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# A study of two protocols combining aglepristone and cloprostenol to treat open cervix pyometra in the bitch

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**Abstract**

To compare the efficacy and safety of two protocols using a combination of aglepristone and cloprostenol for the treatment of open cervix pyometra in the bitch and to describe the progesterone ($P_4$) serum profiles before and during treatments, 15 bitches were randomly allocated into two treatment groups: I ($n = 8$): aglepristone was administered at 10 mg/kg, s.c., on Days 1, 3, 8, and 15 (if not cured), combined with cloprostenol at the dose of 1 μg/kg, s.c., on Days 3 and 8, and II ($n = 7$): received the same treatment with aglepristone as Treatment I but cloprostenol on Days 3, 5, 8 10, 12, and 15 (if not cured). Before the beginning of the treatments and then on Days 8, 15, and 29 all bitches were evaluated for clinical signs, side effects, hemogram, serum $P_4$ concentrations, and uterus diameters. Bitches in both treatment groups, with ($n = 6$) or without ($n = 9$; $\geq$1.2 ng/ml) initial basal $P_4$ serum concentrations, achieved treatment success without side effects and no significant differences, either on Day 15 (6/8 for Treatment I and 4/7 for Treatment II) or on Day 29 (2/8 for Treatment I and 3/7 for Treatment II). In both treatments groups, clinical signs, blood parameters, and uterine diameters improved to normal values throughout the experiments. A significant interaction between day and treatment was found for percentage change in $P_4$ when all bitches were considered together. Redevelopment of pyometra in the next estrous cycle occurred in 20% of the bitches. One nonrecurrent bitch was mated and whelped a normal litter. It is concluded that these two combined protocols proved to be efficient and safe in reversing clinical signs of open cervix pyometra independently of initial $P_4$ concentrations and

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that the number of cloprostenol administrations seemed to have an effect on $P_4$ serum changes throughout treatments.


*Keywords:* Pyometra; Bitch; Aglepristone; Cloprostenol

## 1. Introduction

The cystic endometrial hyperplasia—pyometra complex (CEH-P) is a common progesterone ($P_4$)-dependent disease of the genital tract that appears clinically either in the diestrous or anestrous period of the canine estrous cycle [1–4]. Medical treatment of CEH-P is usually required for bitches intended for breeding. Either prostaglandins (PG) or antiprogestins have been proved to be effective for this purpose [3,5–8]. Prostaglandins increase myometrial contractions, may enhance cervical relaxation and have a luteolytic effect after Day 5 of diestrus, decreasing serum $P_4$ concentrations [2]. Cloprostenol sodium is a synthetic PG F2α analogue that has a luteolytic effect and potent uterotonic activity [2,8].

Antiprogestins are synthetic steroids which bind with great affinity to $P_4$ receptors without any effects of $P_4$ [9]. Aglepristone (RU 534) is an antiprogestin, recently marketed for veterinary use, which competes for uterine receptors with a fixating rate three-fold that of $P_4$ in the bitch [10]. In human beings, a combination of antiprogestin (mifepristone, RU 486) and PGs have been successfully used to induce abortion [11]. A combination of PG and aglepristone has shown the best results for the treatment of canine CEH-P as recently reported in two studies [12,13]. In the first study, an improvement in success rate of 22 and 32% was found on Days 14 and 28, respectively, when compared with antiprogestin-treated bitches [12]. Progesterone serum concentrations during these combined protocols, although potentially useful to describe their mechanism of action, have not been reported. Moreover, further work is necessary to determine the optimum administration of these combined treatments.

Therefore, the objective of this study was two-fold: to compare the clinical efficacy and safety of two different administration intervals for cloprostenol combined with aglepristone for the treatment of open cervix CEH-P, and to describe the $P_4$ serum profiles before and during treatment in this species.

## 2. Materials and methods

### 2.1. Animals

Fifteen mixed and purebred bitches, ranging from 16 months to 15 years of age, weighing 4–50 kg, with open cervix CEH-P (defined as an enlarged fluid-filled uterus and vaginal discharge) were recruited and included in this study. Diagnosis of CEH-P was confirmed by routine uterine ultrasound findings [14]. All the bitches had serum urea and creatinine <60 and 1.5 mg/dl, respectively, after restoration of normal hydration.

