Introduction

In dogs, corpora lutea are the primary source of progesterone during pregnancy, as the placenta does not secrete progesterone (Kiso and Yamauchi, 1984). The ovaries are required for maintenance of pregnancy throughout gestation (Sokolowski, 1971; Tsutsui, 1983). In both pregnant and non-pregnant dogs, mechanisms regulating corpus luteum function remain unclear. Previous reports indicated that both LH and prolactin are luteotrophic hormones (Concannon et al., 1987), and that the uterus is not involved in regulating the lifespan of the canine corpus luteum (Okkens et al., 1985; Hoffmann et al., 1992).

The respective roles of LH and prolactin remain uncertain. In rats, luteotrophic requirements change during pregnancy (Morishige and Rothchild, 1974); prolactin and oestradiol constitute the luteotrophic complex and LH is needed for oestradiol production (Murphy and Silavin, 1989; Gibori, 1993). In ferrets, prolactin (but not LH) has an acute dose-dependent effect on progesterone secretion (McKibbin et al., 1984), but LH is required for luteal maintenance in pseudopregnancy (Agu et al., 1986). In rats, prolactin appears to sustain and increase the number of luteal LH receptors, and subsequently promotes progesterone secretion either directly or indirectly (Grinwich et al., 1976; Holt et al., 1976; Casper and Erickson, 1981; Segaloff et al., 1990). Lam and Rothchild (1977) reported that prolactin was a prerequisite for LH to play a luteotrophic role. More recently, the already complex role of LH as a luteotrophin during the luteal phase has been questioned further. Studies in cattle (Peters et al., 1994), pigs (Buhr, 1987), rabbits (Townson et al., 1995) and to some extent, sheep (McNeilly and Fraser, 1987) provide evidence that LH may not be essential for all aspects of luteal function.

In dogs, luteal progesterone secretion in both pregnant and non-pregnant bitches appears to be dependent on both LH and prolactin throughout the luteal phase (Concannon et al., 1987). The role of LH in luteal function in pregnant dogs was investigated at two different periods during pregnancy: (i) the transitional period from apparent total independence of the corpus luteum to relative hormonal dependence (days 20–35); and (ii) the period of full hormonal dependence (days 35–40). At both periods, LH neutralization, LH inhibition and LH administration studies were conducted. At both periods LH immunoneutralization had no significant effect on the secretion pattern of progesterone or prolactin. GnRH antagonist treatment (Nal-Glu) decreased plasma LH below the detection limit in all treatment periods. Nal-Glu had no effect on prolactin. When GnRH antagonist osmotic pumps were implanted, a transient decrease in plasma progesterone concentrations occurred on days 21–22 but not during the remaining implantation period. When GnRH antagonist was injected, plasma progesterone temporarily decreased (24 h) after the beginning of treatment starting on day 20, but decreased for 5 days when the treatment started on day 35. When purified pig LH was injected i.v. twice a day for 2 consecutive days either from day 30 or from day 40, plasma progesterone concentrations remained constant during treatment. However, on days 40 and 41, an increase in prolactin was observed. These results indicate that LH immunoneutralization may not impair corpus luteum function. In addition, GnRH antagonist induces dose- and time-dependent effects. Only high doses resulted in a decrease in progesterone, the duration of which increased as pregnancy progressed. Continuous GnRH antagonist administration, even when associated with complete LH inhibition, was not associated with detectable effects on progesterone. Finally, LH administration does not stimulate progesterone but may modify prolactin in the last third of pregnancy. Other studies indicated a corpus luteum prolactin dependency. The present study indicates that, in pregnant bitches, LH may not be necessary to sustain progesterone synthesis but that its role may vary in a time-dependent manner.

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Progesterone secretion is inhibited by prolactin secretion by dopamine agonists (Conley and Evans, 1984; Concannon et al., 1987; Post et al., 1988; Oncin et al., 1993) or LH passive immunization (Concannon et al., 1987). LH suppression by the GnRH antagonist Detirelix was sufficiently luteolytic to terminate pregnancy when administered at high doses to late pregnant bitches (Vickery et al., 1989). However, the essential role of LH has been brought into question by studies in which LH inhibition had no effect on plasma progesterone concentrations whereas prolactin inhibition caused an abrupt fall in plasma progesterone, indicating that only prolactin is luteotropic in cyclic dogs (Ockens et al., 1990). Studies using dopamine agonist or pure canine prolactin administration confirmed the luteotropic importance of prolactin (Conley and Evans, 1984; Post et al., 1988; Oncin et al., 1993; Oncin and Verstegen, 1997a). On the basis of these results and others (Conley and Evans, 1984; Concannon et al., 1987; Post et al., 1988; Ockens et al., 1990), it appears that prolactin is an essential luteotrophin in pregnancy, particularly after days 25–30. This corresponds to the situation in mink and in ferrets in which similar results were obtained with animals treated with bromocriptine (Donovan, 1967; Murphy, 1979; Murphy et al., 1981). However, prolactin in dogs appears as a ‘permissive’ but not stimulating luteotrophin, as proposed by Rothchild (1981) in other species, as no direct stimulatory effect of prolactin on progesterone production was observed in vivo (Oncin and Verstegen, 1997a).

