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RESEARCH ARTICLE

Use of GnRH Agonist (Desloreline) in Combination with PGF2α on the Termination of Pregnancy in Bitches [1]

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Summary

Ten bitches whose sexual cycle periods were determined with vaginal cytology to be mated at appropriate time were used in this study. Desloreline implant (Suprelorin®) was inserted under the dorsal skin of bitches that were determined to be pregnant using ultrasonography 20 days after the first day of mating. Ten days after the implant insertion, single dose PGF2α (cloprostenol, 20 µg/kg, IM) was administered. The pregnancy was terminated on all bitches that received this procedure. It was determined that the level of progesterone decreased from an average of 28.87±11.86 ng/ml on the day of implant insertion (day 20-21 of pregnancy) to 0.43±0.31 ng/ml when the abortion was completed. It was observed that after the implant insertion, the abortion was completed in an average of 3.9±0.7 days following PGF2α administration. It has been observed that abortions was completed in an average of 2,3±0.48 days after the first vaginal discharge was seen. In conclusion, it was determined that Desloreline, a GnRH agonist, successfully could terminate pregnancy when it is combined with a single dose of PGF2α administration in bitches.

Keywords: *Bitch, Pregnancy termination, Desloreline, PGF2α*

Köpeklerde Gebeliğin Sonlandırılması Amacıyla GnRH Agonisti (Desloreline) ile PGF2α Kombinasyonunun Kullanımı

Özet

Araştırmada seksüel siklus evresi vaginal sitoloji ile belirlenerek en uygun zamanda çiftleştirilen on dişi köpek kullanıldı. İlk çiftleştirme gününü takiben 20. günden itibaren yapılan ultrases muayenesi ile gebe olduğu belirlenen köpeklere Suprelorin® boyun derisi altına yerleştirildi. İmplantın yerleştirilmesini takiben 10. günde bir kereye mahsus olmak üzere PGF2α (cloprostenol, 20 µg/kg,) kas içi yolla uygulandı. Uygulama yapılan köpeklerin tümünde gebelik sonlandı. Progesteron değerinin implantın uygulandığı gün (gebeliğin 20-21. günü), ortalama 28.87±11.86 ng/ml iken, abortion tamamlandığında ortalama 0.43±0.31 ng/ml'ye düştüğü belirlendi. İmplant uygulamasından 10 gün sonra enjekte edilen PGF2α'yı takiben ortalama 3.9±0,7 günde köpeklerde abortionun tamamlandığı görüldü. Abortionun vajinal akıntının ilk kez görülmesinden sonra 2.3±0.48 gün içinde tamamlandığı belirlendi. Sonuç olarak, uzun etkili GnRH anoloğu olan Deslorelin'in köpeklerde tek doz PGF2α uygulaması ile birlikte kombine edildiğinde gebeliği başarılı olarak sonlandırabildiği belirlendi

Anahtar sözcükler: *Köpek, Gebeliğin sonlandırılması, Desloreline, PGF2α*

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INTRODUCTION

Pregnancy in bitches lasts an average of 63 days after ovulation. However, this period varies based on the number of puppies, the time of mating and the breed [1]. In bitches, the corpus luteum (CL) is the primary source of the progesterone (P4) hormone, which is necessary for sustaining pregnancy [2], and the placenta does not secrete P4 [2,3]. In order to sustain pregnancy in bitches, P4 must maintain a level of at least 2 ng/ml, otherwise pregnancy will be terminated [4]. The level of progesterone begins to increase before ovulation and peaks about 15 to 25 days after estrus, reaching a level of 15-90 ng/ml [5]. P4 concentrations increases a second time at 25-35 days. If pregnancy develops, P4 concentrations tend to drop in the last week of pregnancy [6].