### 2.2. Procedure

The bitches were randomly allocated to one of the following groups: Treatment I ($n = 8$): aglepristone (Alizine®, Virbac, Carros, France) was administered at 10 mg/kg, s.c., on Days 1, 3, 8, and 15 (if not cured) and cloprostenol (Estrumate®, Schering Plough, Bs. As, Argentina) far from feeding time was administered at the dose of 1 μg/kg, s.c., on Days 3 and 8; or Treatment II ($n = 7$): the bitches received the same treatment with aglepristone as for Treatment I, but the cloprostenol (same dose) was administered in the same way on Days 3, 5, 8, 10, 12, and 15 (if not cured). Day 1 was considered as the day of presentation of the bitch.

A combination of amoxycillin–clavulanate at 12.5 mg/kg (bid p.o., Clavamox®, Pfizer, Bs. As, Argentina) and supportive hydration were administered during both therapeutic protocols. Before the beginning of the treatments on Day 1 and then on Days 3, 8, 15, and 29 (if not cured), all bitches were evaluated for body weight and temperature, hydration, anorexia, polyuria/polydipsia, uterus total, and lumen diameters assessed by ultrasonography (Pie Medical S100, 5 MHz transducer, Maastricht, The Netherlands), and vulvar discharge. On Days 1, 8, 15, and 29 (if not cured), blood samples were taken for hemogram and serum $P_4$ concentrations (Coat-A-Count, DPC®, Los Angeles, CA) determinations. Animals were observed for possible side effects during the treatments. All the bitches were followed up to their next estrous cycle.

### 2.3. Statistical analyses

Categorical data for the frequency of bitches achieving clinical success (defined as recovery to general health and ultrasonographic observation of normal uterus) and side effects either for Treatments I and II, or for bitches with initial basal or nonbasal $P_4$ serum concentrations were analyzed by PROC FREQ [15] on Days 15 or 29 (bitches not cured on Day 15). For this purpose, $P_4$ serum concentrations on Day 1 were categorized as basal or nonbasal ($<$ or $\geq$1.2 ng/ml, respectively).

Percent change of serum $P_4$ concentrations ((final value [Days 8, 15, or 29]−initial value [Day 1]/initial value) × 100) was analyzed by least-squares analysis of variance using the General Linear Model procedure PROC GLM [15]. The mathematical model included the main effects of treatment (I or II) and day (8, 15, or 29) and the treatment by day interaction. Descriptive statistics of all parameters assessed were analyzed by PROC MEANS [15] and expressed as LSM ± S.E.M. The level of significance was set at $P < 0.05$.

## 3. Results

All bitches in Treatments I (8/8) and II (7/7) achieved treatment success either on Day 15 (6/8 for Treatment I and 4/7 for Treatment II) or 29 (2/8 for Group I and 3/7 for Treatment II) of the protocols. No significant differences in achieving success was found between treatments or initial $P_4$ concentrations either on Day 15 or 29. None of the bitches showed either systemic or local side effects in relation to the treatment (0/15).

Vulvar discharge was increased in all the bitches within the 24–48 h after the first administration of aglepristone with an improvement in general health condition. Body temperature, hydration, appetite, and polyuria/polydipsia began to improve markedly to normalcy from Day 3 in both groups. Hemogram parameters had a clear tendency toward normal values at the end of the treatments, being the white blood cells within physiological range at that time (Fig. 1). Uterine diameters diminished to normal size and the lumen was undetectable or without contents on Days 15 or 29 in both groups (Fig. 2).

Progesterone serum concentrations before and during Treatments I and II in bitches either with basal or nonbasal initial $P_4$ are represented in Fig. 3. Progesterone showed a decreasing tendency in three of the four subgroups.

A significant interaction between day and treatment was found for $P_4$ percentage change when all the bitches were considered together and when only the bitches with nonbasal initial $P_4$ concentrations ($n = 9$) were analyzed ($P > 0.05$). The number of bitches ($n = 6$) with basal initial $P_4$ concentrations was statistically insufficient to be analyzed.

Redevelopment of CEH-P in the next estrous cycle occurred in 3/15 old bitches (7–15 years old); two of these had had more than two previous episodes of CEH-P. One bitch was ovariohysterectomized before her next cycle. Nonrecurrence in the next cycle occurred in 11/15 bitches; four of these were treated with amoxicillin during the next open cervix period (proestrus and estrus). One (2 years old) of these four bitches was mated and whelped a normal litter.

