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Evaluation of Cabergoline and Buserelin Efficacy for Oestrous Induction in the Bitch

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Contents

The purpose of this work was to compare two different protocols of oestrous induction, using either a dopamine agonist (cabergoline) or a GnRH agonist (buserelin) in anoestrus bitches. The clinical trial involved 22 Beagle bitches, randomly allotted to two treatment groups: group A ($n = 12$) was orally administered cabergoline (Galastop®; Centralvet-Vetem, Milan, Italy; 5 μg/kg SID), until the onset of cytological oestrus or for a maximum of 30 days and group B ($n = 10$) was treated with buserelin acetate, (Suprefact®; Aventis Pharma, Milan, Italy), administered subcutaneously t.i.d., at 1.5 μg/kg for 11 days and 0.75 μg/kg for the following 3 days. Blood samples were collected twice a week to measure progesterone and prolactin concentration. Both cabergoline and buserelin produced a significant early decline in prolactin concentration ($p < 0.01$), but the effect of cabergoline lasted longer. Progesterone concentration was significantly affected by buserelin administration, showing a significant increase ($p < 0.01$) from day 3 to day 6 of treatment. Cabergoline confirmed its effectiveness in inducing oestrus as 10 of 12 bitches responded to the treatment, were mated and whelped. On the contrary, oestrus was observed in only three of 10 buserelin-treated bitches and in two of them 7 and 13 days after the end of treatment. These same two bitches accepted mating and conceived. The results suggest that in a clinical setting, dopaminergic treatment is the treatment of choice as it yields more consistent results and involves a much easier administration protocol.

Introduction

The possibility of shortening the reproductive cycle of the bitch has been largely investigated, but the success of the treatment is far from being satisfactory (Shille et al. 1984; Concannon and Verstegen 1997; Jeukenne and Verstegen, 1997). Oestrous induction protocols involving administration of FSH and oestrogens, GnRH agonists, prostaglandins or dopamine agonists give variable results, largely depending on the phase of the cycle when treatment begins (Concannon, 1989; Verstegen et al. 1999; Zoldag et al. 2001). Anoestrus in the bitch is characterized by a concurrent pulsatile secretion of FSH and LH, with basal FSH concentration increasing during anoestrus progression, while basal LH values remain unchanged. The administration of some dopamine agonists, like bromocriptine or cabergoline, is associated with an increase in basal plasma FSH concentration, resulting in the shortening of the interoestrus interval (Okkens et al. 1997; Kooistra et al. 1999a). However, basal LH plasma level and pulse frequency increase during the follicular phase of the oestrus cycle (Kooistra et al. 1999b). The administration of LH alone can terminate anoestrus (Verstegen et al. 1997) in a manner similar to the administration of GnRH and GnRH agonists that induce LH release. Oestrus can be induced administering GnRH agonists or purified LH t.i.d. (Concannon and Verstegen, 1997; Verstegen et al. 1997), with sustained-release GnRH agonist formulations (Inaba et al. 1998) or with GnRH agonist implants (Kutzler et al. 2002).

The present study was designed to evaluate the clinical efficacy of two methods of oestrous induction in the bitch, to find a clinically applicable method aiming to improve the efficiency of breeding management in a large breeding facility. Two treatments were compared – the administration of a dopamine agonist (cabergoline) and the administration of a GnRH agonist (buserelin) in different anoestrus conditions.

Materials and Methods

Twenty-two Beagle bitches aged 4.2 ± 0.4 years (mean ± SEM), weighing 10 ± 2 kg (22.2 ± 4.4 lb), born and housed in a dog-breeding facility in Northern Italy, were included in a clinical trial of oestrous induction, carried out during the months of October and November. The bitches were housed in groups of five to six animals, exposed to natural light, fed a commercial dry canine diet and given water *ad libitum*. The reproductive history of the animals, gathered from breeding records, indicated that they were in middle to late anoestrus, with the exception of nine bitches, which showed, at the time of the study, a delay in the expected pro-oestrus onset ranging from 3 to 12 months (Table 1). The bitches were randomly allotted to two treatment groups, apart from the ones with a prolonged interoestrus interval, which were evenly distributed between groups (group A: $n = 12$ and B: $n = 10$). Group A was treated with the dopamine agonist cabergoline (Galastop®), administered orally s.i.d., at 5 μg/kg, until the progression of pro-oestrus into oestrus (as indicated by vaginal cytology) or for a maximum of 30 days. Group B was treated with the GnRH agonist buserelin acetate, in aqueous solution (Suprefact®), administered subcutaneously t.i.d., at 1.5 μg/kg for 11 days and 0.75 μg/kg for 3 days.

