

HORMONAL PROFILE OF IRAQI BITEHES DURING VARIOUS PHYSIOLOGICAL STAGES.

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ABSTRACT

The study was conducted to investigate the success of induced estrus and to track the hormonal levels during the estrus cycle, pregnancy and post-parturition in dogs. Ten females, aged 2-3 years and weighted 18-22 kg, were kept in cages at the College of Veterinary Medicine-University of Baghdad. Dogs were mated and tracked till they become conceive. Then, after one month of parturition, they treated with Bromocriptine (Parlodel)[®] a dose of (0.05mg/kg/dog) twice daily to induce estrus. Eight dogs showed estrus and mated again with the same mature dogs. Blood serum was taken once a week from cephalic vein. Follicular Stimulating Hormone (FSH), Luteinizing hormone (LH), Estrogen and Prolactin were measured by using canine Gamma counter kits. In both, normal and induced estrus periods, FSH and LH hormone levels started to elevate in proestrus and estrus phases then they declined in pregnancy months and post-parturition. Estrogen and prolactin hormones had significant differences during the second month in the other non-responsive dogs. Estrogen levels indicated that the proestrus, estrus and treatment period showed higher significant differences ($P<0.01$) than anestrus period.

Results indicated that the FSH and LH had coordination in their levels starting from high rate during proestrus and estrus then declining after wards to reach their

minimal levels during post-parturition in normal and induced estrus periods, while the estrogen and Prolactin had an important role during all these periods.

INTRODUCTION

The ovarian function in dogs and the hormonal levels had a significant value for evaluation of fertility (1). The Canids species are monoestrous, polytocous and spontaneous ovulates with a spontaneous luteal function (2). Bitches ovulate only once or twice at any time of the year (3). Prolactin seems to play a role in canine inter-oestrus interval, possibly by affecting gonadotropin secretion and (or) ovarian responsiveness to gonadotropins (4). Prolactin secretion was suppressed by administration of dopamine agonists to shorten the duration of anoestrus (5). Domestic dogs were pronounced progesterone secretion that lasts for 55-75 days duration of pregnancy with inter-estrus intervals range from 5 to 13 months and endogenous circannual cycle (1). The pre-ovulatory LH surge and the ovulation 2 days later, occur after a 1-3 weeks period of proestrus, which characterized by cornification of the vaginal epithelium, discharge of fluid and erythrocytes, and pheromonal secretion causing increased attraction of males, and anatomically visible edema and turgor of the vulva and vaginal stroma (2). These events raise estradiol concentrations then estrus begins typically in 0-1 days after the LH surge and behaviorally lasts seven days in average (1). Luteal phase typically ends in 7-9 days after the LH surge (6). Dogs were highly fertile, with rates often reaches 95% of mated bitches. Oocyte maturation completed in the uterine tubes 2-3 days after ovulation (7). Mating involves a copulatory lock that lasts from one to 20 min. Fertile mating can occur in an early as five days before ovulation or as late as six days after that which can be referred to the prolonged life span of intrauterine sperms, which lasts up to 7 days, and of oviductal oocytes (up to 7-8) (8). Parturition occurs 65 ± 1

days after the LH surge or 64 days after first mating, and 43 days after implantation, which occurs at day 21-22. The corpus luteum was dependent on both LH and prolactin secretion (1). Prolactin secretion several-fold more during pregnancy than non pregnant period to stimulated luteal function (9). Several studies were conducted in Iraq to evaluate the induction of oestrus in dogs using gonadotropins hormones (10; 11 and 12). This study is the first of its kind to use Dopamine agonist for inducing estrus and track hormonal levels before and after estrus induction.

MATERIAL AND METHODS

Ten female dogs aged 2-3 years and weighted 18-22 kg was used. All the dogs were kept in the cages at the College of Veterinary Medicine University of Baghdad in 2015. The dogs were monitored and tracked till they entered the estrus cycle then became pregnant. After one month of parturition, these female dogs were given Bromocriptine (Parlodel)[®] at a dose of 0.05 mg/kg/dog twice daily, orally for 28 continuous days to induce estrus. Females were mated with two mature males aged three years and weighted 22kg and 25kg respectively (the same ones from the first mating) before and after the induction of estrus. Blood serum was collected from all female dogs once a week from the cephalic vein. The FSH, LH, estrogen and prolactin hormone were assayed using Gamma counter canine kits (Biomedical). The data was analysed by using ANOVA, and the LSD was used to determine the significant differences between the means of the groups (13).

