Progesterone and Ultrasound Comparative Study of Estrus and Pregnancy between induced estrus and normal cyclic Dogs

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Abstract

This study was designed to study the progesterone level in normal and induced estrus dogs and the accuracy of ultrasound examination in these animals. Twenty female dogs aged 2-5 and weighted 20-25 kg with five mature male dogs aged 2-5 and weighted 18-22 kg were used during 2015. The females were separated into two groups (normal and treated). The treated group was injected IM/ with PMSG 20 IU/Kg/dog for 6 days followed by single IM/ injection of hCG 500 IU/dog on day 7. Serum samples were obtained for progesterone assay once weekly. The examination by using ultrasound was done to all females once weekly also. The result of duration of response to estrus after treatment was 19.3±1.45 days, with no significant differences between two groups in estrus cycle length. There were a significant decrease ($P<0.01$) in estrus and conception percentage in treated dogs, while the pregnancy length and puppies number show no significant differences. Progesterone assay had no significant differences between the two groups before or during estrus cycle, pregnancy months and post partum. The embryonic vesicle could be detected by using ultrasound in 20±0.75 days and fetus at 38±0.98 days, and there were no significant differences between the numbers which detected during pregnancy and after birth, with accuracy reach 100%. From the results we conclude that the using of progesterone and ultrasound examination can be used with highly accuracy to detect pregnancy in female dogs, and induction of estrus can be done in female dogs in Iraq with lower estrus and pregnancy percentage than normal group.

Key ward: dogs, induced estrus, progesterone, ultrasound.

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دراسة مقارنة للبروجستيرون والموجات فوق الصوتية للشبق والحمل في الكلاب المستحدثة الشبق والطبيعية

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الخلاصة

صممت هذه الدراسة لدراسة مستوى البروجستيرون في الكلاب الطبيعية والمستحدثة الشبق ودقة فحص الموجات فوق الصوتية لهذه الحيوانات. عشرون كلبة بعمر 2–5 وبوزن 20–25 كغم مع خمسة ذكور بالغة بعمر 2–5 وبوزن 18–22 كغم استخدمت خلال 2015. الإناث قسمت إلى مجموعتين (الطبيعية والمعالجة). تم علاج المجموعة المعالجة بهرمون مصل الفرس الحامل بجرعة عشرون وحدة دولية لكل كغم لكل كلبة عضلياً لمدة ستة أيام ومن ثم عقبت بجرعة مفردة 500 وحدة دولية لكل كلبة من الهرمون المشيمي الغذائي البشري في اليوم السابع. تم جمع المصل للفحص الهرموني مرة أسبوعياً من كل الإناث. وكذلك تم فحص الإناث بالموجات فوق الصوتية مرة أسبوعياً. أظهرت نتائج الدراسة الحالية أن فترة الاستجابة للشبق في المجموعة المعالجة كانت 19.3±1.45 يوم، وعدم وجود فرق معنوي في طول دورة الشبق بين كلا المجموعتين. وجد انخفاض معنوي ($P<0.01$) في نسبة الشبق والحمل في المجموعة المعالجة، بينما لم يظهر طول فترة الحمل وعدد الأجنة أي فرقاً معنوياً. لم يلاحظ وجود فرق معنوي في مستوى هرمون البروجيستيرون بين المجموعتين خلال الفترات قبل الشبق واثناء دورة

الشبق، أشهر الحمل وبعد الولادة. شوهدت الحوصلة الجنينية في اليوم 20±0.75 يوم والجنين في 38±0.98 يوم، ولم يلاحظ فرقاً معنوياً ما بين عدد الأجنة أثناء الحمل وما بعد الولادة، مع نسبة دقة وصلت إلى 100%. من هذه النتائج أمكننا أن نستنتج بأنه استخدام تحليل البروجستيرون وفحص الموجات فوق الصوتية أثناء الحمل وبدقة عالية في إناث الكلاب، ومن الممكن أن نستحدث الشبق في إناث الكلاب في العراق مع نسبة شبق وحمل أوطئ من المجموعة الطبيعية.

