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Effects of Gonadotrophin Releasing Hormone Administration on the Pituitary-Ovarian Axis in Anoestrous vs Ovariectomized Bitches

JJCWM Buijtels[1,*], NJ Beijerink[1,*], HS Kooistra[1], SJ Dieleman[2] and AC Okkens[1]

[1]*Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine;* [2]*Department of Farm Animal Health, Section of Reproduction, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

Contents

The aim of this study was to determine the effects of gonadotrophin releasing hormone (GnRH) administration on the plasma concentrations of reproductive hormones in intact and ovariectomized (OVX) bitches. Therefore, blood samples were collected at multiple times before and after the administration of 10 μg/kg GnRH (Fertagyl®) for the determination of the plasma concentrations of luteinizing hormone (LH), oestradiol, progesterone and testosterone in six anoestrus and in six OVX bitches. The mean plasma LH concentrations before and 60 min after GnRH administration were significantly lower in the anoestrous bitches than in the OVX bitches. In both groups GnRH administration resulted in a significant increase in the plasma LH concentration. The highest plasma LH concentrations were found at 10 min after GnRH administration and these values did not differ significantly between the two groups. Only in the anoestrous bitches a significant increase in plasma oestradiol concentrations was found after GnRH administration and these values were significantly higher than those in the OVX bitches. The plasma concentrations of progesterone and testosterone were low (close to or below the limit of quantitation) both before and after GnRH administration and the differences between anoestrous and OVX bitches were not significant. It can be concluded that (i) basal plasma LH concentration is significantly higher in OVX bitches than in anoestrous bitches, (ii) plasma LH concentration increases after GnRH administration in both anoestrous and OVX bitches, (iii) GnRH administration causes a significant rise in plasma oestradiol concentration only if ovarian tissue is present and (iv) measurement of plasma progesterone and testosterone concentrations before and after GnRH administration does not aid in distinguishing between anoestrous and OVX bitches. The results of this study may provide a basis for the diagnosis of remnant ovarian tissue and verification of neuter status in the bitch.

Introduction

It can be difficult to verify the neuter status of dogs with an unknown reproductive status. In addition, it is occasionally difficult to differentiate ovariectomized (OVX) dogs having remnant ovarian tissue from completely OVX dogs. The presence of ovarian tissue can be confirmed by cytological evaluation of a vaginal smear and vaginoscopy during the follicular phase, measurement of the plasma progesterone concentration during the progression to the late follicular phase, ovulation and luteal phase, abdominal ultrasonography and exploratory laparotomy (Wallace 1991; Root and Spaulding 1994; Schaefers-Okkens 2005).

In the intact bitch, the stages of the oestrous cycle can be classified on the basis of ovarian function as the follicular phase, the phase of pre-ovulatory luteinization and ovulation, the luteal phase and non-seasonal anoestrus (Schaefers-Okkens 2005). During the follicular phase, demonstration of the presence of ovarian tissue is straightforward. The concentration of oestradiol, which is mainly synthesized and secreted by granulosa cells in developing ovarian follicles, rises during this phase and reaches peak levels around the pre-ovulatory luteinizing hormone (LH) surge (Olson et al. 1982; Schaefers-Okkens 2005; De Gier et al. 2006). Vaginoscopy and vaginal cytology can be used to recognize the influence of oestrogens (Schutte 1976; Schaefers-Okkens 2005). The use of cytological evaluation of a vaginal smear as a bioassay for oestrogen influence is even more reliable than a single plasma oestradiol determination (Shille and Olson 1989). The plasma concentration of progesterone, which is secreted by partially luteinizing granulosa cells before ovulation and by mature luteal cells after ovulation, is increased in the progression to the late follicular phase, ovulation and during the luteal phase (Schaefers-Okkens 2005). Elevated plasma progesterone concentrations provide evidence for the presence of ovarian tissue (Okkens et al. 1981).

