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RESEARCH ARTICLE

Effectiveness of Aglepristone at Lower-Than-Standard Doses in Prevention of Pregnancy in Mismated Bitches

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Summary

The objective of this study was to evaluate the clinical efficacy of three different doses of aglepristone administered in early diestrus for prevention of pregnancy in mismated dogs. A total of 48 mismated, copulation confirmed bitches were used as material. Bitches were randomly allocated into one of four groups and treatment groups received 5 mg/kg (group I, n=12), 7.5 mg/kg (group II, n=12) or 10 mg/kg (group III, n=12) of aglepristone by two injections, 24 h apart within 10 days of diestrus. Twelve bitches (group IV) served as controls. Pregnancy was assessed by ultrasonography on day 30 of diestrus. Blood samples were collected for determination of plasma progesterone concentrations by radioimmunoassay on days 0, 3 and 7 of first aglepristone administration and on the day of ultrasonographic examination. None of aglepristone treated bitches except for one bitch in group I became pregnant. Pregnancy rate in the control group was 83.3%. There was no significant difference between mean plasma progesterone concentrations in study groups on day 0 and 3 (P>0.05). On day 30 of diestrus, plasma concentrations of progesterone were significantly lower in aglepristone treated bitches (group I: 92.60±5.74 nmol/L; group II: 85.66±3.69 nmol/L; group III: 78.87±4.86 nmol/L) compared to controls (110.62±6.24 nmol/L, P<0.01). Aglepristone administration resulted in shortening of interestrus interval in all groups (group I: 15.33±5.29 days; group II: 12.78±4.94 days and group III: 14.45±4.29 days, P>0.05). Twenty aglepristone treated bitches were bred in the subsequent estrus and overall pregnancy rate obtained was 90%. In conclusion, aglepristone at lower-than-standard doses administered in early diestrus effectively prevents pregnancy in mismated bitches and potentially offers a cost-effective treatment option.

Keywords: *Aglepristone, Bitch, Mismating, Prevention of pregnancy*

Köpeklerde İstenmeyen Çiftleşmeleri Takiben Gebeliğin Engellenmesinde Standarttan Düşük Dozlarda Aglepristonun Etkinliğinin Araştırılması

Özet

Bu çalışmanın amacı, köpeklerde istenmeyen çiftleşmeler sonrası gebeliğin engellenmesi amacıyla farklı dozlarda uygulanan aglepristonun klinik etkinliğinin araştırılmasıdır. Bu amaçla, çiftleşmenin doğrulandığı 48 dişi köpek materyal olarak kullanıldı. Köpekler rastgele 4 gruba ayrılarak uygulama gruplarında, diöstrusun ilk 10 günü içerisinde 24 saat arayla 2 kez 5 mg/kg (grup I, n=12), 7.5 mg/kg (grup II, n=12) veya 10 mg/kg (grup III, n=12) dozunda aglepriston uygulandı. Oniki köpek (grup IV) kontrol grubunu oluşturdu. Gebelik muayenesi diöstrusun 30. gününde ultrasonografik yöntemle gerçekleştirildi. İlk ilaç uygulamasının 0, 3 ve 7. günleri ile ultrasonografik muayene günü alınan kan örneklerinde plazma progesteron konsantrasyonları radioimmunoassay yöntemiyle belirlendi. Grup I'de bir köpek dışında aglepriston uygulanan köpeklerin hiçbirinde gebelik belirlenmedi. Kontrol grubunda gebelik oranı %83.3 olarak belirlendi. Çalışma gruplarında ortalama plazma progesteron konsantrasyonları 0 ve 3. günlerde belirgin değişiklik göstermedi (P<0,05). Diöstrusun 30. gününde ortalama plazma progesteron konsantrasyonları aglepriston uygulanan köpeklerde (grup I: 92.60±5.74 nmol/L; grup II: 85.66±3.69 nmol/L; grup III: 78.87±4.86 nmol/L) kontrol grubundaki köpeklere (110.62±6.24 nmol/L) kıyasla düşük bulundu (P>0,01). Aglepriston uygulaması grupların tümünde interöstrus aralığının kısalması ile sonuçlandı (grup I: 15.33±5.29 gün; grup II: 12.78±4.94 gün; grup III: 14.45±4.29 gün, P>0.05). Aglepriston uygulanan 20 adet köpek takip eden östrusta çiftleştirildi ve %90 gebelik oranı saptandı. Standarttan daha düşük dozlarda uygulanan aglepristonun istenmeyen çiftleşmeler sonrası gebeliğin engellenmesinde etkili olduğu ve uygun maliyetli bir tedavi seçeneği sunduğu sonucuna varıldı.