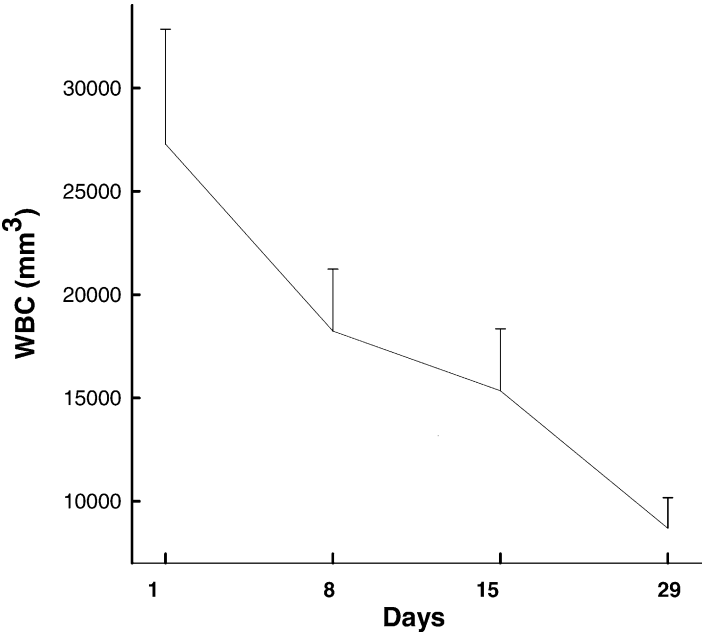


Fig. 1. Least square means of the white blood cells (WBC) of 15 bitches suffering cystic endometrial hyperplasia—pyometra complex treated with two combined protocols of PG and aglepristone before and during treatments. No significant differences in WBC were found between treatment, so they were represented together. Bars on symbols represent the corresponding S.E.M.

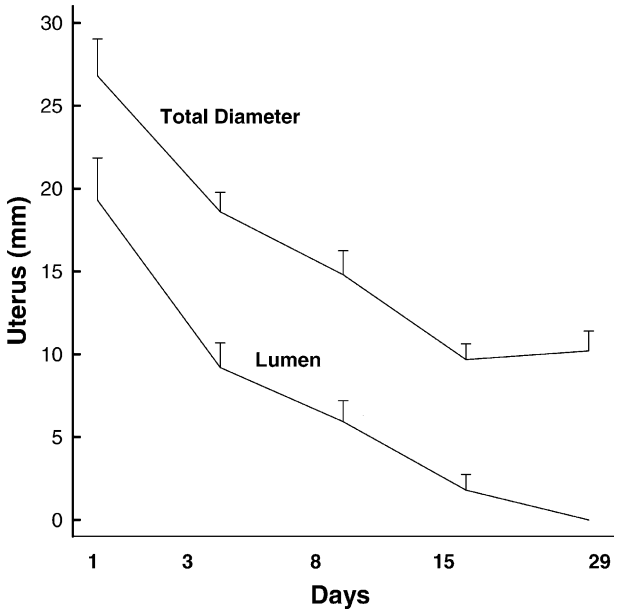


Fig. 2. Least square means of uterine total and lumen diameters assessed by ultrasonography of the same animals ($n = 15$) before and during the treatments. Bars on symbols represent the corresponding S.E.M.