During anoestrus, which lasts from 2 to 10 months, the plasma progesterone concentration is in general below 3 nmol/l (Okkens et al. 1985a). Plasma oestradiol concentration is also usually low and does not begin to rise again until late anoestrus, although sporadic elevations have been reported (Olson et al. 1982). During anoestrus, LH is secreted in a pulsatile fashion but the basal plasma LH concentration is low (Olson et al. 1982; Kooistra et al. 1999). Plasma testosterone concentrations are also low during anoestrus in the bitch. Plasma testosterone concentration begins to rise near the end of anoestrus and reaches peak levels near the time of the pre-ovulatory LH surge (Olson et al. 1984; Concannon and Castracane 1985).

There are no obvious clinical or behavioural differences between anoestrous and OVX bitches. Vaginal cytology also has no diagnostic value in this differentiation and ultrasonographic visualization of ovarian tissue in anoestrous bitches is sometimes difficult (England and Allen 1989; Root and Spaulding 1994). Production of the ovarian-derived hormones oestradiol and progesterone ceases with OVX, but their plasma concentrations in anoestrous and OVX bitches overlap (Jeffcoate 1993a; Frank et al. 2003). The loss of negative feedback of ovarian steroids causes a rapid increase in

*Both authors contributed equally to this article.

the concentration of circulating gonadotrophins (Chaffaux et al. 1981; Olson et al. 1992; Concannon 1993; Jeffcoate 1993a; Löfstedt and Vanleeuwen 2002; Reichler et al. 2004), while their secretion pattern remains pulsatile (Concannon 1993). Baseline gonadotrophin levels may provide some useful information, but as a result of the pulsatile secretion pattern a considerable overlap in the plasma concentrations may be expected between intact and OVX animals and thus the diagnostic value of a single hormone measurement is questionable (Jeffcoate 1993a; Löfstedt and Vanleeuwen 2002).

To differentiate between bitches with and without ovarian tissue, a provocative test of the pituitary-ovarian axis using gonadotrophin releasing hormone (GnRH) may be helpful. In the intact bitch, GnRH administration during anoestrus causes an increment in the circulating plasma concentrations of LH and oestradiol (Van Haaften et al. 1994). The GnRH induced plasma LH and oestradiol responses are higher in late anoestrus than in early anoestrus (Van Haaften et al. 1994). Information regarding the response to exogenous GnRH after ovariectomy is limited to a few studies. One study found a rise in LH after GnRH administration in OVX bitches (Chaffaux et al. 1981), while another found an unpredictable response (Jeffcoate 1993a). The discrepancy may be because of differences in sampling times and doses of GnRH that were used. Only one study investigated the effect of a GnRH challenge on plasma oestradiol concentration in OVX bitches (Jeffcoate 1993a). The low dose of GnRH that was used in the latter study produced no increase in plasma oestradiol concentration. No data have been reported on the response of the plasma testosterone concentration to GnRH stimulation in intact and OVX bitches.

The aim of this study was to determine the effects of GnRH administration on the plasma concentrations of reproductive hormones in intact and OVX bitches. Therefore, blood samples were collected at multiple times before and after the administration of GnRH iv for the determination of the plasma concentrations of LH, oestradiol, progesterone and testosterone in anoestrous and in OVX bitches.

Materials and Methods

Animals, treatment and collection of blood samples

Twelve bitches, consisting of ten Beagles, one Border Collie, and one mongrel, were used in this study. Six of them, 3–7 years of age and weighing 13.6–16.9 kg, were sexually intact. The other six, 5–12 years of age and weighing 13.2–27.0 kg, were OVX. The Beagles were whelped and raised in the Department of Clinical Sciences of Companion Animals and were accustomed to the laboratory environment and handling such as collection of blood samples. They were housed in pairs in indoor-outdoor runs, fed a standard commercial dog food once daily, and given water *ad libitum*. The Border Collie and the mongrel dog were owned by veterinarians working at the Department of Clinical Sciences of Companion Animals and were also accustomed to the collection of blood samples.