الكلمات المفتاحية: الكلاب، احداث الشبق، البروجستيرون، الموجات فوق الصوتية.

Introduction

Inter estrous interval was the time from the onset of proestrus to the subsequent onset of proestrus and includes proestrus, estrus, diestrus and anestrus (1). These phases considered, respectively, follicular phase which characterized by rising in estrogen, and then the luteal phase which depict by rise in progesterone and decline in estrogen, the remains of the luteal phase, and the interval between loss of luteal function and onset of next cycle (2). A number of methods had been used to induce estrus in dogs, but most of them were not appropriate for application (3, 4). The administration of one or more exogenous gonadotrophic hormone to stimulate an ovarian response that results in proestrus followed by fertile estrus was use followed by ovulation either spontaneously or induced by additional hormones (hCG or GnRH) administration (5). Many protocols depend on using of hormone preparations was mentioned (6). In Iraq the using of eCG followed by hCG to induce ovulation was studied by (7). Endocrine differences have been reported in pregnant dogs in comparison to non pregnant female (8). The pregnancy-related increases in progesterone and estradiol secretion between days 25 and 40 were not completely reflected in serum concentrations due to increased steroid metabolism (9). Progesterone hormone play primary roles in female to stimulate of endometrial secretion, inhibition gonadotropin releasing hormone (GnRH) release, inhibition of reproductive behavior synergistically with dropping estrogen and the maintenance of pregnancy (10). Pregnancy diagnosis methods which used include abdominal palpation, radiography and ultrasound (11, 12, 13, 14). By using ultrasound the uterus contents (normal or abnormal) could be identified, black appearance of the fluid-filled structures on the screen of ultrasound, as well as embryonic vesicles could be display 15 to 20 days after ovulation (11, 15). The size increasing of embryonic vesicles until 32 to 34 days after ovulation, when they were no longer determined as individual swellings (16). This study represent the first one of its kinds to follow up estrus cycle and pregnancy in induced estrus dogs in Iraq by using progesterone and ultrasound examination.

Materials and Methods

Our study was conducted during 2015. Twenty female dogs of local breeds aged 2-5 years and weighted 20-25 kg with five fertile males aged 2-5 years and weighted 18-22 kg were used for estrus detection and natural insemination. The female dogs randomly were divided into two groups (normal and treated). The treated group was injected IM with PMSG (Folligon/Intervet/UK) 20 IU/Kg/dog for 6 days followed by single IM injection of hCG (Chorulon/Intervet/UK) 500 IU/dog on day 7). Blood collection (10 ml) was done from jugular vein from the groups of the experiment. Separate serum were obtained in centrifuge (3000 rpm at 30 minutes) and incubated in epindroff tube for hormonal assay at -20C°. Progesterone assay in serum samples were done by Radio Immune Assay by using Antibodies online com (USA) kit. Examination of female by using ultrasound was done once weekly according to the method which described by (12). T test, ANOVA and LSD test was used for statistical analysis for mean groups. This was done according to (17).

Results

The comparison between the results of normal (that cyclic normally) and treated groups were (9.4±0.34, 8.5±0.45 days) and (9.5±0.50, 8.3±0.49 days) in proestrus and estrus phase respectively, and showed no differences between the two groups. Whereas duration of the response for observation proestrus bleeding and onset to proestrus of treated group was 19.3±1.45 days (Table 1).

Table (1) Length of proestrus, estrus, and duration of response (days) in the normal and induced estrus dogs

Parameters	Duration of response	Proestrus	Estrus
Normal estrus	-	9.4±0.34 a	8.5±0.45 a
Induced estrus	19.3±1.45	9.5±0.50 a	8.3±0.49 a

-The number represents mean ±SE.

-The similar small letters represent no significant differences at the same raw and column.

