USE OF A DECREASED DOSE OF CABERGOLINE TO TREAT SECONDARY ANOESTRUS IN BITCHES

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Received for publication May 17, 2006.

Abstract

The aim of the study was to determine whether a dose (0.6 µg/kg/d) quite lower than the prolactin-lowering dose of cabergoline, prepared for humans, would be a safe and effective method for the stimulation of oestrus in bitches at secondary anoestrus or late anoestrus. Twenty-four pure blood bitches from various breeds were used in the study at their already determined periods of anoestrus. The treatment group included bitches at late and prolonged anoestrus. Eight bitches that had not shown any signs of oestrus for the preceding 370 to 485 d formed the secondary anoestrus group. Eight of the 16 bitches at late anoestrus (days 165-280) have accomplished the late anoestrus group and another 8 have been chosen randomly for the control group (untreated). Cabergoline was orally administrated until day 2 after the onset of pro-oestrus or for a maximum of 42 d. Blood samples were taken daily from each bitch during the first 5 d of behavioural oestrus to measure progesterone concentrations. In the secondary anoestrus, late anoestrus, and control groups, oestrus was induced on days 4-14 and 12-45 at a ratio of 75.0% (6/8) and 87.5% (7/8), respectively.

The mean pro-oestrus and behaviour oestrus durations, serum progesterone concentrations on day 5 of oestrus, ovulation rates, pregnancy rates, and the mean litter sizes in secondary anoestrus, late anoestrus, and control groups were found to be similar. None of the dogs had any adverse gastrointestinal effects associated with cabergoline administration. The results of the present study suggest that the administration of 0.6 µg/kg/d of cabergoline is a safe and effective treatment for secondary anoestrus in bitches.

Key words: bitch, secondary anoestrus, late anoestrus, oestrus induction, cabergoline.

Dogs are mono-oestrous and experience an obligate anoestrus of 2 to 10 months following the luteal phase with an average duration of about 75 d (4). The normal inter-oestrus interval is therefore typically between 5 and 12 months (6). Abnormalities of the anoestrus period are common in bitches. Persistent anoestrus is classified as primary or secondary, with primary anoestrus defined as lack of oestrus by 18 to 24 months of age and secondary anoestrus defined as lack of oestrus within 12 months after preceding oestrus period (8, 9). Treatment of primary and secondary anoestrus should be directed towards identifying and treating the underlying cause. However, oestrus induction (EI) may be attempted when an underlying cause is not found (9). Owners of pure breed bitches with long interval between cycles often request to shorten inter-oestrus intervals, so the number of litters per year can be increased. Several protocols with exogenous gonadotropins, synthetic oestrogens, GnRH agonists, and dopamine agonists (DA) have been tried for oestrus induction (EI) in anoestrous bitches (3, 11, 13, 14). These methods differ widely in the efficacy of inducing oestrus, as well as in the fertility of the induced oestrus (18). Gonadotropins for EI can result in low fertility and abnormal luteal function (12, 13). The use of DA to prematurely terminate anoestrus typically results in a physiological oestrus and spontaneous ovulation (5, 9, 11, 21).

Dopamine agonists such as cabergoline, bromocriptine or metergoline are ergoderivative alkaloids that exert an anti-prolactinergic effect. However, both bromocriptine and metergoline are not selective DA. Cabergoline has a high specificity for D2 receptors, long specific activity on pituitary lactotrophic cells, and fewer central nervous system effects than bromocriptine (19). Cabergoline (Galastop, Vetem, Italy), marketed as a veterinary drug in some European countries, successfully induces fertile oestrus in most bitches but can be cost prohibitive in the other countries, where this drug is not readily available for veterinary use (18). Cabergoline (Dostinex, Pharmacia, Italy) is also used in the treatment of hyperprolactinaemia in women (23) and can be purchased easily.

Most of the previous studies on the use of DA to induce oestrus have involved healthy bitches (2, 5, 11, 12, 21). There are limited studies about the use of DA in the treatment of persistent anoestrus. Cabergoline has been traditionally used at a dose of 5 µg/kg/d in...
suppression of lactation, termination of pregnancy or EI
(1, 9, 10, 21). In a recent study of ours it has been shown
that normal and fertile oestrus can be induced more
economically in bitches at the anoestrus period using a
quite lower dose (0.6 µg/kg/d) than the prolactin-
lowering dose (5 µg/kg/d) of cabergoline (5). The
purpose of the study; therefore, was to determine the
effects of a decreased dose (0.6 µg/kg/d) of cabergoline
prepared for humans on the oestrus induction, oestrus
and pro-oestrus durations, ovulation rates, pregnancy
rates, and litter size in bitches with secondary anoestrus
by comparing treatment bitches with the control ones at
late anoestrus.