The six intact Beagles were examined three times weekly for swelling of the vulva and a serosanguinous vaginal discharge, which were considered to signify the onset of pro-oestrus. Plasma concentrations of progesterone were determined three times weekly from the start of pro-oestrus until plasma progesterone concentration exceeded 16 nmol/l, at which time ovulation was assumed to occur (Concannon et al. 1977; Wildt et al. 1979; Okkens et al. 1985b). During this study the six Beagle bitches were in anoestrus (123–203 days after ovulation), as confirmed by plasma progesterone concentrations below 3 nmol/l (Okkens et al. 1985a). Six dogs had been OVX at the Department of Clinical Sciences of Companion Animals at Utrecht University at least 1 year before the start of the experiment and there had been no subsequent signs of oestrus as demonstrated by the absence of swelling of the vulva, vaginal discharge and attractiveness of male dogs.

Blood samples were collected for measurement of the plasma concentrations of LH (at −40, 0, 10 and 60 min), oestradiol (at −40, 0, 60 and 120 min), progesterone (at −40, 0, 60 and 120 min) and testosterone (at 0, 60 and 90 min) after the iv administration of 10 μg GnRH (Fertagyl®; Intervet, Boxmeer, the Netherlands) per kg body weight at 0 min. GnRH was administered via the cephalic vein. Blood samples were collected from the jugular vein, placed immediately in chilled heparin-coated tubes, and centrifuged at 4°C for 10 min at $1500 \times \boldsymbol{g}$. Plasma was stored at −25°C until analysis.

Hormone measurements

Plasma LH concentration was measured by use of a heterologous radioimmunoassay (RIA) as described previously (Nett et al. 1975) with the following modifications. A rabbit antiserum raised against ovine LH (CSU-204, kindly supplied by G.D. Niswender, Colorado State University, CO, USA), radioiodinated bLH-7981 as prepared for our bovine LH assay (Dieleman and Bevers 1987) and canine pituitary standard LER 1685-1 (a gift from Dr L.E. Reichert, Albany Medical College, NY, USA) were used in this assay. The intra- and inter-assay coefficients of variation (CV) for values above 0.5 μg/l were 2.3% and 10.5%, respectively. The lower limit of quantitation was 0.3 μg/l.

Plasma oestradiol concentration was measured by use of a solid-phase RIA (Count-A-Count® TKE; Diagnostic Products Corporation, Los Angeles, CA) according to the manufacturer's instructions with modifications as described previously (Dieleman and Schoenmakers 1979) and validated for the dog (Van Haaften et al. 1994). The intra- and inter-assay CV were 14% and 11.8%, respectively. The lower limit of quantitation was 7 pmol/l.

Plasma progesterone concentration was measured by a previously validated RIA (Dieleman and Schoenmakers 1979; Okkens et al. 1985a). The intra- and inter-assay CV were 11% and 14%, respectively. The lower limit of quantitation was 0.13 nmol/l.

Plasma testosterone concentration was measured by RIA (Coat-A-Count Total Testosterone, Diagnostic Product Corporation) according to the manufacturer's protocol with the following two modifications to

increase the sensitivity. First, three extra standard points were included corresponding with 51, 255 and 1020 pmol/l producing standard curves with an average estimated dose of 1643 (CV 11.4%, n = 10 RIAs), 230 (7.1%) and 37 (6.8%) pmol/tube at 20, 50 and 80% relative binding, respectively. Second, aliquots of 1 ml plasma were extracted with 2.5 ml diethylether. The residue after evaporating under nitrogen was dissolved in 125 μl A-serum (Diagnostic Products Corporation) of which 50 μl duplicates were used for RIA. The average extraction efficiency was 85% (CV 1.8%, n = 10 RIAs) as determined on the basis of 3H-testosterone added to parallel series of plasma samples (n = 6 per RIA). Values of the samples obtained in pmol/tube were calculated into pmol/l by correction for volume and extraction efficiency. The intra- and inter-assay CV were 5% and 6%, respectively. The lower limit of quantitation was 51 pmol/l.