Anahtar sözcükler: *Aglepriston, Dişi köpek, İstenmeyen çiftleşme, Gebeliğin engellenmesi*

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INTRODUCTION

Mismating in dogs is one of the most frequently presented reproductive problems in veterinary practice. Two different treatment approaches exist for accidental matings of bitches whose owners are unwilling to pursue surgical sterilization. Prevention of nidation and implantation should be considered as an early treatment for mismating or pregnancy should be terminated after confirmation in mid-term. Due to ethical considerations prevention of nidation and implantation is requested by many owners [1].

In the past, for more than 20 years, estrogens have been the only pharmacological alternatives in the treatment of mismating and have been used within 1-5 days of breeding in an attempt to disrupt embryonic implantation [1]. However, this treatment has been associated with many undesirable affects that include prolongation of the estrus signs, aplastic anemia due to bone marrow suppression and an increased risk for uterine pathologies [2,3]. Hence, the use of estrogens as an immediate treatment for an unwanted mating in dogs is no longer recommended or considered ethical [1,4]. Prostaglandins (PGs) have also been used to prevent pregnancies in mated bitches in the early period. However, the canine corpora lutea (CL) are resistant to the luteolytic effect of $PGF_{2\alpha}$ in early diestrus and inconsistent results were reported in terms of efficiency. Furthermore, the association of prostaglandins with significant side effects and the need for multiple administrations for a relatively long period hamper the use of prostaglandins in mismated bitches in early diestrus [5].

Administration of aglepristone, a progesterone receptor antagonist, is the current method of choice in treatment for mismating in dogs. Maintenance of canine pregnancy is dependent on progesterone (P_4) secreted by CL throughout gestation [6]. Progesterone receptor antagonists are synthetic steroids that bind with great affinity to P_4 receptors, preventing P_4 from exerting its biological effects [7]. Aglepristone, available in the veterinary market of some American and European countries with an indication for pregnancy termination, has proved to be safe and effective in early and midgestation pregnancy termination in the bitch [8-10]. However, the product remains very expensive and its use in early prevention of pregnancy is limited to a greater extent due to high treatment costs.

The current recommended protocol includes two administrations, 24 h apart (10 mg/kg body weight, SC). The early administration of aglepristone at 0 to 25 days after mating always resulted in prevention of pregnancy. However, slightly lower success rates (95.7% and 94.4%) were reported after aglepristone treatment at days 26-45 [11]. The biological effects of P_4 are to a great extent dependant on progesterone receptor (PR) expression. In a recent study, it was reported that relative expression of PR mRNA in canine pregnant uterus was significantly higher in the preimplantation period compared to later stages of pregnancy [12] suggesting a higher PR availability in early diestrus. In addition, plasma concentrations of P_4, with which aglepristone competes for PR, are lower in early diestrus compared to later stages of pregnancy [13]. Collectively, we supposed that aglepristone administrations at lower than the recommended dose in early diestrus in mismated dogs might effectively prevent pregnancy.

The objective of this study was to compare the clinical efficacy of three different doses of aglepristone (5.0, 7.5 and 10 mg/kg BW, SC, two consecutive days) administered in early diestrus for prevention of pregnancy in mismated dogs. Effects on interestrus interval and subsequent fertility were evaluated as well.

MATERIAL and METHODS

Animal experimentation was approved by the respective local authority (Local Ethics Committee on Animal Experiments of Ankara University, Ankara, Turkey; Approval No: 2011-123-482).

