

Hormonal Assay for Estimation of Progesterone Levels in Normally and Induced Estrus Bitches

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ABSTRACT

The present study was conducted to measure the concentration of progesterone throughout gestation and parturition in control and treated bitches. Thirty anestrus bitches aged 3-6 years and weighed 19.5-26 kg were used in this study. The bitches were divided equally into three groups as follows: the 1st group as a control group without treatment, the 2nd group as a treated group anestrus bitches treated orally with 0.125 mg/kg BW bromocriptine (Parlodel) twice daily, and the 3rd group treated orally by 0.012 mg/kg BW cabergoline (Dostinex) until the appearance of estrus signs. The results showed that the number of responded animals were in proestrus bleeding and became pregnant and newborn 70%, 60%, and 50% for control, bromocriptine, and cabergoline treated groups, respectively. In addition, there were no significant differences ($P \leq 0.01$) in progesterone concentration between the control and treated pregnant bitches. It can be concluded that both medicines bromocriptine and cabergoline do not affect progesterone levels during pregnancy period in bitches.

Keywords: Hormone, Progesterone, Estrus, Bitch, Pregnant, Assay

Introduction

The bitch has a prolonged luteal phase with the persistence of the corpora lutea for 70-80 days in the non-pregnant animals (1, 2).

Progesterone concentrations in the peripheral circulation of pregnant bitches are similar to those of non-pregnant individuals and for this reason, unlike other species cannot be used to diagnose pregnancy (3, 4, 5). A progesterone concentration begins to rise at the last 2 to 48 hours of proestrus above the critical 0.5 ng/ml plateau (6, 7). Plasma progesterone concentration is typically 0.5 ng/ml in mid proestrus, rising above 0.5 ng/ml prior to the onset of standing heat or before the LH peak

and continuing to increase for the following 15 to 25 days (8).

However, there was a lot of individual variation with peak values obtained between the 8th and 29th days after the LH peak in pregnant bitches. There is some evidence that at the time of implantation or just after implantation, progesterone concentrations increase, due possibly to the effect of placental gonadotropin (9).

From about 30 days of gestation there is a gradual decrease in progesterone so that by about day 60, the values are 5 ng/ml are obtained followed by a sudden decline just before parturition to zero just afterward (10). Pregnancy lasts 63 ± 1 days, if measured from the day of ovulation, and corpora lutea are the only source of progesterone and individual progesterone profiles may vary considerably including the first day of progesterone declining < 2 ng/ml, often accepted as the cut-off value regarding impending parturition or pregnancy loss (11, 12).

A progesterone concentration of 2 ng/ml was documented 36-48 hours prior to the whelping (7). In one study, Concannon et al. (4) reported that whelping bitches maternal progesterone concentrations fall below 2 ng/ml. This study was

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carried out to investigate the progesterone level in both normal and induced estrus bitches during pregnancy periods in bitches.

Materials and Methods

Thirty adult female dogs have previous birth were subjected to this study in Collage of Veterinary Medicine, University of Baghdad and carried out during February, March, and April 2018. These female dogs aged between 3-6 years weighting 19.5-26 kg and 4 fertile males aged 3-5 years and weighing 22-28 kg were used for estrus detection and natural insemination.

The age of these animals was determined by teeth formula according to the techniques described by (13). The animals were kept under quarantine with veterinary observation for one month. Any sign of illness, a reproductive problem during previous cycles, or whelping of any animals was eliminated. The bitches were divided into three equal groups the 1st as a control group and the 2nd group as a treated group anestrus bitches treated with Bromocriptine (Parlodel) a dose of 0.125 mg/kg/BW orally twice daily and the 3rd group treated by Cabergoline (Dostinex) a dose of 0.012 mg/kg/BW orally until the appearance of estrus signs. Blood samples were then obtained for the estimation of serum progesterone concentration.