RESULTS

All ten females showed proestrus signs and mated with males to become pregnant before the induction of estrus (Table 1). After the induced estrus, only eight females showed response by proestrus using Bromocriptine and mated again and

became pregnant (Table 1). Response duration to the treatment ranged from 19 to 40 days with 27.6 ± 6.4 days mean (Table 1).

Table 1: Degree and duration of response and degree of conception in natural and induced estrus dogs.

Type of estrus	Response percentage		Duration of response (days)	Conception percentage	
Naturally	10	100%	-	10	100%
Induced	8	80%	27.6 ± 6.4	8	80%

The results indicated that there were significant differences ($P < 0.05$) in FSH between the proestrus phase and anestrus, post-partum and in non responsive animals before and after treatment (Table 2). The highest FSH hormone was noticed during proestrus phase (26.5 ± 2.8 , 25.0 ± 2.0 ng/ml) before and after treatment respectively (Table 2). Less hormonal levels were noticed in the anestrus, post-partum and in non-responsive dogs (3.9 ± 1.4 , 4.2 ± 2.1 , 4.2 ± 2.1 , 5.0 ± 0.5 and 3.0 ± 0.1 ng/ml) respectively (Table 2).

The LH hormone showed significant differences ($P < 0.01$) between the estrus phase (before and after treatment) with other periods (Table 2). High levels of the hormone were noticed during estrus phase before treatment (101 ± 25 ng/L) and after treatment (90 ± 32 ng/L) (Table 2). The same hormonal level (32 ± 16 ng/L) was noticed during anestrus, post-partum (before and after treatment) and in non-responsive females (Table 2).

Estrogen hormone had clearly indicated that there were a significant differences ($P < 0.05$) between proestrus and estrus phase in the treated dogs with non-responsive dogs (Table 2). There was a slight elevation in estrogen levels during proestrus, estrus before and after treatment and; a small dropping continued through pregnancy and post-partum period and also before and after treatment (Table 2).

The prolactin hormone test demonstrated significant differences ($P < 0.01$) between proestrus and estrus phase (before and after treatment) with anestrus, second month in non-responsive dogs and post-partum period in induced estrus (Table 2).

Table 2: FSH, LH, estrogen, prolactin hormones levels during estrus cycle, pregnancy months and the postpartum period in female dogs before, during and after treatment.

Reproductive period		Hormonal level				
		(ng/ml)	LH (ng/L)	Estrogen (ng/ml)	(ng/L)	
Naturally cyclic estrus	Anestrus phase For all animals	3.9±1.4 c	32±16 d	43.3±8.4 abc	110±32 c	
	Proestrus phase For all animals	26.5±2.8 a	57±9 cd	68.0±18.4 abc	267±157 a	
	Estrus phase For all animals	13.5±5.6 abc	101±25 a	64.0±10.7 abc	236±63 a	
	1 st month of pregnancy For all animals	9.3±1.9 bc	54±19 cd	52.8±16.4 abc	235.85±37.72 ab	
	2 nd month of pregnancy For all animals	12.6±2.2 abc	63±32 bcd	58.5±13.5 abc	220±32 abc	
	Post-partum period (one month) For all animals	4.2±2.1 c	32±16 d	35.5±7.8 bc	189±63 abc	
Induced estrus treatment	Non-responsive	Treatment period (four weeks) For all animals	13.7±5.5 abc	54±19 cd	64.5±9.4 abc	267±16 a
		1 st month after treatment for non-responsive animals	5.0±0.5 c	32±16 d	41.6±8.1 abc	157±25 abc
	Responsive to treatment	2 nd month after treatment for non-responsive animals	3.0±0.1 c	32±16 d	33.5±1.3 c	104±16 c
		Proestrus phase For responsive animals	25.0±2.0 ab	57±9 cd	69.8±16.3 ab	220±32 abc
		Estrus phase For responsive animals	11.9±4.2 abc	90±32 ab	71.5±3.7 a	267±32 a
		1 st month of pregnancy For responsive animals	9.9±2.9 bc	50±16 cd	63.0±18.6 abc	204±63 abc
		2 nd month of pregnancy For responsive animals	11.7±2.9 abc	79±16 abc	58.5±13.6 abc	220±32 abc
Post-partum period (one month) For responsive animals	4.2±2.1 c	32±16 d	46.4±16.8 abc	126±32 bc		
LSD		16.2*	32**	34.7*	122**	