A total of 48 mismated, copulation confirmed bitches were included. Bitches were randomly allocated into one of four groups: group I (n=12, 5.0 mg/kg, BW), group II (n=12, 7.5 mg/kg, BW) and group III (n=12, 10 mg/kg, BW). Twelve bitches (group IV) served as controls. Physical examination of the bitches was performed as soon after the breeding as possible. Stage of sexual cycle as well as the first day of diestrus was determined by evaluation of Papanicolaou stained daily vaginal cytology specimen. Copulation was confirmed by presence of spermatozoa in vagina of bred bitches using a previously described technique [14]. Briefly, a cotton-tipped swap used for collection of vaginal specimen was placed in physiological saline solution for 10 min. The saline is centrifuged and the pellet was evaluated microscopically at 400x.

Aglepristone was administered twice, 24 h apart within first 10 days of diestrus. The subcutaneous injection site in the neck was massaged immediately after the injection. The injection site was checked for local swelling within 7 days following injection. Animals were examined to test for side effects and changes in general condition. Pregnancy was assessed by ultrasound using a Piemedical Falco 100 scanner (Maastricht, The Netherlands) equipped with a 5 MHz linear-array transducer on day 30 of diestrus.

Blood samples were collected from each dog on days 0 (day of first aglepristone injection), 3, 7 and on the day of ultrasonographic examination by venipuncture of the cephalic vein. Upon collection, blood samples, drawn into tubes without anticoagulant, were centrifuged (3.000 g for 15 min) and sera stored at -20°C until assayed for progesterone.

Plasma P_4 concentrations were determined by radioimmunoassay (RIA) using a gamma counter (Mini-Assay type 6-20; Mini Instruments Ltd., London, England). A commercial progesterone RIA kit (Immunotech®) was used as described by the manufacturer. Maximum intra- and inter-assay coefficients of variation, and minimum detectable dose were 6.5%, 7.2%, and 0.12 nmol/L. It was confirmed in a previous study [15] that the test shows a high linear correlation to the established radioimmunoassay.

The interestrous interval was defined as the period between the onset of one proestrus and that of the next. According to the clinical history of each dog, the interestrus intervals of previous cycles were normal. The owners were interviewed face to face or by telephone and asked for the timing of the onset of next proestrus and whether the bitch had been successfully bred in the subsequent estrus.

Statistical analyses were performed using the SPSS software® (Version 14.01 for Windows, SPSS Inc., Chicago, IL, USA). Data are presented as means ± standard errors. The changes of P_4 concentrations among the groups were determined by one-way analysis of variance and follow up analysis were determined by Duncan test. The shortening of interestrus interval in treatment groups were compared by using Kruskal Wallis Test. P-values of <0.05 were considered statistically significant.

RESULTS

Of the aglepristone treated dogs, only one bitch in group I was diagnosed pregnant and whelped healthy puppies on term. Clinical efficacy of aglepristone treatment in prevention of pregnancy in group I, II and III were 91.6%, 100% and 100%, respectively. Pregnancy rate in control group was 83.3%.

The mean plasma concentrations of progesterone on day 0, 3 and 7 of aglepristone administration and on day 30 of diestrus are given in *Table 1*. Plasma progesterone concentrations increased on day 3 and 7 of aglepristone injections and slightly lower concentrations were observed on d 30 of diestrus in all groups. There was no significant difference between mean plasma progesterone concentrations in study groups on day 0 and 3 (P>0.05). The mean progesterone concentration in control group (127.04±6.14 nmol/L) was higher than in group III on day 7 (99.48±5.09 nmol/L, P<0.05). On day 30 of diestrus, plasma concentrations of progesterone were significantly lower in aglepristone treated bitches (group I: 92.60±5.74 nmol/L; group II: 85.66±3.69 nmol/L; group III: 78.87±4.86 nmol/L) compared to controls (110.62±6.24 nmol/L, P<0.01).

