovulation or day of the preovulatory LH surge was not estimated with accuracy. The prepartum decline in progesterone is presumably the cause of the acute rise in prolactin immediately prior to whelping. The decline in progesterone also removes the suppressive effects of progesterone on lactation and thus initiates milk production. Clinically, body temperature declines by 1 °C or more during the 12 to 24 h prepartum interval, occurring 12–18 h after the abrupt decline of progesterone.

6. Conclusions

Pregnancy regulation and endocrine control of pregnancy in the dog is slowly being elucidated. However many questions still remain and warrant further studies; these include the respective role of FSH, LH and prolactin during pregnant versus non-pregnant luteal phases, the roles of relaxin in controlling prolactin and CL function, and the presence of embryonic factors regulating pre-implantation events and CL function. Answers to these questions should allow us to improve the way we manage pregnancy in the dog, diagnose pregnancy, and facilitate maintenance of pregnancy in infertile animals.

References


