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Fertility outcome after medically treated pyometra in dogs

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ABSTRACT

Cystic endometrial hyperplasia-pyometra complex (CEH/P) is a challenge in canine reproduction. Present study aimed to assess fertility after medical treatment. One-hundred-seventy-four bitches affected by CEH/P received aglepristone on days 1, 2, 8, then every 7 days until blood progesterone < 1.2 ng/mL; cloprostenol was administered on days 3 to 5. Records were grouped according to bodyweight (BW): small (< 10 kg, n = 33), medium (10 ≥ BW < 25 kg, n = 44), large (25 ≥ BW < 40 kg, n = 52), and giant bitches (BW ≥ 40 kg, n = 45). Age; success rate; aglepristone treatments number; relapse, pregnancy rates; diagnosis-relapse, -first, -last litter intervals; litters number after treatment, and LS were analyzed by ANOVA. Overall age was 5.14 ± 1.75 years, without difference among groups. Treatment was 100% successful, without difference in treatments number (4.75 ± 1.18), relapse (15/174, 8.62%) and pregnancy (129/140 litters, 92.14%) rates, intervals diagnosis-relapse (409.63 ± 254.9 days) or -last litter (418.62 ± 129.03 days). The interval diagnosis-first litter was significantly shorter (163.52 ± 51.47 days) and longer (225.17 ± 90.97 days) in small and giant bitches, respectively. Overall, 1.47 ± 0.65 litters were born after treatment. Expected LS was achieved in each group, as shown by ΔLS (actual-expected LS by breed, overall −0.40 ± 1.62) without differences among groups. Concluding, CEH/P affects younger dogs than previously described. Relapses were rarer than previously reported. Medical treatment with aglepristone+cloprostenol is effective and safe, preserving subsequent fertility, as demonstrated by negligible changes in LS.

Keywords: Aglepristone; cloprostenol; dog; fertility; pyometra

INTRODUCTION

Cystic endometrial hyperplasia and pyometra complex (CEH/P) represents a common life-threatening challenge in small animal reproduction [1,2]. Pyometra creates a medical emergency, requiring rapid intervention to prevent its progression to sepsis. If untreated, pyometra may be fatal [3]. Its incidence is reported to be up to 19% in the first 10 years of life in dogs [4]. For this reason, for a very long time, practitioners used to suggest that dog owners neuter female dogs [5]. In fact, due to its specific etiology and being progesterone-dependent [6], pyometra cannot occur in a non-cycling bitch [1,7]; normally, it becomes

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clinically evident in diestrus, when the blood progesterone is high and estradiol is basal [8]. On the other hand, to maintain future fertility, dog breeders may desire medical treatments to solve this burdensome problem affecting mature, young [1] and even nulliparous bitches [9]. Moreover, due to the awareness that surgical sterilization of bitches implies possible related side effects, the considerable incidence of CEH/P has led to the development of medical treatment strategies. In the last 20 years, several studies described different medical protocols based on aglepristone administration, alone or combined with cloprostenol, in attempts to avoid ovariohysterectomy [5,10-13]. Aglepristone is a synthetic steroid which binds progesterone receptors with three times higher affinity than, but without the effects of, progesterone [14]. Although the use of aglepristone in the medical treatment of pyometra has been described [5,10-13], consistent results on subsequent fertility are fragmentary [15]. Evidently, conservative medical treatment, such as that provided by aglepristone and cloprostenol, is not recommended in life-threatening situations that require emergency procedures, such as uterine rupture, peritonitis, severe renal failure, or a significant electrolyte imbalance [13]. The present study aimed to assess fertility outcomes in bitches (*i.e.*, relapse rate, pregnancy rate, and litter size [LS]) after medical treatment for CEH/P using a combination of aglepristone and cloprostenol.