- Number represent mean±SE.
- Identical letters represent no significant differences.
- Non-identical letters represent significance at *P<0.05 or **P<0.01 levels.
- Total number of animals=10.
- Number of non-responsive animals=2.
- Numbers of responsive animals=8.

DISCUSSION

Table (1) shows 100% proestrus signs with 100% success of conceiving, and after induction of estrus by using Bromocriptine 80% show fertile estrus with 80% conceive. This result agrees with the findings of (14; 15; 16; 17; 18; 19 and 20) related to the same drug. Results also appear to fit with (10) results, and more than the responsive percentage of (11 and 12) who used gonadotropins hormonal drug in Iraqi dogs. Dopamine agonists increase basal (FSH) secretion, inducing ovarian follicular development; Or, perhaps they sensitise the ovary to respond to FSH and LH. However, dopamine receptors are present in both, ovary and the pituitary. It is not yet known if these effects of dopamine agonists occur at the pituitary or ovarian levels or both (19). Observations suggest that an inhibition of prolactin secretion may regulate the initiation of proestrus (21). It has also been demonstrated that shortening of the inter-oestrus interval by bromocriptine in a dose that also lowers plasma PRL concentration is associated with an increase in plasma FSH concentration without a concomitant increase in plasma LH concentration (18). Oestrus induction and ovulation has been used to shorten the bitch anoestrus period, which is mostly long (4-8 months) (1 and 2). Furthermore, estrus induction is used for the treatment of primary and secondary anoestrus, and for the improvement of other biotechnologies research such as embryo transfer (21; 22 and 23). Dopamine agonists disadvantage is the duration of the estrus induction treatment that can last for more than 40 days (23 and 24). Cabergoline applications were used to induce oestrus cycle in bitches with pseudopregnancy problems or with prolonged lactation (25). The mechanism of action of cabergoline could involve the reinitiating of gonadotropin secretions resulting a

decrease in prolactin secretion since prolactin has shown to inhibit GnRH and Gonadotrophin secretion in human beings and some mammals (25).

Table (2) clearly indicates that FSH and LH have a curve shape during proestrus, estrus, pregnancy months and post-partum period. This finding fitted with (8). The increase in LH is more important than in FSH in the onset of proestrus on canine females which is suggested by (26) in their observations during their study by injecting purified porcine LH that induced fertile estrus in anestrus bitches, which did not occur with administration of FSH. This information is partially in agreement with our study that the FSH is important also as the LH in estrus cycle. This partial agreement may be related to the pulsatile role of LH effect that makes it difficult to detect its role during estrus cycle (27). LH and FSH concentrations are both decreased to the lowest levels of the cycle in late proestrus, and that might be related to the estrogen and inhibin negative feedback (1). Their results also in partial agreement with our recent study. This partial agreement might be related to the increases in FSH and LH during the preovulatory surge, with elevation in FSH in 0.5–1 day after the peak in LH and decline to baseline within 1-2 days longer than that of LH (28). The concentration of FSH is equal to that which observed in anestrus, whereas LH is 10-100 folds higher than anestrus concentrations (1). Likewise, our result agrees in part with his findings.

The estrogen and prolactin hormones show no significant differences in cyclic or induced estrus animals. However, the level of these hormones has a significant dropping during the 2nd month in non-responsive females which agrees with (8). Estradiol increased throughout proestrus, rising from basal 5-10 pg/ml to reach peaks of 45-120 pg/ml (in most instances) 1-3 days before the preovulatory LH peak (1).

