Cabergoline applications in early and late anoestrus periods on German Shepherd dogs

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SUMMARY

This study was performed with 18 German Shepherd female dogs whom previous cycles were recorded. Bitches were separated into three groups: two treated groups and a control one. The first group was selected from 7 bitches in early anoestrous period, i.e., at the stage 108 to 124 days after the beginning of their last pro-oestrus. The second group consisted in 6 bitches in late anoestrous period, i.e., at the stage 160 to 187 days after the beginning of their last pro-oestrus. The control group was constituted by 5 untreated bitches which reach their spontaneous pro-oestrus 173 to 204 days after the beginning of their preceding pro-oestrous.

Cabergoline was orally given to each of the dogs of the first and second groups at a daily dose of 6 mg/kg until the onset of pro-oestrus symptoms or maximally up to 14 days. The mean time separating the beginning of the preceding pro-oestrus and the initiation of the treatment was 116.1±5.8 and 171±9.2 days for the first and the second groups, respectively. The mean duration of cabergoline treatment and the mean number of bitches in pro-oestrus 14 days after the onset of the treatment was 11.3±2.8 days (5/7) and 6.6±0.8 days (6/6) in the first and the second group, respectively.

When considering the cycle following the treatment, the plasma levels of progesterone ranged between 2.5-8 ng/ml on the 4th day of oestrus in all groups. The mean duration of the pro-oestrus (7.6±1.0, 8.7±1.0, 8.0±1.0 days, for the first, second and control groups, respectively) and oestrus (7.0±1.0, 7.0±0.8, 8.0±1.0 days, for the first, second and control groups, respectively), the mean number of pregnant bitches (5/7, 6/6, 5/5, for the first, second and control groups, respectively), the mean time lag between births and the following pro-oestrus (224.2±14.5, 215.5±14.1, 218.2±6.0 days, for the first, second and control groups, respectively) and mean litter size (6.8±1.30, 6.0±0.89, 7.2±1.5, for the first, second and control groups, respectively) did not differ between groups.

In conclusion, cabergoline applications are effective to induce oestrus without deleterious effect in bitches, the efficacy being increased in late anoestrous.

KEY-WORDS : bitches-anoestrus-cabergoline.

Introduction

The reproductive cycle of the bitch is characterised by a long inter-oestrus interval ranging between 5 and 12 months [5,6]. Luteal phase continues for about 65 days in pregnant bitches and 75-90 days in non-pregnant ones. The anoestrous period of the cycle begins when the progesterone plasma concentration falls below 1 ng/ml [6,8].

Primary anoestrus is generally characterised by the non-apparition of oestrus in 18-24 months aged bitches, while secondary anoestrus is characterised when oestrus is not observed although a period of 12 months had passed after the former oestrus. Bitches in anoestrus should be basically treated after the factor leading to anoestrus is identified. However, the estrous cycle can be practically stimulated also in the situations in which the leading factor cannot be understood [9].

Methods based on PMSG and hCG treatments [2,10,13], oestradiol or diethylstilbestrol (DES) applications followed by gonadotrophin treatments [3,10], GnRH treatments [4,10]
were experimented to induce oestrus in bitches without satisfactory results. Nearby, inter oestrus interval was shown to be shortened by the use of cabergoline and bromocriptine, which are dopamine 2 receptor agonists, and with serotonin antagonists, which exhibit anti-prolactin characteristics during luteal and anoestrous periods [18-20, 23]. Hence, cabergoline given to pseudopregnant and/or lactating bitches was shown to cause pro-oestrus bleeding [1]. JÖCHLE et al [16] obtained high pregnancy rates from induced oestrus with cabergoline in the anoestrous bitches [16]. GÜNAY and SOYLU [10], found oestrus can be induced with 6 mg/kg dosage of cabergoline. JEUKENNE and VERSTEGEN [15] reported that the dioestrus period was significantly shortened by applying cabergoline, 30 days later after LH release, although they could not obtain pregnancy.

In this study, we aimed to compare the ratio of oestrus induction resulting from cabergoline treatments according to the stage of anoestrus, early and late anoestrus. The characteristics of the cycle induced by cabergoline (duration of the pro-oestrus and oestrus period, number of pregnant bitches, litter size and time lag between births and following pro-oestrus) was compared with that of the spontaneous cycle of untreated bitches.

Material and Methods

ANIMALS

Eighteen female German Shepherd Dogs were used. The dogs were subjected to clinical examination at the beginning of the trial and healthy dogs were selected. They were fed with dry food once a day and water was supplied ad-libitum. The previous records of the dogs used in the study were reviewed and dogs without any reproductive problem during previous cycles and not mated in their last oestrus were used.

EXPERIMENTAL DESIGN

Bitches were allocated into three groups. The control group was constituted by 5 untreated bitches which terminates anoestrus 173 to 204 days after the beginning of previous pro-oestrus. The first group consisted in 7 bitches which were treated with cabergoline in early anoestrus, i.e., from 108 to 124 days after the beginning of previous pro-oestrus. The second group was formed from 6 bitches which were treated with cabergoline in late anoestrus, i.e., from 160 to 187 days after the beginning of previous pro-oestrus.