All dogs were clinically examined at the beginning of treatment (day 0) and their anoestrus condition was assessed by vulvar observation and vaginal cytology and later on confirmed by plasma progesterone levels. Thereafter, the bitches were examined twice a week, until 18 days after the end of drug administration, or

Table 1. Reproductive history of the two groups of bitches. Group A was treated with the dopamine agonist cabergoline (5 μg/kg s.i.d.); group B with the GnRH agonist buserelin acetate (1.5 μg/kg t.i.d., 0.75 μg/kg t.i.d.) (mean value ± SEM)

Group	No. of animals	Age (years)	No. of previous oestrus cycles	Parity	Anoestrus (months)
A	12	4.7 ± 0.6	4.3 ± 0.9	3.7 ± 0.9	9.7 ± 1.5 range: 4–16
B	10	3.6 ± 0.6	3.9 ± 1.0	2.9 ± 0.8	8.4 ± 1.2 range: 4–16

until the transition into dioestrus, for the late cycling bitches. The presence of vulvar swelling and serosanguineous vaginal discharge was considered as the hallmark of pro-oestrus onset. Each examination of the animals included vaginal smear and blood sample collection by venepuncture of the cephalic vein, always between 2 and 4 p.m. Plasma was separated by centrifugation at $1000 \times g$ for 15 min and stored at −20°C until assayed. Vaginal smears were stained with Diff Quick® (Baxter Scientific Products, Milan, Italy) and were examined for estimation of changes in cell types (percentage increase in superficial cornified/anucleated cells). Oestrus bitches were naturally mated with males of proven fertility, following the standard procedure of the breeding facility. Progesterone concentration was measured to confirm the initial anoestrus condition and the normality of the induced oestrus cycles; prolactin concentration was measured as an indication of response to treatment.

For progesterone determination, extracted plasma was assayed by microtitre RIA (Battocchio et al. 1999). The assay was validated for canine plasma by parallelism ($y = 247.8 \times -4.07$; $R^2 = 0.99$) and recovery tests ($y = 247.8 \times -4.07$; $R^2 = 0.99$). The intra- and inter-assay CVs were 9.3 and 12.4% for control low (2.5 nmol/l), 6.8 and 7.0% for control medium (19.87 nmol/l) and 7.3 and 11.1% for control high (159 ng/ml). Sensitivity of the method was 1.65 pM per well as determined by the software package RiaSmart (Packard Instrument Company, Meriden, USA).

Prolactin concentrations were measured by immunoenzymatic assay using a commercial kit (MKVCP1; Milenia Biotec, Bad Nauheim, Germany), validated for canine plasma (Onclin and Verstegen, 1997). The intra- and inter-assay CVs were 6.8 and 14.4%, respectively, and the lowest limit of detection was 17.4 pmol/l (RiaSmart).

Data were analysed by a two-way analysis of variance (ANOVA) followed by a Bonferroni adjusted multiple comparison between experimental groups for plasma prolactin and progesterone concentrations, and percentual changes in serum prolactin during treatments. The degrees of interaction among initial prolactin concentration, age of the animals, treatment outcome and interval to pro-oestrus onset, were evaluated statistically using Spearman's correlation coefficient. The level of statistical significance was set at $p < 0.05$. Data are given as mean ± SEM.

Results

Table 2 shows the results of the oestrous induction treatments. Ten of 12 group A bitches responded to the treatment and showed pro-oestrus signs on average after 24 days of cabergoline administration (23.5 ± 3.2 days). In all the bitches pro-oestrus progressed to oestrus; and ovulation, as confirmed by progesterone data (and by pregnancies), occurred in all the animals. In group B, two animals showed pro-oestrus signs on day 7 and day 13 after the end of treatment, respectively and the oestrus cycle was confirmed to be normal by progesterone analysis first and by pregnancies later. One bitch showed the onset of pro-oestrus 10 days after the beginning of buserelin administration. However, the induced oestrus lasted more than 20 days and during this period the bitch never accepted mating. The progression into dioestrus was confirmed both by vaginal cytology and progesterone concentration, which increased to 66.8 nmol/l on day 43 after pro-oestrus onset. All the mated animals of the two groups conceived and whelped litters of average size.