These observations indicate that the role of LH in regulating luteal function in canine pregnancy warrants additional investigation. Therefore, the present study was performed to examine the importance of LH in the maintenance of corpus luteum function using a controlled experimental approach involving immunological LH neutralization, pharmacological blockage of LH release and LH administration. The studies were designed to provide the first information on the time-related changes (approximate periods days 20–30 and 40) in the luteotropic role of LH during pregnancy in dogs. LH neutralization as well as inhibition of LH release were conducted to determine whether significant plasma LH concentrations are essential for corpus luteum maintenance and function, whereas LH administration was carried out to detect a possible direct or indirect luteotropic effect for this hormone, which may be responsible for the pregnancy related increase in progesterone secretion (Gudermuth et al., 1998).

Materials and Methods

Animals, housing and collection of blood samples

Forty mature Beagle bitches aged from 2 to 8 years (mean ± SD: 4.6 ± 2.0) and weighing 10 ± 2 kg were used in this study. All animals were born and housed at the animal facilities of the Small Animal Reproduction Department at the University of Liege. Animal housing, care and experimentation were in accordance with Belgian regulations and with the NIH Guide for the Care and Use of Laboratory Animals. Routine veterinary examinations were carried out each week as well as before and during treatment. Bitches were housed in groups of two to five in indoor–outdoor runs (2.5 m × 10 m) and exposed to natural lighting. The animals were fed a commercial dry canine diet (Canine Maintenance Hill’s Science Plan, veterinary formulated) once a day in sufficient amounts to maintain body weight and were given water ad libitum. For each bitch, the oestrous cycle under consideration as well as the previous cycle was characterized in terms of vaginal cytology, plasma progesterone and LH concentrations during prooestrus, oestrous, and early metaoestrus.

All dogs were examined twice a week for the presence of vulvar swelling and serosanguineous vaginal discharges as indicators of the onset of prooestrus. Day 0 of the cycle was defined as the day of the pre-ovulatory LH surge, measured by an heterologous radioimmunoassay (De Coster et al., 1979). Bitches were either mated naturally on the first day of acceptance of the male and every day thereafter until refusal, or inseminated with fresh or frozen semen on days 3 and 5 after the LH peak. Both natural and artificial service was used for purposes of efficient management. The insemination method had no effect on pregnancy rate or luteal phase progesterone concentration, and was not considered in the experimental design. Pregnancy was diagnosed and monitored by ultrasonography (model EUB-415, 7.5 MHz linear transducer; Hitachi Medical Corporation, Tokyo) every 3–4 days from day 20 until parturition as described by Verstegen et al. (1993). Fifty pregnancies were studied; none of the bitches used in the anti-LH or LH administration studies were used more than once.

Blood samples (5 ml) were collected by jugular venepuncture into heparinized tubes, centrifuged at 3000 g for 15 min at 4°C within 30 min of collection, and multiple plasma aliquots (500 µl) were stored at –20°C until assayed. Blood samples were obtained every day from the onset of prooestrus until cytological metaoestrus, then twice a week until the experiments began, after which they were collected as described below. No change in the mean cell volume due to frequent collection of blood samples was observed (data not shown).

Immunoassays

Plasma progesterone concentrations were measured by radioimmunoassay using a commercial kit (Progesterone Coat-a-Count, Diagnostic Products Corporation, Humbeek-Grimbergen), previously validated for canine plasma (Srikandakumar et al., 1986). The sensitivity at 95% binding was 0.1 ng ml⁻¹ and the intra- and interassay coefficients of variation were 5.1% and 8.8%, respectively.