The bitches were weighed at the beginning of the trial and the first day of drug application was considered as day 0. Cabergoline (Galastop.Vetem, Milano) was orally given with the help of a pipette at a daily dose of 6 mg/kg. This application was continued until the beginning of pro-oestrus symptoms or maximally up to 14 days. Drug administration was applied at the same hour every day before feeding when the bitches were hungry, considering the possibility of vomiting. All groups were daily subjected to vaginal cytology from the onset of pro-oestrus to the metoestrous periods of the cycle following cabergoline treatment. The dominance of parabasal cells besides intermediate cells in vaginal cytology was evaluated as anoestrus. Increase in the rate of intermediate cells, observation of erythrocytes, vaginal bleeding and vulva oedema were appraised as pro-oestrus, whereas the dominance of superficial cells (above approximately 80%) and the acceptance of mating the bitches were evaluated as oestrus. Bitches who accepted mating during oestrus were mated with male dogs whose spermatological characteristics were controlled beforehand every other day. Blood samples were obtained before the initiation of cabergoline treatment and from the mated bitches on the 4th day following the onset of oestrus. Bitches whose progesterone levels were below 1 ng/ml were considered to be in anoestrus according the reports of GOBELLO et al [9]. A sudden decline in the rate of superficial cells below 20 %, the prevalence of parabasal cells in the smear samples and refusal of being mated by bitches were accepted as the metoestrous period.

The study was carried out nearly within two years, in different months of the year. The characteristics of the cycle and of the pregnancy initiated during the cycle induced by cabergoline treatment (duration of pro-oestrus, oestrus, number of pregnant bitches, litter size and time lag between births and following pro-oestrus) were monitored and recorded.

SMEAR SAMPLING AND DRUG APPLICATIONS

Vaginal smear was dyed with Giemsa (Merck, Darmstadt). Progesterone levels were evaluated using EIA techniques (Target, Canine Ovulation Timing Kit, BioMetallics, USA).

STATISTICAL ANALYSIS

A Chi-square test was used to compare the number of bitches in pro-oestrus 14 days after the onset of experiment and the number pregnant bitches between groups. The characteristics of the cycle and of the pregnancy initiated during the cycle induced by cabergoline treatment (mean duration of the pro-oestrous and oestrous periods, mean number of pregnant bitches, mean litter size and mean time lag between births and following pro-oestrus) were compared with that of the spontaneous cycle of untreated using a Student T-test. All data are presented as mean ±SD.

Results

The mean values (± SD) of the time lag between the onset of the last pro-oestrus and the onset of cabergoline treatment, the number of bitches in pro-oestrous, duration of pro-oestrus and oestrous periods, the number of pregnant bitches, the time lag between births and the following pro-oestrus and litter size were shown in Table I.

Any side effects such as vomiting were observed following cabergoline applications. It was observed that drugs were taken easily by bitches. Five of the seven treated bitches of the first group (early anoestrus) came into pro-oestrus after a mean of 11.28 ± 2.8 days of treatment. In two bitches of the first group (early anoestrus) came into pro-oestrus after 14 days of treatment. In two bitches of the first group (early anoestrus) came into pro-oestrus after 14 days of treatment.
days since pro-oestrus could not be induced. The mean duration of cabergoline treatment was lower in late anoestrus treated bitches, ranging between 6 and 8 days (6.6 ± 0.8 days).

No statistical difference was observed between groups when considering the duration of pro-oestrus and oestrous periods of the following cycle. When considering the pregnancy initiated during the following cycle, the number of pregnant bitches, the litter size and the time lag between births and following pro-oestrus did not differ among groups. (Table I).

The leukocyte ratio determined in vaginal cytology at the early pro-oestrus stage of the bitches in the first group was little bit more intensive than control. No marked difference was observed in the vaginal smear results of the three groups during the oestrus period. The plasma concentration of progesterone ranged between 2.5-8 ng/ml in 18 bitches at the 4th day of the first oestrus. All litters were determined to be healthy after birth.

### Discussion

Satisfactory results could not be obtained with treatment based on PMSG, GnRH, DES and Gonadotrophin applications when compared to cabergoline applications in terms of practical application and adverse effects for stimulating oestrus and ovulation in bitches [10]. In our study, positive results were obtained with cabergoline in female German Shepherd Dogs with regard to stimulating oestrus and ovulation and obtaining pregnancy. Some researchers [1,16] reported that positive results could not be obtained from cabergoline during anoestrus in normal cycle, but that it could be used for ending the abnormally long anoestrus periods.

In previous studies, cabergoline applications were used to induce oestrus cycle in bitches with pseudopregnancy problem or with prolonged lactation [16], in bitches with prolonged anoestrus (1,9) and in bitches at the dioestrus stage [15]. Some previous studies were focused on the efficacy of cabergoline treatment according to the stage of anoestrus [1, 9, 10, 24] and have shown that the stage of anoestrus an important criterion affecting the results [24].