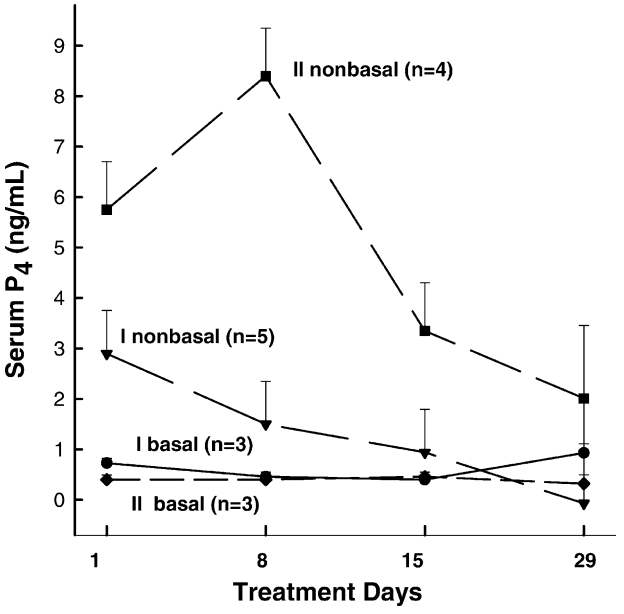


Fig. 3. Least square means of serum $P_4$ concentrations of the same animals ($n = 15$) divided into four subgroups according to treatment (I or II) and initial (Day 1) $P_4$ concentrations (basal or nonbasal, < or $\geq$1.2 ng/ml, respectively) before and during treatment. Bars on symbols represent the corresponding S.E.M.

## 4. Discussion

The proportion of bitches with initial basal $P_4$ concentrations in the present trial was higher than that reported in two previous studies [3,12]. Although this finding was probably incidental, it does confirm that this complex can become clinically evident either during diestrus or anestrus [1,6].

In line with a recent study but in contradiction with a previous one, in which only aglepristone was used, no relationship between initial basal $P_4$ concentrations and treatment success was found in this study [12,16].

Some bitches were cured without achieving basal $P_4$ serum concentrations at the end of the treatments. Moreover, pregnancy termination with aglepristone in the bitch occurs in the presence of high $P_4$ serum concentrations [17]. These findings seem to confirm that antiprogestins decrease intrauterine $P_4$ through the number or sensitivity of $P_4$ receptors and not through the serum concentrations of this hormone [12]. The initial serum rise (on Day 8) of a nonbasal group could be explained by the blocking of uterine receptors and the consequent elevation of $P_4$ in blood. Ecbolic PG effect could have also contributed to success in these cases.

More difficult to explain is the clinical improvement in bitches with basal $P_4$ concentrations throughout treatments. Although the uterotonic effect of PG could have caused the success in these cases, clinical improvement and the increase in the vaginal discharge was seen before the first treatment with PG on Day 3.

Although in the present study, cloprostenol was administered at 48 h or longer intervals, the results on Day 15 (6/8 and 4/7 for Treatments I and II, respectively) were better than those reported in another study (50%), using the same drug and doses on a daily basis [12]. In the current study, based on two treatment protocols for cloprostenol, the number of treatments did not seem to be crucial in the resolution of the clinical cases, although a larger number of treated bitches would be necessary to either confirm or reject this initial finding. Conversely, the number of PG treatments seemed to influence $P_4$ serum changes during the different days of the study.

In disagreement with two recent studies [2,12], but in line with a previous report in which only aglepristone was used [5], no side effects were reported in this study. The administration of a low dose of a potent PG at a time far from feeding could have accounted for these differences. Moreover, all the bitches were in a better condition on the day of the first cloprostenol injection (Day 3) than at the beginning of the treatments.

In this study, recurrence rate was higher than in a similar previous study in which no redevelopment appeared in 12 bitches that were followed up to the next estrous cycle [12], and also higher than in one study in which only aglepristone was used [16]. The higher recurrence rate may be due to the advanced age of most of the bitches used in the present study. Conversely, the present results were in the lower limit range of recurrence for bitches treated only with PG (10–77%) [6,7]. These findings suggest that the uterotonic effect of PG does not reverse CEH and many dogs may have a decrease in clinical signs towards subclinical levels which then becomes undetectable. We hypothesized that antiprogestin treatment might better manage endometrial abnormalities depending on the depth of inflammatory infiltration measured using Dow's classification [18]. Conversely, a study in

which histological examination of uteri was carried out revealed no differences between 6 days-antiprogestin-treated and control bitches [3].

Consistent with a previous report [5], fertility after treatment did not seem to be affected by endometrial pathology in the young bitch that was mated. This young bitch was one of the most representative of the population for which these combined protocols are mainly intended in clinical practice. Further studies including larger number of bitches of different ages, with or with out past episodes of CEH-P are required to evaluate fertility after treatment.

We concluded that these two combined protocols proved to be efficient and safe in reversing clinical signs and abnormal uterine ultrasonographic findings in bitches suffering CEH-P, independently of initial $P_4$ serum concentrations. Also, the number of PG administrations seemed to have an effect on $P_4$ serum concentrations throughout treatments. Further work with a larger number of bitches is necessary to confirm these initial findings.

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