Plasma prolactin concentrations were measured using a commercial canine prolactin immuno-enzymatic assay (Milenia canine Prolactin assay, Diagnostic Products Corporation, Humbeek-Grimbergen), as described by the manufacturer and validated for canine plasma (Oncin et al., 1995). The sensitivity at 95% binding was 0.20 ng ml⁻¹. The intra-assay coefficient of variation was 3.8% for three pools in
Experiment 1: immunoneutralization of circulating LH

The effect of treatment with an immune serum against ovine LH (oLH) on LH, progesterone and prolactin concentrations was evaluated in two groups of five pregnant bitches and compared with a control group of five animals treated with saline. The rabbit anti-oLH serum used was that described above for the radioimmunoassay. The dosage used in the day 30 treatment group was determined on the basis of the circulating LH concentrations during oestrus in bitches (Concannon et al., 1975; Jeffcoate and Lindsay, 1989) and on the study of Malecki et al. (1986) indicating that one i.v. injection of anti-oLH serum can immunoneutralize circulating LH for at least 12 h and that some binding activity is still detectable after 5 days. Injections (i.v.) of 0.5 ml crude undiluted anti-oLH serum were administered twice a day from day 30 to day 34 after the LH surge. In the day 40 treatment group, one i.m. injection of 10 ml of the rabbit anti-oLH serum was administered on day 40 after the LH surge. In the control group, five pregnant animals were injected with physiological saline from day 30 to day 34 and on day 40 using the same protocols. Blood samples were collected from each bitch once a day for 5 days before treatment, twice a day for 5 days and finally once a day for 5 days.

An immunoaffinity study was carried out on the samples collected once a day from animals in the day 30 treatment group to demonstrate canine LH (cLH) immunoneutralization by the injected anti-oLH serum. After column conditioning according to the manufacturer’s instructions, 0.5 ml plasma followed by 0.8 ml PBS was injected on the immunoaffinity column (Hitrap protein A 1 ml, Pharmacia Biotech International, Roosendaal). The free and cLH-bound antibodies were trapped by the protein A, whereas the plasma fraction with free cLH passed freely through the matrix and was collected as fractions A. The columns were washed with 2 ml PBS and the protein A-bound antibodies were eluted with 2 ml of 0.1 mol citric acid l⁻¹ and collected as fractions B. The fractions A were assayed directly for LH by radioimmunoassay to detect free cLH. The fractions B were assayed with iodinated bLH to evaluate their antibody content. The ability of the injected rabbit anti-oLH serum to neutralize circulating cLH was expected since this antibody was used in a cLH radioimmunoassay (De Coster et al., 1979) and since various anti-LH sera from different mammalian species neutralized LH in several species (Morishige and Rothchild, 1974; Greenwald and Terranova, 1981; Concannon et al., 1987).

Experiment 2: inhibition of LH secretion

The effects on progesterone, LH and prolactin concentrations of constant administration of a GnRH antagonist to pregnant bitches (n = 5) from day 20 to day 40 after the LH surge were investigated and compared with a control group given saline injections (n = 5). From day 20 to day 40 after the LH surge, osmotic pumps (osmotic pump, model 2ml4, Alza Corporation, Palo Alto) delivering 480 μg Nal-Glu (Salk Institute under Contract N01-HD-0-2906 with NIH) per day in physiological saline were implanted s.c. In addition, on the day of implantation, 10 μg Nal-Glu kg⁻¹ was injected i.m. to obtain an immediate effect of the GnRH antagonist. The control bitches were injected with physiological saline but were not sham-implanted with saline pumps. Blood samples were obtained once a day for 2 days before treatment, twice a day from day 20 to day 40, and once a day until day 50 after the LH surge. In addition, a group of five pregnant bitches was given 50 μg Nal-Glu kg⁻¹ i.v. twice a day from day 20 to day 24 and from day 35 to day 39 to compare with the beginning and the end of the pump implantation period and test for a possible effect of the mode of administration. Blood samples were obtained once a day for 2 days before treatment, between the two periods of treatment and for 10 days after the end of the second treatment, and were taken twice a day from day 20 to day 24, and from day 35 to day 39, that is during the treatment.