MATERIALS AND METHODS

Ethics

The study was performed in accordance with the Animal Welfare Committee's ethical guidelines, and all procedures were carried out pursuant to the Italian legislation on animal care (DL 116, 27/01/1992) and the European Guidelines on Animal Welfare (Directive 2010/63/EU). Owners' informed consents were obtained for treatment and for use of the data for research purposes.

Animals

This retrospective multicenter study included patients presented to the authors' veterinary care centers, at which the activities are mainly focused on small animal reproduction. Bitches were enrolled if they met the inclusion criteria described below and had a 3-year-follow-up [16]. Data drawn from their clinical history were evaluated retrospectively.

The data for analysis were obtained from the records of 174 bitches that were clinically confirmed to be affected by CEH/P, based on ultrasonography and hemato-biochemical analyses [1,7], and submitted for medical treatment.

Bitches enrolled in breeding programs after medical treatment for CEH/P underwent the following procedures, routinely used in the study's medical centers, for canine breeding management: estrus monitoring with vaginal smears and serum progesterone dosages, artificial insemination with semen evaluation, advanced ultrasonographic monitoring of pregnancy, and prediction of parturition to ensure the best assistance at delivery [17-21].

Procedure

The treatment protocol consisted of aglepristone (10 mg/kg SC, Alizin, Virbac Italia Srl, Italy) administration on days 1 (day of diagnosis), 2, 8, and then repeated every 7 days. Following manufacturer's instruction, in large- and giant-size bitches, the total drug amount (10 mg/kg) was inoculated in different subcutaneous sites, with a maximum 5 mL volume for each injection

site. The repeated administration of aglepristone was ceased when the treatment end-point was achieved, fulfilling the following objectives: recovery of good general health conditions (with leukocytes, transaminase, creatinine, blood urea nitrogen, protein, and electrolyte levels restored to within normal ranges); no uterine lumen enlargement detected on ultrasonographic examination; absence of vulvar discharge, and blood progesterone level < 1.2 ng/mL (basal level) [22]. Cloprostenol (1 μg/kg SC, Estrumate, Schering-Plough Animal Health, USA) was administered daily on days 3–5. Even in cases of repeated administration of aglepristone after day 8, cloprostenol was not administered after day 5. Appropriate symptomatic therapies (*e.g.*, fluid therapy, antibiotics, nonsteroidal anti-inflammatory drugs, and anti-emetic drugs) were given to patients according to their individual conditions. According to previous literature, fluid therapy was administered to correct dehydration and prevent toxic shock [3], choosing a fluid composition based on the specific hydro-electrolytic imbalance.

Treatment of pyometra typically includes the immediate initiation of antibiotic therapy [23]. Based on previous reports [24,25], the antibiotics clavulanate amoxycillin (12.5 mg/kg PO - BID, Synulox, Pfizer Italia Srl, Italy) [7,26] and enrofloxacin (5 mg/kg PO - SID, Enrox Flavour, Virbac Italia Srl) [27,28] were administered for at least 14 days.

Bitches were divided into 4 groups according to body weight (BW): small (BW < 10 kg, n = 33), medium (10 ≥ BW < 25 kg, n = 44), large (25 ≥ BW < 40 kg, n = 52), and giant (BW ≥ 40 kg, n = 45) bitches, and relevant data were recorded. The following data were drawn from the medical records of each bitch: age at diagnosis; number of aglepristone treatments; intervals between diagnosis and relapse, as well as to first and last litters; number of litters after treatment, and LS. The difference from the expected LS, ΔLS, in each patient was calculated as the difference between the actual and the expected LS for the specific breed [29]. Rates of success, relapse, and pregnancy were calculated for each group as follows: successfully treated/treated bitches, relapsed/treated bitches, and pregnant/mated bitches, respectively, expressed as ratios.