All procedures carried out to terminate unwanted pregnancies in bitches depend on either directly or indirectly eliminating the influence of the P4 and prolactin (PRL) hormones. The abortive agents that are most frequently used to terminate pregnancy are prostaglandins (dinoprost, cloprostenol), dopamine agonists (cabergoline, bromocriptine), corticosteroids (dexamethasone, prednisolone), antiprogestins (mifepristone, aglepristone) and agents that inhibit protein synthesis (epostan) [3,6-12]. Because the use of those drugs are more difficult due to their side effects such as breast tumors, pyometra, acromegalic changes, diabetes, adrenocortical suppression, masculinity in female fetuses, local skin reactions, bradycardia, hypersalivation and vomiting, GnRH agonists (desloreline, nafareline), which indirectly suppress the secretion of LH and FSH, are frequently used to suppress cyclic activity in bitches [12-15]. In bitches, LH is necessary as a luteotropic hormone for the luteal secretion of P4 throughout pregnancy [9]. For this reason, it is thought that slowly released GnRH agonists could be used to terminate pregnancy because it suppresses the LH. It has been thought that desloreline could eliminate P4 secretion for the maintenance of pregnancy by suppressing the secretion of LH.

Short-acting and long-acting GnRH agonists have been available in implant form [16]. Long-acting GnRH agonists have been tested to control the reproduction of wild animals with positive results [13]. Moreover, desloreline, a slow-release GnRH agonist with no side effects, has been used to delay the estrous cycle in bitches. However, there is no study with regard to use of long-acting GnRH agonists for the termination of pregnancy in bitches. Therefore, the objective of this study is to investigate whether previously untested GnRH agonists could be used to terminate unwanted pregnancies in bitches.

MATERIAL and METHODS

This study was carried on ten bitches whose stages of sexual cycle was determined with vaginal cytology to be mated at a suitable time. Ethical Board approval of this investigation has taken from Erciyes University Veterinary Faculty (date:26/12/2006, number:041). During the study, the bitches were kept at the department of clinical sciences of the Kafkas University, Faculty of Veterinary Medicine. A schema of the experiments design of the study has been shown in *Fig. 1*.

Pregnancy diagnosis was carried out by using transabdominal ultrasonography (Pie Medical, 5 MHz) 20-25 days after the first day of mating. After the dog was found to be pregnant with ultrasound (20-25 days after mating), desloreline (Suprelorin®, 4.7 mg [6-D-tryptophan-9(N-ethyl-L-prolinamide)-10deglycinamde)], GnRH, Peptech Animal Health, Australia) was inserted under the skin on the back between the two scapula.

In order to record changes in P4 levels, 3 ml of blood was taken immediately prior to the insertion of the implant and then once every 2 days until the termination of pregnancy. Ten days after the implant insertion, a single IM dose of $PGF_{2\alpha}$ (Cloprostenol sodium, 20 µg/kg, Estrumate, DIF, Turkey) was administered after taking blood. The blood plasma was separated with a centrifuge (Nuve NF-615) at 3000 rpm for 20 min and then the samples were stored at -20°C until to be

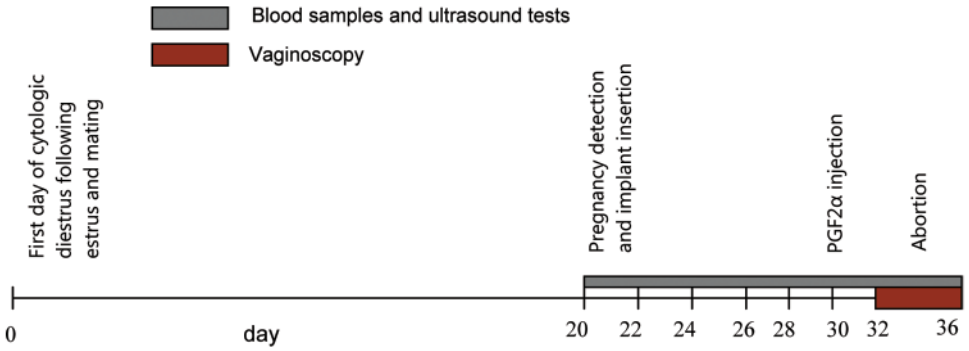

Fig 1. A schematic of the experiment's design

Şekil 1. Deney dizaynının şematik sunumu

assayed for P4. Commercially available kits were used to determine the plasma P4 level (Progesterone EIA kit, DRG International, Inc., USA). After administering Suprelorin®, a vaginal examination and ultrasonographic examination were performed every other day. During the vaginal examination, the condition of the cervix and any abnormal discharge were recorded.

At ultrasonographic examination, the size of the uterus and the viability of the fetus and the amount and quality of amniotic fluid were examined to monitore abortion.