Material and Methods

The study was carried out at a breeder kennel
between July and November of the same year. Twenty-
four pure blood bitches of various breeds and different
age, and weighing from 15 to 45 kg, were used in the
study. The determination of the anoestrus day was based
on the breeding records. Anoestrus was confirmed in
each bitch on the ground of serum progesterone level <1
ng/mL (22), determined by radioimmunoasay (DSL-
3900, DSL, Texas, USA), and the absence of
intermediate or superficial cells in vaginal smears.
Bitches were housed in individual indoor-outdoor runs,
exposed to natural light, provided with water ad libitum
and fed a commercial dry food once daily in amounts
sufficient to maintain body weight. The treatment group
included bitches at late and prolonged anoestrus. Eight
bitches that did not show any signs of oestrus for the
preceding 370 to 485 d formed the secondary anoestrus
group. Eight of the 16 bitches at late anoestrus (days
165-280) were assigned to the late anoestrus group, and
another 8 were chosen randomly for the control group
untreated). Cabergoline tablets (Dostinex, Pharmacia,
Italy) with 0.5 mg of the active substance were dissolved
in distilled water (10 µg/mL) at 37°C. This aqueous
solution was prepared fresh and administered orally
using a medical dropper within 15 min after preparation.
Cabergoline administration was continued until the 2 d
after the onset of the first signs of pro-oestrus, when the
progress of pro-oestrus was confirmed by both external
signs (i.e. vulvar swelling and serosanguinous
 discharge) and vaginal cytology (i.e. >50% intermediate
or superficial type epithelial cells), or until day 42
without signs of pro-oestrus. All the bitches were
examined daily for the presence of vulvar swelling and
serosanguinous vaginal discharges until the onset of pro-
oestrus. Vaginal smears were taken every 4 d until the
onset of pro-oestrus and daily during the pro-oestrus and
anoestrus until the 2 d of meta-oestrus. Vaginal smears
were stained with Giemsa (Merck, Darmstadt, Germany)
and were evaluated for cell types and approximate
percentages of epithelial cells, as previously described
(7).

Female dogs were placed together with an active
male dog every day from day 2 of pro-oestrus and their
behaviour was observed. A bitch was considered to be in
oestrus when showing tail deflection, allowing the male
to mount and initiating coitus. Oestrus was confirmed
also by epithelial exfoliative cytology (>90% cornification).
The 1 d of meta-oestrus was the day characterised by refusal of male and was confirmed by an
abrupt 10 to 60% decrease in superficial cells and the
appearance of intermediate and parabasal cells in
vaginal smears (21). For the determination of the
behavioural oestrus period, bitches were either mated
naturally or inseminated with fresh semen on the 1 d of
acceptance of the male and every day thereafter until
refusal. Breeder male dogs with proven fertility, pre-
determined by the company, were used in all mating and
artificial inseminations.

Blood samples (7 to 10 mL) were collected by
venipuncture of the jugular vein, centrifuged at 3 000 x
for 15 min within 30 min after collection, and multiple
plasma aliquots (500 µL) were stored at -20°C until
assay. Blood samples were taken from each animal daily
during the first 5 d of behavioural oestrus and serum
progesterone (P4) concentrations were measured by
radioimmunoasay using a commercial kit (DSL-3900,
DSL, USA). Ovulation was considered to have taken
place if P4 concentrations were ≥5 ng/mL or if
pregnancy occurred (24). Following mating, the dogs
were observed until delivery and the litter size was
reported. Pregnancy rate was determined as the ratio of
delivered bitches to those showing behavioural oestrus
signs and mated.

Statistical analysis. Pregnancy, ovulation, and
induced oestrus rates were analysed by the Chi-square
test. The mean pro-oestrus time, the behavioural oestrus
time, the duration of treatment, and the serum P4 levels
were analysed by means of one-way ANOVA in the
SPSS patch programme and significance assessment
between the groups was carried out by the Duncan-test.
The results are presented as mean ± standard deviation
(mean ± SD).

Results

Six of 8 dogs (75.0%) and 7 of 8 dogs (87.5%) in
persistent anoestrus and late anoestrus groups showed
pro-oestrus, respectively (P=0.05). The mean duration of
treatment was found significantly shorter in bitches with
anoestrus (range 4 to 14 and mean 10.3±4.23
d) than that in those with late anoestrus (range 12 to 45
and mean 30.6±13.02 d, P<0.0001, Table 1). Signs of
pro-oestrus were observed in two late anoestrus bitches
on the 3 d from the end of treatment (day 45). In all
constantly responded bitches, pro-oestrus progressed to
behavioural oestrus. Mean pro-oestrus and behavioural
oestrus durations, serum P4 concentrations on day 5 of
oestrus, ovulation rates, pregnancy rates, and mean litter
sizes in secondary anoestrus, late anoestrus, and control
groups were found to be similar (Tables 1 and 2,
P=0.05). The mean time intervals between the beginning
of the treatment and pro-oestrus in the treatment groups
were found to be significantly shorter, compared to
those in the control group (Table 1, P<0.0001). None of
the dogs had any adverse gastrointestinal effects
associated with cabergoline administration.
Table 1
Comparison of data obtained from dogs in the secondary anoestrus, late anoestrus, and control groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Duration of pro-oestrus (d)</th>
<th>Onset of experiment to pro-oestrus (d)</th>
<th>Dogs responding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary anoestrus</td>
<td>8</td>
<td>7.0±1.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.3±4.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.0% (6/8)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Late anoestrus</td>
<td>8</td>
<td>8.3±2.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.6±13.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.5% (7/8)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>8.1±2.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.5±16.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NA</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup>: within columns, means with no common letters are statistically different (P<0.0001).
NA: does not apply.