The anoestrus condition of all the bitches, clinically assessed at the beginning of treatment, was confirmed by the low plasma progesterone concentration measured (Olson et al. 1982) (Fig. 1). At the beginning of the experiment, plasma prolactin levels were very variable among bitches and, considering the median value (195.6 pmol/l), two sub-sets of animals could be iden-

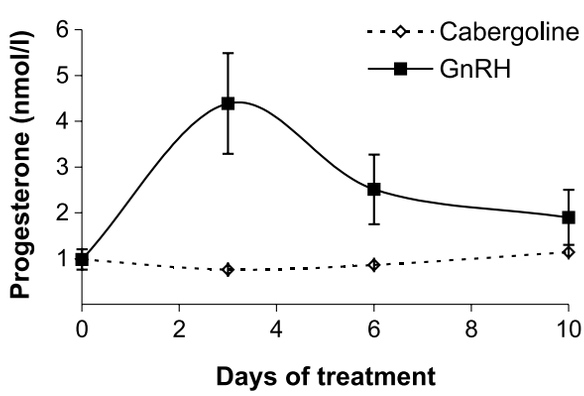

Fig. 1. Plasma progesterone concentration in cabergoline-treated (group A; n = 12) and GnRH agonist-treated animals (group B; n = 10) (mean value ± SEM)

Table 2. Treatment outcome: group A (cabergoline 5 μg/kg s.i.d.); group B (buserelin acetate 1.5 μg/kg t.i.d., 0.75 μg/kg t.i.d.) (mean value ± SEM)

Group	No. of animals	No. of responding bitches	Days to pro-oestrus onset	No. of mated bitches	No. of pregnant bitches	Litter size
A	12	10	23.5 ± 3.2 range: 15–40	10	10	5.2 ± 0.5
B	10	3	18.6 ± 4.7 range: 10–26	2	2	4.5 ± 0.5

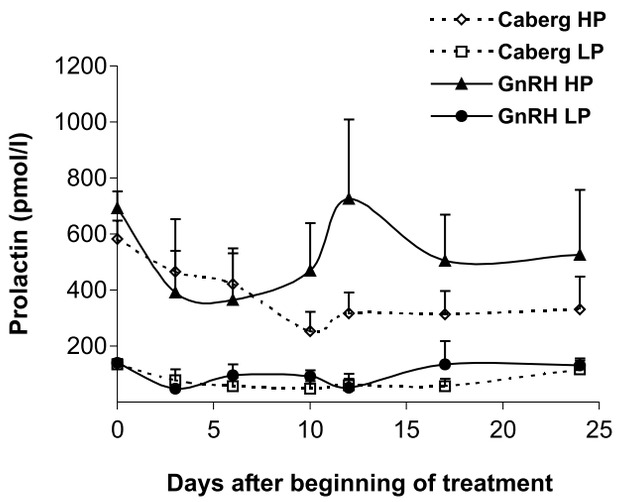

Fig. 2. Plasma prolactin concentration following cabergoline and GnRH administration. Pattern of variation in the two sub-sets of animals (HP, high prolactin level; LP, low prolactin level) (mean value ± SEM)

tified accordingly: a high prolactin group (HP) (626.1 ± 47.8 pmol/l; n = 10) and a low prolactin group (LP) (134.8 ± 13.0 pmol/l; n = 12). Animals belonging to both the hormonal sub-sets were evenly distributed in each treatment group.

Plasma progesterone concentration during the first days of treatment showed a significant increase following GnRH administration (group B bitches) between day 3 and day 6 ($p < 0.01$) (Fig. 1).

As shown in Fig. 2, both cabergoline and the GnRH agonist produced a significant decline in plasma prolactin concentrations ($p < 0.01$). The rate of decline was not statistically different between treatments, whereas its duration was longer and more consistent in the cabergoline treated group (cabergoline effect lasted from day 3 to day 17, while GnRH caused a decline from day 3 to day 6, and then on day 12), irrespective of the initial prolactin concentration.