The descriptive analysis was performed by using the SPSS 11.0 statistics program.

RESULTS

It was found that all pregnancies were terminated in the dogs that were administered desloreline together with $PGF_{2\alpha}$. It was noted that the pregnancies of the dogs continued normally until ten days following implant insertion. The P4 level was in an average of 28.87±11.87 ng/ml on the day the implant insertion (day 20-21 of the pregnancy), and it dropped to an average of 0.43±0.31 ng/ml when the abortion was completed. Following implant insertion, the P4 level initially increased for two days, and then it began dropping. It was in an average of 1 ng/ml on day of $PGF_{2\alpha}$ administeration. In two dogs, P4 level dropped below 2 ng/ml eight days after the initiation of procedure. Two days after $PGF_{2\alpha}$ administration, P4 dropped to an average of 0.6±0.4 ng/ml and it dropped to below 0.5 ng/ml on day four *(Table 1, Fig. 2)*.

An average of 1.6±0.5 days after $PGF_{2\alpha}$ was administered, it was noted that the cervix of the dogs opened and a bloody reddish-green vaginal flow began to appear. Five min after $PGF_{2\alpha}$ was administered, the dogs exhibited very pronounced salivation and vomiting, slightly pronounced sweating and pupillar dilation, and some of them exhibited diarrhea. However, these side effects of $PGF_{2\alpha}$ disappeared within one hour. It was found that the abortion was completed 2.3±0.48 days after the first flow began to appear *(Table 2, Fig. 3)*.

Table 1. *Plasma P4 (ng/ml) levels following induction of abortion (day 0) in bitches*
Tablo 1. *Abortion indüklenmesini (0. gün) takiben köpeklerdeki plasma P4 değerleri (ng/ml)*

Dog's Name	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Odie	14.56	18.56	3.31	15.123	5.75	0.98	0.69	0.41
Marsu	42.54	46.156	45.33	37.8	4.01	2.36	1.1	0.46
Müdür	28.0353	45.33202	37.79863	4.007461	2.3563	1.101186	0.502742	0.27
Zalim	32.01	28.04	19.27	5.2	11.72	0.91	0.69	0.5
Vetkopat	16.65	14.55	9.43	9.66	4.34	0.35	0.05	0.05
Lessi	14.54985	9.429895	9.662517	4.335242	0.347185	0.047346	0.05	0.38
Boncuk	49.02084	20.73122	24.74777	45.39696	2.183514	0.516569	0.379006	0.19
Ares	33.62	26.95	26.65	2.93	1.54	1.6	1.31	1.21
Afro	22.547	46.972	2.2	13.138	2.25	0.886	0.532	0.4165
Zen	35.2	47.834	6.632	18.594	3.93	1.122	0.661	0.4465
Average	28.87 ± 11.86^{a}	30.46 ± 14.88^{ab}	18.50 ± 14.89^{bc}	15.62 ± 14.76^{c}	3.84 ± 3.18^{d}	0.99 ± 0.65^{e}	0.60 ± 0.40^{f}	0.43 ± 0.31^{f}

$^{a, b, c, d, e, f}$ *: Supercripts within a same row indicates significance (P<0.05) (Aynı satırdaki farklı harfler P<0.01 olduğunu gösterir)*