Statistical Analysis

Analysis of variance was used to analyze the data using SPSS software version 22 (IBM SPSS Inc., Chicago, IL, USA). Fisher's least significant difference (LSD) test was used to separate means at $P \leq 0.05$ (14).

Results and Discussion

The results of progesterone concentrations during pregnancy are presented in Table 1. The means of progesterone concentration through 0-15 days of pregnancy in treated and control groups were 5.1 ± 0.215 , 5.57 ± 0.07 , and 5.33 ± 0.15 ng/ml, respectively, and increased during 16-30 days of pregnancy as levels (ng/ml) were 8.83 ± 0.11 - 9.08 ± 0.08 and 8.92 ± 0.085 , respectively, while reached high levels throughout the mid-gestation, 31-45 days in treated and the control group levels (ng/ml) were 17.82 ± 0.01 - 18.76 ± 0.07 and 18.21 ± 0.176 respectively. So, it is increased

gradually and reached the highest level on day 46 to 60 between the pregnant treated and normal animals levels (ng/ml) were 13.99 ± 0.015 - 14.26 ± 0.11 and 14.14 ± 0.129 respectively. Then, maternal progesterone concentration decreased gradually and was obtained (ng/ml) 5.0 ± 0.03 - 5.31 ± 0.04 in treated and 5.186 ± 0.11 in control groups on the day of parturition.

After whelping, progesterone concentration returned to the basal level after two weeks as levels (ng/ml), 0.73 ± 0.02 - 0.82 ± 0.01 for treated groups and 0.76 ± 0.02 for the control group and there were no significant differences in progesterone concentration in treated and control groups during pregnancy and parturition.

The current study showed that the peak level of progesterone during 31-45 days in treated and pregnant animals were 17.82 ± 0.01 - 18.76 ± 0.07 and 18.21 ± 0.176 ng/ml, respectively and reached high levels (ng/ml) 13.99 ± 0.015 - 14.26 ± 0.11 and 14.14 ± 0.129 in day 46 to 60, respectively, while decreased at birth gradually to return the same level as in early pregnancy.

Progesterone levels were noticed to increase remarkably 10 days or more after ovulation and reach a peak at the stage of implantation, or about 20 days after ovulation (3).

The magnitude of the preovulatory progesterone rise in the bitch is reported to be around 1 ng/ml at the beginning of the LH surge to 3 ng/ml during the surge and subsequently continuing to rise to around 5 to 7 ng/ml at the time of ovulation, the rate at which the mating levels of progesterone concentration increased from 2.50 to 5.30 ng/ml was variable (8, 15).

The present study agreed with England and Concannon, (16) reported that progesterone increased rapidly on day 10 and a maximum of 22.9 ± 2.7 ng/ml on day 25, and remained elevated until day 30 at 19.9 ± 2.7 ng/ml. While Ayhan *et al.*, (3) showed that mean progesterone concentrations increased gradually by day 10 to 55 days of diestrus (5.7 ng/ml) and maintained this level up to about 45 days after mating. The level decreased rapidly later after 45 days of mating.

The results of the recent study are similar to (17) who reported that the mean of the predicted values for serum progesterone concentration was 11.9 to 12.3 ng/ml in the pregnant bitches as evaluated at 4 weeks after mating.

The prepartum period did not show the typical dramatic decrease in progesterone concentrations

caused by the final prepartum luteolysis. This is reflected by the lack of significance progesterone values during weeks 7, 8, and 9, and is likely due to differences in times of blood collection between the studies (18).

The variability can be explained by the variation in the days of blood sampling. Pregnancy specific mechanisms exist ensuring both maintenance of elevated plasma progesterone levels throughout gestation and their decline to less than 1 ng/ml at parturition (19). Günzel-Apel et al. (20) reported

that the establish data, which are significant for each week of pregnancy, assess the probability of individual deviations from an average curve which may be caused by affiliation to a breed different from those included in this study or by bitches older than 6 years and canine population is composed of hundreds of different breeds, and it is unlikely that a single pattern of progesterone concentration may work for all and these differences might be due to breed variation.