Experiment 3: effects of LH administration on progesterone production

Pregnant bitches were administered twice a day with i.v. injections of 750 μg pure pig LH (pLH: Closset et al., 1972; Ectors et al., 1974) in physiological saline for 2 days beginning on day 30 (n = 5) or day 40 (n = 5) and the effects on progesterone, LH and prolactin secretion were evaluated. Control bitches (n = 5) were treated during the same periods with twice daily i.v. injections of 0.5 ml physiological saline. Blood samples were taken once a day for 2 days before treatment, twice a day for 5 days, and once day for 5 days in all groups.
Statistical analysis

Results are reported as means ± SEM and calculated and analysed using Statview 4.02 software (Abacus Concepts Inc., Berkeley, CA). Repeated measures ANOVA were used to compare changes in plasma hormone concentrations within and between groups. When a significant treatment effect was identified, an ANOVA test was used to determine, between groups, the specific time at which the changes took place. Paired Student’s t tests were used to evaluate mean hormone concentrations at specific time points within a group. Results were considered significant when $P \leq 0.05$ (Lentner, 1982).

Results

Experiment 1: effects of LH immunoneutralization

Immuo-affinity analysis showed that free immunoreactive LH was detectable in plasma collected before treatment but was not detectable in plasma collected 24 h after the beginning of the antiseraum treatment to day 41 (Fig. 1). LH concentration decreased from a mean of 1.8 ± 0.2 ng ml$^{-1}$ before treatment to below the detection limit of the assay (0.5 ng ml$^{-1}$ at 90% binding) during and after treatment. Plasma LH concentrations began to increase from day 42 and reached 7.3 ± 3.0 ng ml$^{-1}$ on days 48–50. Iodine bound to anti-LH antibodies was detected in the eluted plasma fraction from day 31 to day 42; the peak occurred on days 33–34 (Fig. 1). Iodinated LH was not found in the fractions B before day 31 and was not detected after day 44. Maximum passive immunization against cLH was considered to be from day 31 to day 40 after the LH surge in all animals treated on days 30–34. Plasma progesterone concentrations were never significantly different from those of the control group in bitches treated on days 30–34 or in bitches treated on day 40 (Fig. 2). Plasma prolactin concentrations in treated and control animals were not statistically different (data not shown).

Experiment 2: inhibition of LH secretion by constant administration of GnRH antagonist

Constant Nal-Glu administration. Plasma LH concentrations varied markedly before antagonist treatment in both treated and control groups. The concentrations were generally low, in the order of 1.5 ng ml$^{-1}$ (1.4 ± 0.6), and did not differ between groups at day 20. LH decreased to concentrations below the detection limit of the assay in Nal-Glu-treated bitches throughout the period of infusion, from the evening of day 20 to the evening of day 40. During that period, the difference between treated and control animals was highly significant ($P < 0.0001$, Fig. 3). On days 21–22, a significant

Fig. 1. Mean (± SEM) LH concentrations (ng ml$^{-1}$) in the unbound A fractions (C) and binding of $^{125}$I-labelled bovine LH (c.p.m.) in the eluted B fractions (■) for the immunoaffinity column study of plasma samples from bitches ($n = 5$) treated from day 30 to day 34 after the LH surge with injections of 0.5 ml anti-oLH serum twice a day. Data are presented in relation to the days after the pre-ovulatory LH surge. The solid bar corresponds to the period of anti-LH treatment.

Fig. 2. Mean (± SEM) plasma progesterone concentrations in pregnant bitches treated with saline (●) or with a rabbit anti-ovine LH serum (□) from (a) day 30 to day 34 after the LH peak ($n = 5$) or (b) on day 40 after the LH peak ($n = 5$). The solid bar represents the duration of the 0.5 ml anti-ovine LH treatment given twice a day and the arrow corresponds to the single injection of 10 ml anti-ovine LH serum in the group treated on day 40.

Fig. 3. Mean (± SEM) plasma LH concentrations in groups of five pregnant bitches treated with saline (●) or with the GnRH antagonist Nal-Glu (□) by constant s.c. administration via an osmotic pump from day 20 to day 40 (solid bar). The arrow indicates the time of the i.m. injection of 10 μg Nal-Glu kg$^{-1}$. 
decrease ($P < 0.03$) in plasma progesterone was observed in the Nal-Glu-treated group (Fig. 4). This decrease was transient and had no observable effect on pregnancy. Thereafter, plasma progesterone concentrations were similar in treated and control animals. Plasma prolactin patterns for Nal-Glu-treated and control animals were similar throughout the observation period (data not shown).