The comparison of showing estrus between normal group and treated group exhibits a significant (P<0.01) differences at rate 100% for normal group and 60% for treated group, whereas conception rate showed a significant (P<0.01) differences at rate 100% for normal group and 40% for treated group. And there are no differences in gestational period and numbers of puppies between the two groups. When comparison of the normal cyclic and treated groups the results were 62.7±0.98 and 61.5±1.19 days for gestation period respectively (Table 2).

Table (2) Percentage of estrus animal, conception percentage, gestation period and numbers of born puppies

Groups	Numbers of animals	Estrus percentage	Conception percentage	Gestation period	Numbers of born puppies
Normal estrus	10	100% a	100% a	62.7±0.98 a	47 a
Induced estrus	10	60% b	40% b	61.5±1.19 a	21 a

- **The similar small letters represent no significant differences at the same column.**
- **The different small letters represent significant differences at level P<0.01 at the same column**

The study results for progesterone concentration of blood samples that conducted from 10 normal cyclic dogs during anestrus, proestrus, estrus, metestrus and diestrus phases were (0.26±0.01, 0.4±0.01, 0.44±0.02, 0.8±0.01 and 0.82±0.02) ng/ml respectively and there were a gradual increase in progesterone levels during estrus phases without any significant differences (Table 3). The progesterone concentration for normal cyclic dogs group were pursued during estrus cycle and gestation period display significant (P<0.01) increase in first and second months of pregnancy (4.88±0.26 to 3.3±0.12) ng/ml, comparing with the progesterone level during the estrus phases (Table 3). While results of progesterone hormone collected from 10 anestrus dogs treated with (PMSG and single dose of hCG) for induction of estrus of treated group were (0.12±0.02, 0.36±0.01 and 0.44±0.02) ng/ml before treatment, proestrus and estrus phase respectively and increased during the first and second months of pregnancy (3.71±0.08 and 4.08±0.11) ng/ml and this showed significant (P<0.01) increasing comparing with the estrus phases, pregnancy and post parturient period (Table 3). No other differences was recorded between the two groups (Table 3).

Table (3) Progesterone hormone (ng/ml) during estrus cycle, pregnancy and post parturient period in the normal and induced estrus dogs

Estrus stage	Normal group	Treated group
Proestrus	0.4±0.01 b	0.36±0.01 B
Estrus	0.44±0.02 b	0.44±0.02 B
Metestrus	0.8±0.01 b	-
Diestrus	0.82±0.02 b	-
Anestrus	0.26±0.01 b	-
Before treatment	-	0.12±0.02 B
Unresponse	-	0.24±0.09 B
1st month of pregnancy	4.88±0.26 a	3.71±0.08 A
2nd month of pregnancy	3.3±0.12 a	4.08±0.11 A
Post parturient period	0.66±0.02 b	0.66±0.02 B

- **The number represents mean ±SE.**
- **The similar small letters represent no significant differences at the same raw and column.**
- **The different small letters represent significant difference at the same raw and column at level of P<0.05.**

The ultrasound accuracy for twenty (20) dogs examined were showed fourteen (14) positive result during pregnancy and parturition and six (6) bitches showed negative result (Fig. 1). The accuracy of ultrasound for these groups was 100%. The observation results for the mean of first day appearing of embryonic vesicles by ultrasound for pregnant dogs (Figure 2) were 20±0.75 days, where as the mean of observation of fetuses (Fig. 3, 4, 5) was 38±0.98 days. The number of puppies by ultrasound examination and number of puppies after birth were 4.14±0.44 and 4.86±0.64 respectively and there was no differences between detecting number of puppies during pregnancy and after birth (Table 4).