VERSTEGEN et al. [24] and ARBEITER and BARSCH [1] reported that vomiting was observed in about 25% of the bitches. However, vomiting or another adverse effect was not observed in our study. Side effects and vomiting effects induced by an action on the central nervous system were reported to be negligible since cabergoline do not easily penetrate the blood-brain barrier [1,11,16].

In our study, we aimed to compare the effect of treating bitches with cabergoline in early or late anoestrus. We have shown that a pro-oestrus was induced in 5 of the 7 bitches treated in early anoestrus and in all the 6 bitches treated in late anoestrus, 14 days after the onset of the cabergoline treatment. The mean duration of cabergoline treatment was shorter in the late anoestrus than early anoestrus (6 vs 11 days).

VERSTEGEN et al. [24] reported that a 14-days cabergoline treatment was not sufficient to induce pro-oestrus in the bitches treated at early and medium stages of anoestrus. Indeed, the former authors divided the anoestrous period into 3 stages and reported that pro-oestrus can be induced after 20, 14 and 6 days in the bitches respectively treated at early, middle and late anoestras stages, respectively. GOBELLO et al. [9] also reported that a new cycle can be induced in 4 of 6 bitches treated in late anoestrus after 4, 5, 8 and 28 days while it was only induced after 18 and 34 days in 2 bitches treated in early anoestrus. JEUKENNE and VERSTEGEN [15] pointed out that cabergoline should not be used at very early stage of anoestrus since the implantation is prevented by an incomplete involution of the uterus.

When comparing the cycle induced by the application of cabergoline to the spontaneous cycle of untreated bitches, we have shown that the duration of the pro-oestrus and oestrus

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**Table I.** — The characteristics of the cycle and of the pregnancy initiated during the cycle (i) induced by cabergoline treatment applied in early anoestrus (group I) and late anoestrus (group II) or (ii) spontaneously occurring in control bitches.

<table>
<thead>
<tr>
<th></th>
<th>Time lag between beginning of the preceding pro-oestrus and the cabergoline treatment (days)</th>
<th>Cabergoline treatment duration (days)</th>
<th>Number of bitches in pro-oestrus 14 days after the onset of the treatment</th>
<th>Duration of pro-oestrus (days)</th>
<th>Duration of oestrus (days)</th>
<th>Number of pregnant bitches</th>
<th>Litter size</th>
<th>Time lag between births and following pro-oestrus (days)</th>
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<tr>
<td><strong>Group I</strong></td>
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<tr>
<td>(Early Anoestrus)</td>
<td>(n=7)</td>
<td>116.1±5.81^a</td>
<td>11.3±2.81^a</td>
<td>5/7</td>
<td>7.6±0.89</td>
<td>7.0±1.00</td>
<td>5/7</td>
<td>6.8±1.30</td>
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<tr>
<td><strong>Group II</strong></td>
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<tr>
<td>(Late Anoestrus)</td>
<td>(n=6)</td>
<td>171.8±9.15^b</td>
<td>6.6±0.81^c</td>
<td>6/6</td>
<td>8.7±1.03</td>
<td>7.0±0.66</td>
<td>6/6</td>
<td>6.0±0.69</td>
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<td><strong>Control</strong></td>
<td></td>
<td>Does not apply</td>
<td>Does not apply</td>
<td>Does not apply</td>
<td>8.0±1.00</td>
<td>8.0±1.00</td>
<td>5/5</td>
<td>7.2±1.48</td>
</tr>
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^a,b Different superscripts in the same column indicate significant differences (p<0.01). Data expressed as mean ± SD.
phases of the cycle were not affected by the treatment. Mean plasma progesterone levels evaluated on the 4th day following the onset of oestrus were found to range between 2.5 and 8 ng/ml. It is generally reported that these values range between 2 and 10 ng/ml [6,7,12,25]. Kirmizi [17] who observed the oestrus cycles in German Shepherd Dogs for 8 years determined that the mean interval between birth and following pro-oestrus is 220 days. In our study, we have shown that this interval is 224 ± 14.5 days in the bitches treated with cabergoline during the early anestrus, 215.5 ± 14.1 days in the bitches treated with cabergoline during the late anestrus and 218.2 ± 6.0 days in control bitches.

The mechanism of action of cabergoline could involve the reinitiation of gonadotropin secretions resulting for a decrease of prolactin secretion since prolactin was shown to inhibit GnRH and Gonadotrophin secretion in human beings and some mammalians [14,22,26].

In conclusion, positive results could be obtained from cabergoline applications to induce oestrus in bitches in early and late anestrus. It was concluded that there was no significant difference between the induced and the spontaneous oestrus cycle. Additionally, cabergoline administration did not affect the pregnancy initiated during the cycle induced by cabergoline treatment (number of pregnant bitches, litter size and time lag between births and the following pro-oestrus).

References