Data analysis

Statistical analysis was performed using SPSS® for Windows, Version 12.0.1 (SPSS Inc., Chicago, IL, USA). When the plasma concentration of oestradiol, progesterone, or testosterone was below the limit of quantitation, the respective lower limit value was assigned to the sample. For each bitch the mean basal plasma hormone concentration was calculated from the hormone concentrations before GnRH administration. The average plasma hormone concentration per group was calculated as the average of the mean values of the individual dogs. The plasma LH, oestradiol, progesterone and testosterone data were made normally distributed by converting them to their natural (or neperian) logarithms (ln) and these were analysed using a multivariate repeated measures model, with 'time' as the within-subject variable and 'group' as the between-subject variable. Subsequently, a contrast study was performed to evaluate time effects within each group in comparison with the mean basal level. $p < 0.05$ was considered significant. Results are presented as mean ± SEM and range.

Ethics of experimentation

This study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Utrecht University.

Results

No adverse effects of GnRH administration on health or behaviour were observed in any of the dogs. Mean plasma LH concentrations before and 60 min after GnRH administration were significantly lower ($p < 0.001$ and 0.01, respectively) in the intact bitches than in the OVX bitches. Moreover, there was no overlap between the two groups when these values were compared (Table 1). In both groups GnRH administration resulted in a significant increase in the plasma LH concentration (Fig. 1). In the protocol used, the highest plasma LH concentrations were found at 10 min after GnRH and these values did not differ significantly

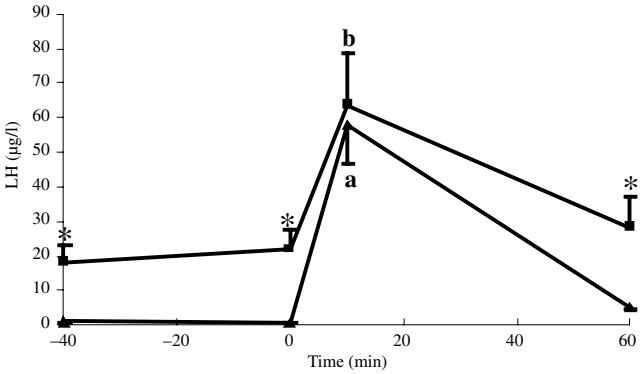

Fig. 1. Mean (±SEM) plasma LH concentration after intravenous administration of GnRH in a dose of 10 μg/kg body weight at t = 0 min in six anoestrous (▲) and six ovariectomized (OVX) (■) bitches. Asterisks indicate a significant difference in mean plasma LH concentration between anoestrous and OVX bitches. (a) indicates a significantly higher mean plasma LH concentration at t = 10 min in the anoestrous dogs compared with the values before and 60 min after GnRH administration. (b) indicates a significantly higher mean plasma LH concentration at t = 10 min in the OVX bitches compared with the values before GnRH administration

Table 1. Mean ± SEM and range of plasma concentrations of luteinizing hormone (LH), oestradiol, progesterone and testosterone in six anoestrous and six ovariectomized (OVX) bitches before and after intravenous administration of 10 μg/kg body weight of GnRH (Fertagyl)