Statistical analysis

All data were recorded in Excel 2010 Office (Microsoft, USA) files and mean ± standard deviation (SD) values were calculated for each parameter in each group. To compare the following parameters among the four study groups, ANOVA was used to examine differences in age at diagnosis; success rate; number of aglepristone treatments; relapse rate; pregnancy rate; intervals from diagnosis to relapse, as well as to first and last litters; number of litters after treatment; LS; and ΔLS. Results were considered significant at $p \leq 0.05$. Statistical analysis was performed with the online tool VassarStats: Website for Statistical Computation (http://vassarstats.net, Vassar College, USA).

RESULTS

Enrolled patients belonged to the following breeds: Akita Inu, American Staffordshire Terrier, Basset Hound, Beagle, Bernese Mountain Dog, Bichon Frisé, Border Collie, Boxer, Cane Corso, Cavalier King Charles Spaniel, Chihuahua, Chow-Chow, Cocker Spaniel, Corgie, Dachshund, Dobermann, Dogue de Bordeaux, English Bulldog, English Setter, French Bulldog, German Shepherd, Golden Retriever, Great Dane, Greyhound, Jack Russell Terrier, Labrador Retriever, Leonberger, Maltese, Medium Schnauzer, Newfoundland, Pinscher, Poodle, Rottweiler, Schnauzer, Setter Gordon, Shetland Sheepdog, Shih-Tzu, Siberian Husky, Yorkshire Terrier.

Table 1. Recorded parameters, expressed as mean ± SD values, in the 4 study groups and in the overall population enrolled in the present study

Parameter	Small dogs	Medium dogs	Large dogs	Giant dogs	Overall
Age (yr)	5.15 ± 1.80*	5.41 ± 1.96*	5.10 ± 1.45*	4.89 ± 1.84*	5.14 ± 1.75
Aglepristone administrations	4.78 ± 1.36*	4.84 ± 0.96*	4.69 ± 1.38*	4.69 ± 1.02*	4.75 ± 1.18
Success rate	33/33 (100)*	44/44 (100)*	52/52 (100)*	45/45 (100)*	174/174 (100)
Relapse rate	4/33 (12)*	2/44 (5)*	5/52 (10)*	4/45 (9)*	15/174 (9)
Interval diagnosis to relapse (days)	416 ± 265.07*	635 ± 629.33*	329 ± 131.74*	258.50 ± 94.22*	409.63 ± 254.90
Pregnancy rate	23/25 (92.00)*	32/35 (91.43)*	38/41 (92.68)*	36/39 (92.31)*	129/140 (92.14)
Litters after treatment	1.52 ± 0.51*	1.30 ± 0.64*	1.29 ± 0.46*	1.77 ± 0.81*	1.47 ± 0.65
Interval diagnosis to 1st litter (days)	163.52 ± 51.47*	210.58 ± 88.50*,†	192.92 ± 83.63*,†	225.17 ± 90.97†	198.05 ± 84.27
Interval diagnosis to last litter (days)	398.25 ± 125.35*	373.25 ± 102.66*	415.88 ± 117.20*	487.11 ± 148.70*	418.62 ± 129.03
Litter size	3.87 ± 1.14*	4.52 ± 1.68*,†	5.26 ± 1.54†	6.86 ± 1.69‡	5.13 ± 1.89
ΔLS	−0.12 ± 0.91*	−0.86 ± 1.79*	−0.64 ± 1.76*	0.47 ± 1.18*	−0.40 ± 1.62

Data are shown as mean ± SD or number (%).
SD, standard deviation.
*,†,‡Different superscripts denote statistically significant differences among columns, at $p < 0.05$.

Table 1 present the results, expressed as mean ± SD, for each recorded parameter in all study groups and in the overall enrolled population. The overall age at diagnosis was 5.14 ± 1.75 years, with no statistically significant differences among the groups. Treatment was 100% successful in all groups, without any statistically significant difference in the number of treatments (overall 4.75 ± 1.18 treatments) among groups. No significant differences were detected for overall relapse (15/174, 8.62%) and pregnancy (129/140 litters, 92.14%) rates, as well as for intervals from diagnosis to relapse (409.63 ± 254.9 days) or to last litter (418.62 ± 129.03 days); however, the interval between diagnosis and first litter was statistically ($p < 0.05$) shorter in small, and longer in giants bitches (163.52 ± 51.47 and 225.17 ± 90.97 days, respectively, vs. 210.58 ± 88.5 days in medium-size bitches and 192.92 ± 83.63 days in large-size bitches). Overall, 1.47 ± 0.65 litters were born after treatment and the expected LS was achieved in each group, as shown by ΔLS (overall change −0.40 ± 1.62), without statistical differences among groups.