The initial prolactin status (HP/LP) was not correlated to the age of the animals, to the outcome of treatment or to the lengths of anoestrus and of the interval before pro-oestrus onset.

Discussion

The dopaminergic drug cabergoline confirmed its efficacy in inducing a normal and fertile oestrus in bitches, during different stages of anoestrus. The results on oestrous induction rate are comparable with previous works (Jeukenne and Verstegen, 1997; Verstegen et al. 1999) but, in the present study, the onset of pro-oestrus occurred after a longer period of treatment, ranging in most cases from 15 to 22 days, but also reaching 40 days; 20 days of treatment is the period reported by Verstegen et al. (1999) for early anoestrus bitches, while 6 days were enough for late anoestrus ones. A possible explanation of the longer period may be that the animals were not as close to the next spontaneous pro-oestrus, as in the earlier study: in fact, although the mean anoestrus length is rather high (Table 1), the breeder's records show a typical pattern for each bitch. Besides, it is very likely that in a field trial the differences among animals are higher than in an experimental setting.

The unsatisfactory results obtained using the GnRH agonist may be due to the luteinization of some follicles, as suggested by the progesterone increase at the beginning of treatment (Fig. 1), still unable to grow and ovulate. It may be speculated that there was a lack of synchrony between LH release and the growth phase of the ovarian follicles. Cain et al. (1990) reported 80% of oestrous induction within 9–11 days of treatment, using the same protocol in anoestrus bitches, albeit with a different GnRH agonist. It is possible that the biopotency of the agonist used in the present study was higher and its administration may have caused a depletion of the pituitary LH stores after few days of treatment and/or down-regulation of GnRH receptors (Ortmann and Diedrich, 1999). The ineffectiveness of the buserelin administration may also be explained by a failure of the TID injection to cause a sustained elevation in LH concentrations. A pulsatile LH pattern does seem less important than a constantly high mean concentration, as demonstrated by the positive results obtained with sustained release LH formulations or implants (Inaba et al. 1998; Kutzler et al. 2002). Assay of LH in similarly treated bitches would have helped to understand the difference in response between the two studies, but blood samples could not have been collected with the due frequency, under our conditions.

However, the few GnRH analogue-induced cycles were normal and fertile, leading to two pregnancies. The data show that concurrent to the progesterone increase induced by the GnRH agonist, there is a decline in prolactin concentration. In the present trial, both cabergoline and the GnRH agonist induced a prolactin decrease of similar intensity, but more prolonged with cabergoline. The decline in prolactin concentration is not the key event triggering anoestrus termination. This appears to be a consequence of other central dopaminergic effects shown by cabergoline but not by other antiprolactin drugs, like metergoline, acting as secotonin antagonists (Kooistra et al. 1999a).

The current data confirm previous observations about the high variability of prolactin mean concentration during anoestrus in the bitch. During this period, the basal profile of prolactin is a fluctuating curve with occasional elevations (Kooistra and Okkens, 2001) and no circadian rhythm of secretion could be detected (Gobello et al. 2001). The bitches, at the beginning of the trial, have a highly variable prolactin concentration, with almost half of them showing values higher than 435 pmol/l (10 ng/ml) and the other half lower than 152.25 pmol/l (3.5 ng/ml), not related to the referred anoestrus length. It seems also unlikely that the higher prolactin concentration in some animals could be stress induced because all the animals were housed in the same environmental conditions and were trained at being manipulated for clinical examination, vaginal smears and blood collection. Gobello et al. (2001), hypothesized that high prolactin concentration could be genetically based, as in different strains of mice with different genetic background. An interesting result, out of the objectives of this work and worth to be investigated further, is the fact that basal prolactin concentration

was not significantly effective on the response to treatment or on the time required for the response.

Under the present clinical conditions, the GnRH agonist treatment cannot be suggested as a valid alternative to cabergoline administration. The outcome of the treatment is unsatisfactory and moreover it cannot be excluded that the oestrus shown by two bitches after the end of treatment was occurring spontaneously rather than induced. Dopaminergic treatment would also be preferable for a much easier way of administration, but a drawback, from a clinical point of view, is that the length of treatment cannot be predicted with any precision.

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