Administration of Nal-Glu twice a day. As in Expt 2, LH decreased to concentrations below the detection limit of the assay in Nal-Glu-treated bitches throughout the period of treatment and during that period, the difference between treated and control animals was highly significant ($P < 0.0001$, data not shown). In the bitches injected twice a day with Nal-Glu at the moderate dose of 50 mg kg$^{-1}$, a decrease in plasma progesterone ($P < 0.03$) lasting 24 h occurred near the beginning of treatment at day 20, whereas a significant decrease ($P < 0.05$) was observed throughout treatment beginning at day 35 (Fig. 5). In both groups, LH concentrations were significantly suppressed during the days of antagonist injection (data not shown). Although significant differences in plasma LH concentrations were not observed among the animals of the same group, in one of the five bitches treated on days 35–40, plasma progesterone concentrations decreased below 2 ng ml$^{-1}$ after 3 days of treatment and the bitch aborted without complications. Thereafter, plasma progesterone concentrations remained at basal values.

Experiment 3: effects of intravenous administration of pLH

In pregnant bitches injected twice a day with pLH, a significant and expected increase in plasma LH was observed during treatment ($P < 0.001$) in both periods studies (Fig 6). Before and after treatment, plasma LH concentrations remained similar in both LH-treated and control animals (Fig. 6). Plasma progesterone concentrations were not modified by the LH treatments and remained similar in both treated and control animals (data not shown). In bitches treated with LH from day 30, plasma prolactin concentrations remained similar to those of the control group throughout the observation period. In bitches treated with LH from day 40, prolactin concentrations were 57.4% higher ($P < 0.05$) than those of the control group (Fig. 7).

Discussion

The principal aim of this study was to use controlled standardized experimental procedures to clarify the time-related changes in the luteotrophic function of LH during pregnancy. Immunoneutralization of LH, inhibition of LH release and LH administration were used to manipulate endogenous LH concentrations at different stages of pregnancy in bitches, and the effects on progesterone, LH, prolactin and pregnancy outcome were examined.

During the transitional period (approximately days 25–30), neither LH immunoneutralization by anti-LH serum nor
constant LH suppression by GnRH antagonist administration caused any consistent changes in plasma progesterone, indicating that normal plasma concentrations of LH are not required for luteal support in the first half of pregnancy. The results showed that both treatments were effective in reducing circulating LH concentrations below the minimum detectable values. However, immunoneutralization does not impede the potential ability of antibody-bound LH to bind to LH receptors and retain biological activity. The finding that the long-term moderate dose of Nal-Glu did not have any effect on progesterone (despite the marked reduction in plasma LH concentrations) indicates that any luteotropic requirement for LH involves luteal sensitivity to LH concentrations below the low detection limit of the assay used in this study (0.5 ng ml⁻¹). Similarly, administration of higher doses of GnRH agonists to suppress circulating LH at the mid-luteal phase in three dogs had no effect on plasma progesterone (Okkens et al., 1990). In the present study, a significant but transient decrease in progesterone was observed immediately after injection of the antagonist Nal-Glu (10 μg kg⁻¹) on day 20 at the start of the infusion, and a similar decrease was detected during administration of higher doses (100 μg kg⁻¹) twice a day from day 20 to day 24. These observations are in agreement with the observation that another GnRH antagonist Detirelix caused only a transient decrease in progesterone in early pregnant bitches at doses that were fully luteolytic and abortifacient at, or after, day 30 (Vickery et al., 1988, 1989). The differences observed between continuous infusion of Nal-Glu and the injection of additional Nal-Glu might be related to the dose or to the mode of treatment. The circulating concentrations of drug achieved by injection were greater than those achieved by infusion. In humans, constant administration of one submaximal suppressive dose of Nal-Glu was less effective than the same dose administered in a pulsatile manner (Salameh et al., 1994). This effect may be due to downregulation of the receptors, but in the present study an initial effect of greater duration would have been expected before desensitization. The observation that large doses of Nal-Glu are needed to induce an effect on plasma progesterone and, as shown in the present study and other studies, LH secretion is blocked at the pituitary at significantly lower doses, indicate that high doses of GnRH antagonist may have effects at other sites in the reproductive-endocrine axis, affecting steroidogenesis directly or indirectly. Direct effects at the ovary through GnRH receptors have been observed in other species (Kakar et al., 1994; Brus et al., 1997) and it is possible that they also occur in dogs. Indirect actions at unknown sites (including the placenta and the oocytes) by modifying other hormones or the secretion of paracrine and autocrine factors are also possible and may interfere with the endocrine homeostasis needed for corpus luteum function. This possibility warrants investigation in dogs and in other species. Nevertheless, in the pregnant bitch before day 30, it is apparent that LH is merely a facultative luteotrophin contributing to a luteal function that is, in part, independent of LH support. Alternatively, it is also possible that LH is not luteotropic at this time, and that the GnRH-antagonist effect is not due to depressed LH secretion but to other imprecise interactions, including a possible direct gonadal effect.