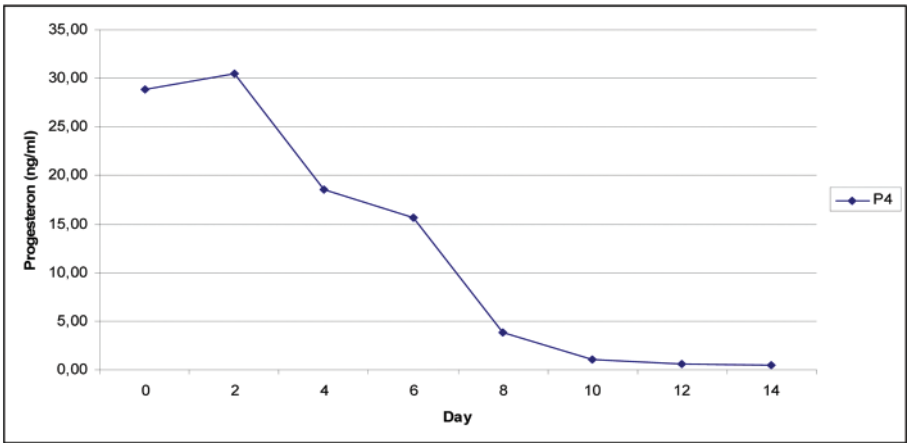

Fig 2. Average P4 levels during induction of abortion (Day 0) in bitches

Şekil 2. Köpeklerde abort indüksiyonu (0.gün) süresince ortalama P4 değerleri

Table 2. *Initiation (day), completion (day) and duration (days) of abortion following Desloreline and PGF2α administrations in dogs*
Tablo 2. *Köpeklere Desloreline ve PGF2α uygulamasını takiben abortun başlama, bitiş ve oluş süreleri (gün)*

Dog's Name	Initiation of Abortion Following Desloreline Administration	Initiation of Abortion Following PGF2α Administration	Completion of Abortion Following Desloreline Administration	Completion of Abortion Following PGF2α Administration	Duration of Abortion
Odie	12	2	15	5	3
Marsu	12	2	14	4	2
Müdür	11	1	13	3	2
Zalim	12	2	15	5	3
Vetkopat	12	2	14	4	2
Lessi	12	2	14	4	2
Boncuk	11	1	14	4	3
Ares	12	2	14	4	2
Afro	11	1	13	3	2
Zen	11	1	13	3	2
Average	11.6±0.5	1.6±0.5	13.9±0.7	3.9±0.7	2.3±0.48

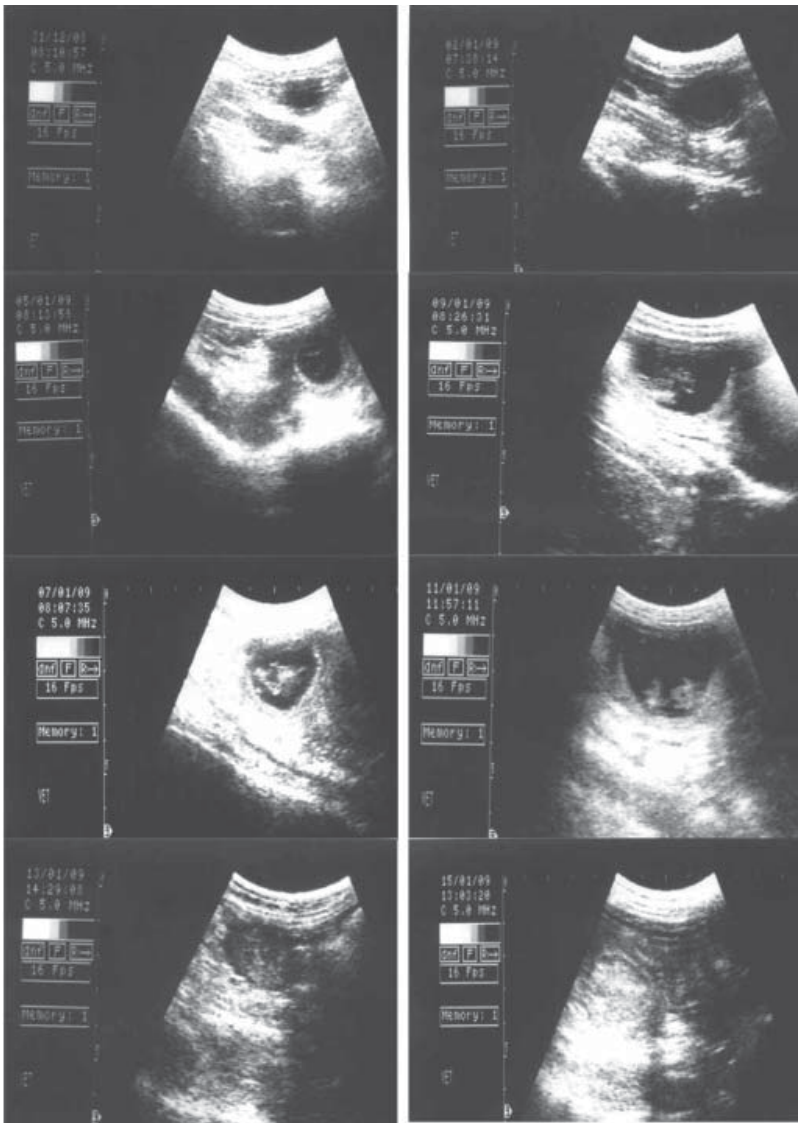

Fig 3. Ultrasound images taken from the beginning of the induction of abortion until the end of the abortion in a bitch