	Anoestrous bitches		Ovariectomized bitches	
	Mean ± SEM	Range	Mean ± SEM	Range
Basal LH (μg/l)	0.64 ± 0.04*,[a]	0.48–0.93	20.2 ± 3.6*,[a]	6.2–41.0
LH 10 min after GnRH (μg/l)	58.0 ± 11.1[b]	25.4–101	63.9 ± 15.3[b]	30.3–113
LH 60 min after GnRH (μg/l)	4.9 ± 0.5*,[a]	2.8–6.2	28.4 ± 8.7*,[ab]	10.9–68.9
Basal oestradiol (pmol/l)	NC[a]	< 7–14.1	NC	< 7
Oestradiol 60 min after GnRH (pmol/l)	23.0 ± 4.0*,b	14.1–35.7	NC*	< 7
Oestradiol 120 min after GnRH (pmol/l)	24.4 ± 3.5*,b	12.3–32.4	NC*	< 7
Basal progesterone (nmol/l)	NC	< 0.13–2.2	NC	< 0.13–0.95
Progesterone 60 min after GnRH (nmol/l)	NC	< 0.13–1.9	NC	< 0.13
Progesterone 120 min after GnRH (nmol/l)	NC	< 0.13–1.6	NC	< 0.13
Basal testosterone (pmol/l)	NC	< 51–75.6	NC	< 51.0–70.0
Testosterone 60 min after GnRH (pmol/l)	74.7 ± 7.8	56.2–102.5	NC	< 51.0–55.7
Testosterone 90 min after GnRH (pmol/l)	80.4 ± 7.8	56.7–101.8	NC	< 51.0

NC: mean not calculated because not all plasma hormone concentrations were above the detection limit. When a plasma hormone concentration was below the limit of quantitation, in order to perform statistical analysis, the respective lower limit value was assigned to the sample.
*Indicates a significant difference in mean hormone concentration between anoestrous and OVX bitches.
[a,b,ab]Different letters within a column per hormone indicate significant differences.

between the two groups. The mean plasma LH concentration at 10 min after GnRH was significantly higher than those before ($p < 0.001$) and 60 min after GnRH ($p < 0.001$) in the anoestrous dogs. In the OVX dogs, the mean plasma LH concentration at 10 min after GnRH was significantly higher ($p < 0.05$) than the mean basal plasma LH concentration.

Basal plasma oestradiol concentration in all dogs except one anoestrous bitch was below the detection limit of the assay. The difference in the basal plasma oestradiol concentration between the two groups was not significant. After GnRH stimulation, plasma oestradiol concentration was below the detection limit in all OVX dogs but above this level in all anoestrous bitches. In the protocol used, the maximum plasma oestradiol concentration after GnRH administration was observed at 60 min in three of the anoestrous bitches and at 120 min in the other three. At 60 and 120 min after GnRH stimulation, plasma oestradiol concentrations were significantly higher in the anoestrous bitches than in the OVX bitches ($p < 0.05$ in both cases) and the increase above baseline in the anoestrous bitches was also significant ($p < 0.02$ and 0.01, respectively).

Basal plasma progesterone concentration was above the detection limit of the assay in all but three samples from the anoestrous bitches, whereas it was below the detection limit in all but one sample from the OVX bitches. In both groups, no change in plasma progesterone concentration after GnRH administration was significant. Differences in mean plasma progesterone concentration between the two groups before and after GnRH administration were not significant (Table 1).

Basal plasma testosterone concentration was above the detection limit of the assay in three anoestrous bitches and one OVX bitch. After GnRH administration, plasma testosterone concentration was detectable in all anoestrous bitches but only one OVX bitch. Plasma testosterone concentration did not change significantly after GnRH administration and the difference in mean plasma testosterone concentration between anoestrous and OVX dogs was not significant.

Discussion

The plasma concentrations of reproductive hormones were measured before and after GnRH administration in OVX bitches and bitches in anoestrus, to evaluate the applicability of such measurements for ascertaining the presence or absence of ovarian tissue. The anoestrous period was chosen because during this phase neither vaginoscopy nor vaginal cytology nor the basal plasma progesterone concentrations are of value in differentiating between intact and OVX bitches.

The different sampling times for LH and oestradiol were based upon information from previous experiments (Van Haaften et al. 1994; Meij et al. 1996). No data have been reported on the response of the plasma testosterone concentration to GnRH stimulation in the bitch. In intact male dogs administration of 10 μg/kg GnRH results in a maximum testosterone concentration at 60 min after injection (Knol et al. 1993), and therefore we assumed that a GnRH-induced increase in plasma testosterone concentration will be found in blood samples at 60 and 90 min after GnRH administration. Because circulating testosterone concentrations in bitches are described to be low or undetectable (Nickel 1996), and no studies could be found that report diurnal variation of plasma testosterone concentrations in bitches, only one pre-GnRH trial sample for testosterone was collected.