Table (4) Observation of embryonic vesicles and fetus (days) and puppies' numbers (per dam) by ultrasound during dogs pregnancy

Parameters	Embryonic vesicles	Fetus	Puppies at pregnancy	Puppies after birth
Day of observation	20±0.75	38±0.98	4.14±0.44	4.86±0.64

- **There is no significant difference between numbers of puppies during pregnancy and after birth.**

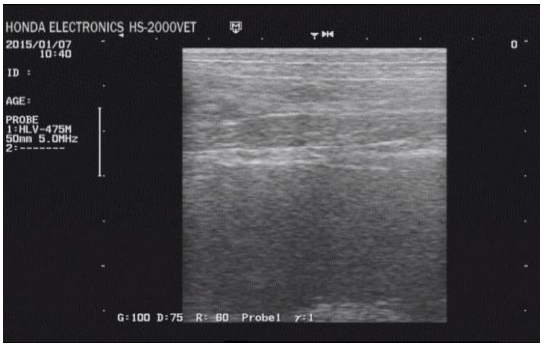

Fig (1) Ultrasonographic image of uterine horns in non pregnant bitch.

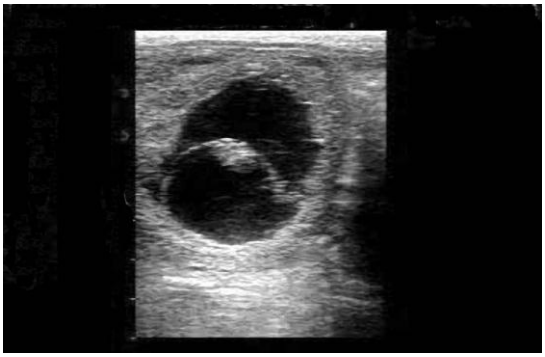

Fig. (2) Observation of embryonic vesicle at 21 days of pregnancy.

Fig. (3) Ultrasonographic image of presence of fetuses skeleton in pregnant bitch.

Fig. (4) Observation of fetus at 59 days of pregnancy.

Fig. (5) Fetuses at 45 days of pregnancy.

Discussion

The study results (Table 1) appointed that the proestrus and estrus duration is (8.5±0.45 and 8.3±0.49) in non treated group come with duration average of proestrus phase in mature bitches is 9 days, with a range of 0 to 27 days while the estrus phase at average 9 days, with range of 4 to 24 days conducted by (18). The present result of response agreed near with (7, 19). Present results disagreed with (7) which showed estrus and conception percentage for group of bitches treated with PMSG and HCG were 100% and 83.3% respectively. And this study is typical with the results of (19). The average of gestation period agreed with other researchers that showed parturition occurs 65±1 days next the LH surge (day 0) (16). While 64-66 day pregnancy measured from the LH surge to parturition, since the ovulation has been estimated to occur 2 days after the surge in LH (20). The study results similar with other study that showed serum progesterone concentrations during proestrus are at basal levels (<0.5 ng/ml) (19 and 21) and then start a gradual rise at the finishing of proestrus and rapid and detectable increase around the period of LH surge (22). While disagreed with other study that showed progesterone concentration at estrus phase about (1.0 ng/ml) (23) and (2 ng/ml) during anestrus (1). There were slightly decrease in progesterone level from 1st pregnancy month compare with the 2nd month of pregnancy. This agreed with other studies of progesterone level during the first and second months of pregnancy which were (4.5± 0.6 and 3.1 ± 0.4 ng/ml) (19, 24). The decline in progesterone before parturition was noticed previously for bitches sampled (22, 25). Such results support the idea that progesterone withdrawal may play a major role in the mechanism of parturition induction in the dog (3). (26) Assessed the accuracy of ultrasound of the gestational age prediction within 65±1 day and ±2 days were 70.8% and 86%. While ultrasound provides an accurate estimate of parturition date when the examination

conducted at day 30 (16). This result agreed with other study that showed the appearance of embryonic vesicles in dogs at 18-23 days (27) and 20 days after ovulation (28). Whereas embryonic vesicles were observed between 13-21 days after the first mating, this study according to the observations of (15) on pregnancies dogs of various breeds. Conclusion, progesterone and ultrasound examination can be used with highly accuracy to detect pregnancy in female dogs, while the estrus induction with lower estrus and pregnancy percentage can be done in dogs than normal group in Iraq.

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