DISCUSSION

Results from the present study suggest that CEH/P does not affect older patients, as the mean age at diagnosis found in the present study (5.14 ± 1.75 years) was younger than those previously reported by Pretzer [1] and Jitpean et al. [4]. (7.25 and 7.0 years, respectively). The inclusion criteria for medical therapy in the present study could have influenced the overall mean age reported at diagnosis. Other studies have enrolled cases solved by surgical therapy, while the present study focused on the medical option only, which is often more appropriate in younger subjects intended for reproduction [7], but also in older ones if there is an increased anesthesiological risk [3]. However, no significant differences in average age were detected among the four study groups. This may reflect the etiology of the disease, which does not depend on the chronological, but on the repeated cyclic exposure to estrogen and progesterone [23].

The present study detected a 100% success rate (174/174 treated bitches) for the medical treatment, with no significant differences among the study groups. Efficacy does not depend on the type of pyometra [7] because two days after aglepristone administration, the cervix also opens in closed pyometra cases [16]. The action of aglepristone is rapid because it blocks free progesterone receptors and displaces progesterone already bound to receptors [6]. The cervical opening and uterine contractions indirectly induced by aglepristone are followed by the evacuation of a large volume of purulent discharge, with a significant improvement in

general conditions [7]. Systemic conditions also benefit from antibiotic therapy, which has a two-fold action: first, it eliminates bacteria from the uterus, and second, it prevents the diffusion of micro-organisms through the bloodstream [25]. Ideally, culture and sensitivity tests should be performed before the beginning of an empiric antibiotic therapy, but in case of life-threatening diseases, such as pyometra, antibiotic therapy can be started at the time of the diagnosis to ensure a rapid and safe recovery of the bitch [25]. The combination of enrofloxacin and clavulanate amoxicillin widens the antibiotic activity spectrum in order to prevent any super-infection development or the persistence of bacteria that may be resistant to one of the two classes of antibiotics used [3,12,15,25,26,30]. Moreover, fluoroquinolones in general and enrofloxacin, in particular, concentrate very well in the canine myometrium, with uterine fluid concentrations exceeding plasmatic ones [27]: a uterine concentration of 2.2 ug/mL of enrofloxacin was reported 1 h after an oral dose of 2.5 mg/kg, notably overcoming the serum concentration of 1.2 μg/mL 1 h after the oral administration of 5 mg/kg of enrofloxacin [28].