Studies of bitches during the last third of pregnancy when the corpus luteum appears to be dependent on luteotropic support (after days 35–40) indicated that LH may have a role in normal luteal function at this period of pregnancy (Concannon, 1980; Concannon et al., 1987; Vickery et al., 1989). The ability of LH to interfere with progesterone secretion during this period remains to be determined. A significant but transient decrease in progesterone was reported after the administration of a large volume of an equine anti-bovine LH serum in both pregnant and non-pregnant bitches on day 42 (Concannon et al., 1987). However, in the present study, the decrease in progesterone after injection of a large amount of rabbit anti-ovine LH serum at day 40 was transient and not significant. In the present study, significant and sustained decreases in progesterone were only observed when LH was suppressed by injection of high doses of Nal-Glu from day 35 to day 40, or when the GnRH antagonist Detirelix was administered at high doses after day 30 in the study of Vickery et al. (1989). The 100 μg per kg per day dose of Nal-Glu was less luteolytic than the 300 μg per kg per day dose of Detirelix in the earlier study (Vickery et al., 1989), and the difference most likely reflects differences in the biopotency of the two regimens. However, Nal-Glu was directly or indirectly sufficiently luteolytic to terminate pregnancy in one bitch, indicating that higher doses of this antagonist might be more routinely abortifacient. Although the effects of GnRH antagonist treatment are presumed to be due to LH suppression, it is possible that GnRH antagonists act by other mechanisms, including a direct action on ovarian GnRH receptors (Kakar et al., 1994; Brus et al., 1997). Constant infusion of the GnRH antagonist as well as immunoneutralization producing both plasma LH concentrations below the detection limit of the assay (0.5 ng ml⁻¹) were not associated with any significant change in plasma progesterone concentrations.

Administration of exogenous LH failed to alter progesterone in pregnant bitches treated either at day 30 or day 40. This finding is in contrast to the stimulating effect of LH on progesterone secretion between days 35 and 55 reported for non-pregnant bitches (Concannon, 1980), but is in agreement with the observation that an increase of endogenous LH caused by a GnRH agonist did not stimulate progesterone secretion in the second half of the luteal phase.
of three treated bitches (Okkens et al., 1990). It is possible that endogenous progesterone secretion is at a maximum and that further stimulation is not possible. Alternatively, the absence of significant stimulation of progesterone by LH may reflect an insensitivity of the corpus luteum to LH at this stage in pregnancy. There are no reports on the LH receptor content of canine luteal cells during pregnancy. In non-pregnant bitches, the concentration of LH receptors in luteal cells is relatively stable throughout the mid- and late-luteal phases (Fernandes et al., 1987). However, there are differences between the pregnant and non-pregnant corpus luteum, as reflected by the plasma changes in prolactin or progesterone metabolites in pregnant versus non-pregnant animals (Gudermuth et al., 1998; Onclin and Verstegen, 1997b).

The LH treatment on days 40 and 41 significantly increased prolactin concentrations in pregnant bitches. This observation supports the possibility of an indirect luteotropic role for LH mediated by the secretion of prolactin. LH may stimulate ovarian oestradiol secretion. In other species, oestradiol at low doses stimulates prolactin release (Fink, 1988; Soaje and Deis, 1997); however, due to sample volume, oestradiol was not measured in the present study. This hypothesis seems unlikely as secretion of oestradiol during the luteal phase is similar in pregnant and non-pregnant dogs (Gráf, 1978; De Coster et al., 1979; K. Onclin, L. Silva, P. Jeukenne, B. Murphy and J. P. Verstegen, unpublished) and no prolactin increases are detected in non-pregnant dioestrus animals (Onclin and Verstegen, 1996, 1997b).

In summary, the results of the present study show that canine luteal function is more sensitive to LH support in mid- and late pregnancy compared with early pregnancy and that its regulation may change during pregnancy. LH immunoneutralization does not impair corpus luteum function and the effects of the antagonist appear to be time- and dose-dependent during the late stages of pregnancy. LH administration does not stimulate progesterone secretion, but may stimulate prolactin secretion in late pregnancy. In conclusion, LH does not appear to be directly luteotropic in pregnant dogs, in contrast to prolactin, but it may support luteal function indirectly. Further investigation of corpus luteum regulation in pregnant bitches is warranted.

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