We were able to get information about the duration of interestrus interval in 30 aglepristone treated bitches. Aglepristone administration resulted in shortening of interestrus interval in all treatment groups. However, this effect was not observed in all bitches (group I: 7/10; group II: 5/9; group II: 7/11). The difference in the mean shortening of the interestrus interval in treatment groups was not significant (group I: 15.33±5.29 days; group II: 12.78±4.94 days and group III: 14.45±4.29 days, P>0.05). Twenty aglepristone treated bitches were bred in the subsequent estrus and over all pregnancy rate obtained was 90% (group I 86%, 6/7; Group II: 100%, 7/7; group III: 83%, 5/6).

DISCUSSION

Administration of aglepristone, is the current method of choice in pharmacological treatment for prevention of nidation and implantation in bitches. However, its use is mainly limited due to high treatment costs at the recommended dose [16]. Another limiting factor is the fact that only a small proportion of bitches are actually pregnant when admitted to a veterinary hospital for mismating management [5]. The probability of pregnancy from a single mount after misalliance is reportedly only 40% [17] and in a survey only 70% of bitches presented for pregnancy termination were diagnosed pregnant [18]. For this reason, physical examination of the bitches as soon after the unplanned breeding as possible is suggested [5]. Copulation confirmed bitches were included in the current study and the pregnancy rate observed in the control group was 83.3%. This is in accordance with previously reported pregnancy rates of 85% [19], 88.6% [20] and 90% [21] after controlled natural matings in the dog.

Table 1. *Mean plasma progesterone concentrations on day 0, 3 and 7 of aglepristone administration and on day 30 of diestrus*
Tablo 1. *Aglepriston uygulamasının 0, 3 ve 7. günleri ile diöstrusun 30. gününde ortalama plazma progesteron konsantrasyonları*

Group	Day 0 (nmol/L)	Day 3 (nmol/L)	Day 7 (nmol/L)	Day 30 of Diestrus (nmol/L)
Group I	69.83±11.71	104.10±11.71	116.00±8.66[a]	92.60±5.74[a]
Group II	71.50±9.11	94.18±7.22	111.25±4.84[a]	85.66±3.69[a]
Group III	67.43±9.50	88.54±8.05	99.48±5.09[ab]	78.87±4.86[a]
Control	68.95±7.23	98.23±5.85	127.04±6.14[ac]	110.62±6.24[b]
P	P<0.05	P<0.05	P>0.05	P>0.01

Within column values with different superscripts (a,b,c) are significantly different

Copulation was confirmed by presence of spermatozoa in vagina of bitches. The technique used was reported to be effective in identification of spermatozoa in 100% of smears up to 24 h and in 75% of smears up to 48 hours after mating [14]. In addition, stage of sexual cycle and the first day of diestrus were determined by evaluation of daily vaginal cytology specimen in the current study and bitches that were already in diestrus were excluded in order to maximize the probability of pregnancy. Although the onset of cytological diestrus usually occurs about 3 days before the end of behavioural estrus, bitches bred in diestrus are less likely to have conceived than those bred in late proestrus or estrus [5]. Having the first day of diestrus determined was of importance for experimental design of the study, as well.

The biological effects of P_4 are to a great extent dependant on PR expression. Aglepristone binds with great affinity to PRs without any progesterone like activity [7] and competes with progesterone for canine uterine receptors with a fixating rate of 3 [22]. In a recent study, it was reported that relative expression of PR mRNA in canine pregnant uterus was significantly higher in the preimplantation period (days 10 to 12) compared to implantation (days 18-25) and placentation (days 28-45) periods [12] suggesting a higher PR availability in early diestrus. In addition, plasma P_4 increases in first 2-3 weeks of diestrus in the bitch [13] and concentrations of P_4, with which aglepristone competes for PR, are lower in early diestrus compared to later stages of pregnancy. Furthermore, there is a negative correlation between plasma P_4 and PR concentrations and a suppressive effect of P_4 on its own receptors in the bitch was suggested [23]. In the light of abovementioned results we supposed that a dose response study would be interesting. None of aglepristone treated bitches except for one bitch in group I became pregnant confirming our hypothesis that aglepristone at lower than recommended dose administered in early diestrus in mismated dogs is effective in prevention of pregnancy.