The results of this study demonstrate that mean basal plasma LH concentrations were significantly higher in OVX bitches than in anoestrous bitches. Moreover, there was no overlap in basal plasma LH concentrations between the two groups. The higher basal plasma LH concentrations in the OVX dogs are in agreement with the results of other studies (Chaffaux et al. 1981; Olson et al. 1992; Concannon 1993; Jeffcoate 1993a; Löfstedt and Vanleeuwen 2002; Reichler et al. 2004) and can be explained by the loss of negative feedback of the ovarian hormones. However, taking into account the pulsatile nature of LH release (Concannon 1993; Kooistra et al. 1999), overlapping of plasma LH concentrations between larger groups of intact and OVX bitches may be expected, and therefore one single LH result should be interpreted with caution. Indeed, a single measurement of LH has been shown not to be a reliable means of determining whether or not a bitch was intact (Löfstedt and Vanleeuwen 2002). In addition, circulating LH concentrations of < 1 μg/l 10–16 weeks after ovariectomy in some bitches have been found (Reichler et al. 2004). Consequently, the presence or absence of ovarian tissue should not be based solely upon the results of a single basal plasma LH measurement.

In both anoestrous and OVX bitches, GnRH administration provoked a significant increase in the plasma LH concentration. As a result of the complete loss of negative feedback of ovarian hormones a maximal stimulation of pituitary gonadotrophin release may have been expected. However, in post-menopausal women the secretion pattern of LH is still pulsatile, and GnRH administration provokes an increase in the plasma LH concentration (Rossmanith et al. 1991), indicating that stimulation of pituitary LH release is still occurring in ovarian hormone-deprived women. Moreover, pulsatile LH release has also been reported in OVX bitches (Concannon 1993).

In agreement with previous studies (Van Haaften et al. 1994; Meij et al. 1996), in the protocol used plasma LH concentration reached its maximum level at 10 min after GnRH administration in both groups. It also reached its maximum in OVX bitches at 10 min after intra-muscular administration of GnRH (Chaffaux et al. 1981). The overlap between anoestrous OVX bitches in plasma LH values 10 min after GnRH administration makes this measurement an unreliable method for distinguishing between the two groups.

Plasma oestradiol concentration was below the detection limit of the assay in all OVX dogs and five of the six anoestrous dogs before GnRH administration and in the OVX dogs after GnRH administration. There was a significant increase in plasma oestradiol concentration following GnRH stimulation in all the intact bitches but none of the OVX bitches. As found in a previous study (Van Haaften et al. 1994), the dose of GnRH (Fertagyl) of 10 μg/kg was sufficient to induce an increase in

plasma oestradiol concentration in the intact bitches. In other studies (Jeffcoate 1992, 1993b), a dose of 0.16 μg GnRH (Receptal®; Intervet, Boxmeer, the Netherlands) per dog, which is, although Receptal is more efficient than Fertagyl (Chenault 1990), a much lower dose than used in this protocol, failed to induce a clear increase in plasma oestradiol concentration in intact dogs. Luteinizing hormone and oestradiol responses after GnRH administration in anoestrous bitches are significantly dose dependent; a dose of 0.01 μg/kg produced little or no response (Van Haaften et al. 1994). The results of the present study suggest that the plasma oestradiol response to an adequate dose of GnRH (Fertagyl; 10 μg/kg) may be a useful and reliable test with which to distinguish between bitches with and without ovarian tissue. The time at which the plasma oestradiol concentration is maximal after GnRH administration could not been determined in this study. Also in another study, it has been observed that GnRH doses of 0.1–100 μg/kg raise plasma oestradiol concentration for 160 min (Van Haaften et al. 1994).