Table 1. Progesterone concentration (ng/ml) in the control and treated Iraqi bitches during pregnancy and parturition

Parameters	Pregnant				
	Bromocriptine early	Bromocriptine late	Cabergoline early	Cabergoline late	Control group
Total numbers	5	5	5	5	10
Pregnant animals	3	3	2	3	7
0 - 15 days	5.57±0.07 ^{Da}	5.51±0.27 ^{Da}	5.1±0.21 ^{Da}	5.34±0.019 ^{Da}	5.33±0.15 ^{Da}
16 - 30 days	9.08±0.03 ^{Ca}	9.08±0.08 ^{Ca}	8.76±0.02 ^{Ca}	8.83±0.11 ^{Ca}	8.92±0.085 ^{Ca}
31 - 45 days	18.76±0.07 ^{Aa}	18.55±0.28 ^{Aa}	17.8±0.01 ^{Aa}	18.16±0.27 ^{Aa}	18.21±0.176 ^{Aa}
46 - 60 days	14.25±0.08 ^{Ba}	14.26±0.11 ^{Ba}	13.99±0.015 ^{Ba}	14.0±0.12 ^{Ba}	14.14±0.129 ^{Ba}
At birth	5.31±0.04 ^{Da}	5.28±0.09 ^{Da}	5.0±0.03 ^{Da}	5.08±0.12 ^{Da}	5.186±0.11 ^{Da}
2 weeks after birth	0.82±0.01 ^{Ea}	0.814±0.013 ^{Ea}	0.73±0.02 ^{Ea}	0.787±0.021 ^{Ea}	0.76±0.025 ^{Ea}

The numbers represent mean ± standard error.

The similar small letters represent no significant differences between the same rows.

The similar capital letters represent no significant differences between the same columns.

The different small letters represent significant differences at level of $P \leq 0.01$ in the same rows.

The different capital letters represent significant differences at level of $P \leq 0.01$ in the same column.

Conflict of Interest

The authors declare that there is no conflict of interest.

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الفحص الهرموني لتقدير مستويات هرمون البروجسترون في الكلاب غير الشبقية والمستحثة الشبق

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الخلاصة

أجريت هذه الدراسة لتحديد تركيز هرمون البروجسترون في جميع مراحل الحمل والولادة في كلاب السيطرة والمعالجة. إذ تم استخدام ثلاثين كلباً غير شبقية تتراوح أعمارهن بين 3-6 سنوات وبوزن 19.5 - 26 كغم. قُسمت الحيوانات إلى مجاميع العلاج والسيطرة. وشملت مجاميع العلاج عشرين كلباً مقسمة إلى مجموعتين متساويتين، وقد غُولجت فموياً بمادة بروموكريبتين بجرعة 0.125 ملغم / كغم / من وزن الجسم مع مادة الكابيرجولين بجرعة 0.012 ملغم / كغم / من وزن الجسم مرتين يومياً لحين ظهور علامات الشبق، بينما تركت مجموعة السيطرة (10 كلابات) دون معالجة وتمت متابعتها للكشف عن الشبق والحمل. إذ أظهرت النتائج أن عدد الحيوانات المستجيبة لأظهار الشبق والحمل والولادة لمجموعة السيطرة، ومجموعة المعالجة بالبروموكريبتين والمجموعات المعالجة بالكابيرجولين على التوالي كانت 70 %، 60 % و 50 %، ولم تكن هناك فروق ذات دلالة إحصائية في تركيز هرمون البروجسترون بين مجاميع السيطرة و الكلابات الحوامل المعالجة، ونسنتج من هذه الدراسة ان كلا من العقارين البروموكريبتين والكابيرجولين ليس لهما تأثير على مستوى البروجسترون في الكلابات اثناء فترة الحمل.

الكلمات المفتاحية: الهرمون، البروجسترون، الشبق، الكلبة، الحمل، الفحص