The reason for the treatment failure observed in one bitch is unknown. Although no side effect was observed at the injection site, injections are hard to make and they take relatively long times due to oily preparation and injection mistakes are possible. In addition, a lot of individual variation in concentration in plasma and tissues were reported after of progesterone antagonist administrations in women [24]. Little is known about aglepristone regarding its bioavailability, dose-response relationship, kinetics in the blood, plasma half-life, nature of metabolites and pattern of elimination from the body which necessitates basic studies before determining whether a standard reduced dose should be recommended for prevention of pregnancy in the bitch. Similar studies have long been conducted with mifepristone, another progesterone receptor antagonist, which is used and as abortifacient and post-coital contraceptive in women. So far, experiences gathered from these studies suggest a reduction of the dose of mifepristone from the recommended 600 mg to 200 mg or less for termination of early pregnancy, post-coital contraception and contraceptive use [25].

Plasma P_4 concentrations increased on days 3 and 7 of aglepristone administrations and slightly lower concentrations were observed on day 30 of diestrus in all groups which paralleled the pattern in diestrus dogs [13]. Administration of aglepristone did not influence plasma progesterone concentrations during or immediately after treatment. However a decrease was observed on day 7 of treatment in group III and in all treatment groups on day 30 of diestrus. Despite the decrease in plasma P_4 concentrations, luteolysis was not the case as evidenced by still high plasma P_4 concentrations. Therefore, the administration of aglepristone during early diestrus in mated bitches affects P_4 production but not sufficiently to cause luteolysis in the first 30 days of diestrus. Similar results were reported in a previous study after administration of aglepristone to non-pregnant bitches within 12 days after ovulation in which an effect on P_4 secretion but not on the length of luteal phase was observed [26].

In contrast to the situation in early diestrus, aglepristone administration in the midpregnancy induces early luteolysis and it is proposed that luteolysis is associated with increased plasma levels of PGFM, the main metabolite of PGF_{2a}, probably induced by fetal expulsion [11]. For the reason that fetal expulsion was not evident in aglepristone treated dogs in the current study, absence of PGFM increase should be considered as a possible cause of different action of aglepristone on CL function at specific reproductive phases. However, aglepristone administration in midluteal phase is able to induce luteolysis in non-pregnant bitches, as well which suggests that direct intraovarian effects induced by aglepristone treatment or an indirect action on the ovary through modulation of hypothalamic-pituitary-ovarian axis might be involved [27].

Aglepristone administration in early diestrus resulted in shortening of interestrus interval. We were unable to monitor the length of luteal phase in the current study. Yet, luteolysis was not observed within first 30 days of diestrus and it was reasonably presumed that that the duration of the luteal phase was unaffected and the shortening of interestrus interval was due to an early termination of anestrus. The extent of shortening of the interestrus interval was comparable in treatment groups indicating that aglepristone similarly affects interestrus interval at all doses studied. In the bitch, progression from early to late anoestrus and the initiation of a new follicular phase are associated with changes in the hypothalamic-pituitary-ovarian axis [28] and the shortening of the interestrous interval by aglepristone administered in early luteal phase was supposed to be due to an effect on this axis [26]. Interestingly, shortening of interestrus interval was only observed in

some bitches in the current study. The design of the study may be, at least partly, responsible for this individual variation, since different breeds of dogs were used and the evaluation of the effect on interestrus interval was based on the observations of the owners. Whatever the reason is, the dog-owners should be informed about a possible shortening in the interestrus interval after the use of aglepristone in early diestrus bitches especially when breeding in subsequent estrus is intended. Pregnancy rates after breeding in the next estrus in aglepristone treated bitches was high in the current study demonstrating aglepristone administered at early diestrus has no detrimental effect on subsequent fertility.

In conclusion, aglepristone at lower-than-standard doses administered in early diestrus effectively prevents pregnancy in mismated bitches and potentially offers a cost-effective treatment option. Treatment has no negative effect on subsequent fertility, but it may result in shortening of interestrus interval. However, further studies will be necessary to reliably determine and specify a reduced standard dose of aglepristone for treatment of mismating in bitches.

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