Table 2
Some reproductive results of bitches showing behavioural oestrus symptoms in the secondary anoestrus, late anoestrus, and control groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Secondary anoestrus</th>
<th>Late anoestrus</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Duration of behavioural oestrus (d)</td>
<td>12.2±2.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.71±5.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.0±2.67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma P4 at the 5th d of oestrus (ng/mL)</td>
<td>8.4±6.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.0±3.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.6±5.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Litter size</td>
<td>4.8±2.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.8±0.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0±2.38&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bitches ovulated</td>
<td>83.3% (5/6)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100.0% (7/7)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100.0% (8/8)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bitches pregnant</td>
<td>83.3% (5/6)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.7% (6/7)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.5% (7/8)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>: Values within rows do not differ (P>0.05).

Discussion

Although DA can provide a successful physiological tool to improve canine reproduction performance, the precise mechanism of DA action in EI is not yet completely understood. Formerly, it was believed that the induction of oestrus in bitches by DA was the result of the suppression of prolactin (PRL) secretion. But, it has been shown in bitches receiving a low dose of bromocriptine that there was significant shortening of the inter-oestrus interval without a decrease in plasma PRL concentration (2). It has been demonstrated also that shortening of the inter-oestrus interval by bromocriptine in a dose that also lowers plasma PRL concentration is associated with an increase in plasma FSH concentration without a concomitant increase in plasma LH concentration (17).

Our results extend observations that administration of cabergoline is a safe and effective treatment for secondary anoestrus in bitches, as reported previously (9, 15). Our data also demonstrate the efficacy of a dose quite lower (0.6 µg/kg/d) than the prolactin-lowering dose of cabergoline in this regard. Jöchle et al. (16) reported that 1.25 µg/kg/d oral dose of cabergoline was ineffective in suppressing or terminating lactation in bitches. They treated nursing bitches with 0, 1.25, 2.5 or 5.0 µg/kg/d of cabergoline for 5 d, commencing on day 8 of lactation. Treatments with the two higher doses caused a reaction and regression of the mammary glands in a dose-related fashion, resulting in cessation of lactation at the dose of 5 µg/kg body weight (16). Even if we have not evaluated the PRL levels, the results of our study support the hypothesis (2) that the initiation of oestrus is not triggered by a decline in plasma PRL concentration, but are probably due to some other action of DA.

Provoked oestrus in bitches with the administration of gonadotropin and oestrogen preparations failed to result in reliable and reproducible data (20). In a study using human menopausal gonadotropin (hMG), pro-oestrus signs were not marked in 30% of the dogs and lasted only 2-3 d (22). Similarly, compared to spontaneous oestrus, 18% of cases of bromocriptine induced oestrus were characterised by shorter duration, less pronounced vaginal bleeding, and fewer clinically expressed oestrus symptoms (25). We have found that the cycles resulting from cabergoline-induced termination of anoestrus were normal in both late anoestrus and secondary anoestrus groups, since the resulting pro-oestrus and oestrus parameters, serum progesterone levels at the 5 d of oestrus, fertility, and litter sizes were very similar to those of control bitches.

It has been demonstrated that the effect of prolactin-lowering doses of cabergoline on the termination of normal obligate anoestrus in dogs varies according to the stage of anoestrus (11, 21), and that the effect occurs more rapidly in late anoestrus (6±1 d) than in mid (14±3 d) or early (20±3 d) anoestrus (21). In our study, mean duration of treatment in late anoestrus bitches (30.6±13.02 d) was quite longer than that of previous experiments (11, 21). The presumptive reason for this difference can be the lower dose of cabergoline applied in our study (0.6 µg/kg/d) than the dose (5 or 6 µg/kg/d) used by Jöchle et al. (16).
μg/kg/d) used by other researchers (11, 21). It has been suggested that there would be a relation, even in not induced oestrus rate, between cabergoline dose and response time (5). The reason, why the duration of treatment was significantly shorter in bitches with secondary anoestrus than in those at late anoestrus in this study could not be explained. In studies with cabergoline, some side effects such as vomiting in dogs have been reported in the rate of 0%-25% (1, 5, 10, 11). No vomiting or other side effects have been observed in our study.

It is concluded from the present study that administration of 0.6 μg/kg/d of cabergoline is a safe and effective treatment for secondary anoestrus in bitches.

References