Şekil 3. Abortusu indüklenen bir köpeğin, uygulamanın başlangıcından abortus sonuçlanana kadar alınan ultrases görüntüleri

DISCUSSION

Johnston et al.[5] reported that the P4 level begins to rise prior to ovulation in dogs and reaches its the highest level (15-80 ng/ml) 15-25 days following estrus. In this study, the P4 level was found to be high (28.84 ng/ml) due to implant insertion; however, P4 levels were still in the range reported by Johnston et al.[5] following mating.

After GnRH agonists are administered, there is a sudden increase(flareeffect)ingonadotropichormonelevels,followed by drop to their lowest levels [17]. GnRH agonists desensitize adenohypophis receptors via continous stimulation which stops the secretion of FSH and LH [16,18,19]. It is thought that the increase in P4 levels following desloreline insertion could be due to the increase in gonadotropin levels. Subsequently, the secretion of gonadotropin could be ceased due probably to desensitization of the receptors in the adenohypophis following continous stimulation; all together it leads to drop in P4 concentrations in this study.

The P4 level increased during the first two days after the desloreline implant was administered, and then it dropped below 2 ng/ml on days eight, ten and twelve of the procedure in two, seven and one bitch, respectively Note that Johnston et al.[4] and Alaçam [20] state that a level of 2 ng/ml is necessary for the maintenance of pregnancy. As a result, it is thought that desloreline could be the primary agent that could induce the abortion.

As the pregnancy progresses, the puppies also naturally grow. When an abortion is induced after the pregnancy has progressed, the duration of the abortion is extended. This not only has various negative effects on the bitch, but it also causes the puppies to be born live and subsequently die, which has a further negative effect on the psychology of the dog's owner [7]. For this reason, we used $PGF_{2\alpha}$, which is well known agent to stimulate the contractility of the uterus in order to terminate the pregnancy before it progresses [21,22]. After a single dose of $PGF_{2\alpha}$, was administered, the abortion was completed in an average of 3.9±0.7 days, and it is thought that this procedure could speed up the progression of the abortion due to its positive effect on uterine contractility. This is because on the day on which $PGF_{2\alpha}$ was administered, except for one dog, the P4 level was below the value necessary to sustain pregnancy, as reported by other researchers [4,20]. For this reason, it is assumpted that the primary factor that induced the abortion was desloreline, and that $PGF_{2\alpha}$ could help the abortion to occur more quickly by increasing the contractility of the uterus. Furthermore, it has been reported that the administration of a single dose of $PGF_{2\alpha}$ is insufficient to induce an abortion in bitches, and two administrations were needed to achieve positive results [20-23]. Moreover, it would be helpful to administer $PGF_{2\alpha}$ in repeated times until the abortion occurs in combination with the other abortion-inducing agents [20-23]. However, in our study, a single application of $PGF_{2\alpha}$ was sufficient, and more benefit was obtained from its effect on uterine contractility than its luteolytic effect. The side effects that occurred a short time after administering $PGF_{2\alpha}$ disappeared within a few hours, as it was also reported by Wanke et al.[3].

In conclusion, administration of Desloreline, a long-acting GnRH analog, in combination with a single dose of $PGF_{2\alpha}$ lead to decrease in the P4 level below the minimum level required to sustain pregnancy, and termination of the pregnancy in bitches. Furthermore, since a large number of injections are not performed on the dog, it is our opinion that the method is easier, less expensive and causes less negative impact on the bitch than other abortion inducting procedures. Desloreline in combination with $PGF_{2\alpha}$ could be an alternative method to terminate pregnancy because this procedure is easier, less expensive and with less negative impact on the bitch. Termination of pregnancy with use of desloreline warrants further research to be accepted for routine application in bitch.

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