In women, oestradiol is also produced in a number of extra-ovarian sites. These sites include the mesengial cells of adipose tissue including that of the breast, osteoblasts and chondrocytes of bone, vascular endothelium and aortic smooth muscle cells and numerous sites in the brain. However, at these sites oestradiol acts locally as a paracrine or intracrine factor, rather than that it is secreted into the circulation (Simpson 2003). Most probably this also accounts for the dog, and therefore a significant rise in plasma oestradiol concentration after GnRH administration can be expected only if ovarian tissue is present. In addition, Frank et al. (2003) performed an ACTH stimulation test and no increase in circulating oestradiol could be evoked in either OVX or intact bitches, indicating that the adrenal glands do not contribute to the circulating oestradiol concentration.

The difference in plasma progesterone concentration between anoestrous and OVX bitches was not significant and the ranges overlapped both before and after GnRH administration. This is in agreement with the findings of others (Jeffcoate 1993a; Frank et al. 2003). Although the main source of progesterone in the intact bitch is the ovary, progesterone can also be secreted by the adrenal cortex (Frank et al. 2004), which is the most logical explanation for the detectable plasma progesterone level in one of the OVX dogs (Concannon 1986). This could be related to stress, as progesterone increases have been reported to occur as a reaction to fear provocations in dogs (Hydbring-Sandberg et al. 2004). On the other hand, in the male dog LH and testosterone values are not influenced by blood sampling (Knol et al. 1992), implying that under the experimental conditions used the validity of results will not be affected by blood collection. Since it is not completely clear to what extent stress might influence the results, the potential effect of stress has to be considered when examining the function of the hypothalamic-pituitary-ovarian axis.

The differences in plasma testosterone concentrations before and after GnRH administration between intact and OVX bitches were not significant. For baseline testosterone concentrations this is in agreement with the results in another study in the dog (Frank et al. 2003). Although mean plasma testosterone concentration in the anoestrous bitches appeared to reflect the increase in mean plasma oestradiol concentration, the change was not significant ($p = 0.15$). As oestradiol is formed from testosterone by the action of the aromatase system (Edqvist and Forsberg 1997), this non-significant increase in the plasma testosterone concentration most probably reflects oestradiol synthesis in the follicles. Different findings have been reported in women. The basal plasma testosterone concentration in oophorectomized women is significantly lower than that in intact women. Furthermore, stimulation with human chorionic gonadotrophin (hCG) raises plasma testosterone concentration in intact women but not in oophorectomized women (Burger 2002; Piltonen et al. 2002).

It is uncertain whether the plasma LH and oestradiol responses observed in intact bitches can be expected to be completely the same in bitches with remnant ovarian tissue. In a previous study (Okkens et al. 1981) 12 of 47 bitches with remnant ovarian tissue were found to have macroscopic cystic structures in the residual ovarian tissue. The effect of these cystic remnants on the results of the GnRH stimulation test is unknown and the successful use of this test in the detection of remnant ovarian tissue has not yet been reported in animals, as it has in humans (Scott et al. 1995).

It can be concluded that (i) basal plasma LH concentration is significantly higher in OVX bitches than in anoestrous bitches, (ii) plasma LH concentration increases after GnRH administration in both anoestrous and OVX bitches, (iii) GnRH administration causes a significant rise in plasma oestradiol concentration only if ovarian tissue is present and (iv) measurement of plasma progesterone and testosterone concentrations before and after GnRH administration does not aid in distinguishing between anoestrous and OVX bitches. The results of this study provide a basis for the diagnosis of remnant ovarian tissue and verification of neuter status in the bitch. However, further studies remain to be applied in bitches suspected of remnant ovarian tissue.

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Author's address (for correspondence): AC Schaefers-Okkens, Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 8, PO Box 80154, NL-3508 TD Utrecht, The Netherlands. E-mail: A.C.Schaefers-Okkens@vet.uu.nl