The key to the success of the application of medical therapy for CEH/P treatment described in the present work is indicated by the end-point of the aglepristone treatment. Aglepristone binding to progesterone receptors is not only competitive but also reversible [6], thus its administration needs to be repeated at specific time intervals, as reported in the manufacturer's instructions. The number of repeated administrations in this study (4.75 ± 1.18) is higher than that previously reported [13], indicating that treatment can last for up to 1.5 months after diagnosis. The efficient therapeutic effect of repeated aglepristone administrations reflects the pharmacokinetics and pharmacodynamics of the drug molecule. Aglepristone is slowly absorbed after subcutaneous injection and is slowly excreted, requiring up to 24 days until 80% of the administered dose is eliminated [30]. This results in the circulation of the active principle for 3 weeks after the onset of anestrus. The prolonged inhibitory effect on progesterone receptors [14] is exerted not only at uterine level, where it lowers the relapse rate, but also at the central level [31], where it shortens the inter-estrus period [7]; moreover, aglepristone binds with hypothalamic receptors for progesterone, probably counteracting the negative feedback exerted by gonadotropin-releasing hormone, follicle-stimulating hormone, and luteinizing hormone on progesterone release and allowing an earlier follicular maturation [14]. While aglepristone competitively act on progesterone receptors, prostaglandins have a luteolytic effect [12], directly decreasing the blood progesterone concentration [32]. The synergistic actions of these 2 molecules contributed to the high success rate achieved. Since many side effects have been described in association with the use of natural prostaglandins, such as dinoprost, a synthetic molecule, cloprostenol, was chosen in the present study, due to its lower dosage, longer half-life, and greater receptor affinity [33]. No side effects related to cloprostenol were observed in the present study, similar to findings in previous reports [12,16]. The absence of side effects probably depends on the administration of low doses of prostaglandins far from feeding time. Moreover, in this study, cloprostenol was administered starting at 3 days after diagnosis and the initiation of aglepristone treatment and any supporting therapies; thus, all bitches were already showing improved systemic conditions and were less susceptible to potential cloprostenol side effects.

The overall relapse rate in the present study was 8.6% (15/174 treated bitches) with no significant differences among the study groups. Among the relapsed bitches, 71.5% were not mated at first estrus after CEH/P medical treatment, and 28.5% had a successful pregnancy before the relapse. The follow-up period in the present study was longer than the 1- or 2-year

periods previously reported [5,16,30]. Only one previous study had a longer follow-up time for single cases of medically treated pyometra [23], reporting a mean recurrence time of 10.5 months. Thus, the follow-up duration of the present study was considered appropriate for estimation of the effective relapse rate. The relapse rate found in the present study was lower than the 18.9%–30% rate reported previously [13]. This difference can be ascribed to 2 main points. First, in the present study, treatment was periodically repeated until the end of diestrus, recognized when basal progesterone values were achieved. In fact, even if antiprogestins like aglepristone decrease intrauterine progesterone by mainly acting on the number and sensibility of its receptors, rather than on its serum concentration [14], the interruption of treatment at a very low progesterone (< 1.2 ng/mL) prevents immediate relapse due to the residual amounts of progesterone on a recently healed uterus. Second, all patients enrolled were free from ovarian abnormalities, such as ovarian cysts, which are reported to be possible causes of pyometra relapse [5,7,23].

The high pregnancy rate achieved in this study (overall 92.14%, 129/140 mated bitches, with no significant differences among groups) confirms that recovery from an endometrial disease does not affect future fertility [11,34]. Present results also confirm the indication to mate bitches at the first estrus after treatment, since the relapse rate is reported to be higher in non-mated bitches [7]. Most bitches were pregnant at the first estrus after treatment and their fertility was successfully preserved for almost two years, allowing breeders to obtain up to three more litters from a female that would otherwise be excluded from reproduction. Moreover, apart from the high pregnancy rate, fertility was preserved as indicated by the maintenance of the expected LS of each breed [29], without a significant change in ΔLS among the four groups. The overall fertility rate after treating pyometra in this study was notably higher than that previously reported by Ros et al. [23] (69%), suggesting that a longer treatment period may allow a more permanent uterine healing. Regardless, it must be stressed that the bitches enrolled in the present study were referred to specialized centers for veterinary reproduction; thus, estrus monitoring, semen evaluation, and possible artificial insemination events may have contributed to attainment of the high fertility rate [34].

The size-related patterns identified for the interval from diagnosis to first litter and the LS results are in agreement with the reproductive physiology characteristics of different-size bitches [22]; features that were not affected by the medical therapy. Small bitches had smaller litters sooner and with a shorter inter-estral period, whereas giant bitches whelped larger litters later and had a longer inter-estral period.

The present results confirm that treatment of CEH/P with a combination of aglepristone and cloprostenol is effective and safe and allows preservation of subsequent fertility, as demonstrated by the negligible ΔLS among bitches of all sizes.

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