Both, LH surge and estrus sex behaviour began immediately after the peak in estradiol level (9) which agrees with this study.

Normal luteal function in bitches requires both LH and prolactin as luteotrophic hormones (29). After day 25 of pregnancy, there is an increase in prolactin concentration and expands the effective life of luteal tissue to the terminus of gestation (30). Prolactin-stimulated luteal function and increased progesterone production during pregnancy several-fold than that of the non-pregnant females (31; 32 and 33). The luteotrophic role of prolactin is assumed to be similar to that reported in rodents, and it includes increased numbers of LH receptors (34). In dogs, LH and prolactin receptors appear to be maintained throughout luteal cell lifespan (35). In female canids, as in other species, prolactin secretion is primarily under the negative control of dopamine and suppressed by dopamine agonists (36). Only in dogs, the progesterone negatively affects prolactin secretion (37). From all of the previously notes we conclude that the non-significant differences in LH and prolactin hormones in our results are related to the fact that the presence of corpus luteum during cycle or pregnancy is maintained by that two hormones, so they must be stable to protect the survival of Corpus luteum.

Canine corpus luteum can synthesise estrogen and progesterone, and both could have autocrine effects (38). In pregnant, as well as in non-pregnant female dogs, luteal function depends on plasma progesterone profiles after day 20-30 and slowly dropping over a 30-50 day period (39).

CONCLUSION

The FSH and LH showed higher levels during proestrus and estrus and started to decline through pregnancy months and reached their lowest level during post partum period. Estrogen and Prolactin have a principal role in reproduction that maintains

their levels through proestrus, estrus, pregnancy months and the post-partum period, which did not happen in non-responsive animals.

الصورة الهرمونية للكلاب العراقية خلال الحالات الفسلجية المختلفة

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الخلاصة

أجريت هذه الدراسة لتقصي نجاح أستحداث الشبق وتتبع مستويات الهرمونات خلال دورة الشبق، الحمل وما بعد الولادة في الكلاب. تم حجر عشرة كلاب بعمر 2-3 سنة وبوزن 18-22 كغم في محاجر الكلاب التابعة لكلية الطب البيطري-جامعة بغداد. تم تسفيد الكلاب وتم متابعتها حتى أصبحت حوامل. ومن ثم وبعد شهر واحد بعد الولادة، عولجت بمركب البروموكربتين (البارادول) بجرعة (0.05 ملغم/كغم/كلب) فموياً مرتين يومياً لأحداث الشبق. أظهرت ثمانية كلاب الشبق ومن ثم سفدت مرة ثانية من نفس الكلبين البالغين المستخدمين بالتسفيد الأول. سحب مصل الدم مرة واحدة أسبوعياً من الوريد الوداجي. تم قياس مستويات هرمونات المحفز لنمو الجريبات، الهرمون اللوتيني، الأستروجين والبرولاكتين باستخدام عدة هرمونية خاصة بالكلاب بطريقة تحليل عداد الكاما. بدأ هرموني المحفز لنمو الجريبات والهرمون اللوتيني في كلتا الفترتين الطبيعية الشبق والمستحدثة الشبق بالأرتفاع خلال طور قبل الشبق والشبق ثم أنخفض خلال أشهر الحمل وفترة ما بعد الولادة. لوحظ وجود فرقاً معنوياً في هرموني الأستروجين والبرولاكتين خلال الشهر الثاني في الكلاب الغير مستجيبة للعلاج. وأكد مستوى هرمون الأستروجين أنه طور ما قبل الشبق، الشبق والفترة العلاجية أرتفاعاً معنوياً (مستوى أقل من 0.01) مقارنة بفترة أنعدام الشبق.

أظهرت النتائج أنه لهرموني المحفز لنمو الجريبات واللوتيني تناسق في مستوياتهما بدأ بأرتفاعهما خلال ما قبل الشبق والشبق ومن ثم أنخفاضه خلال الفترة اللاحقة ليصل أدنى مستوى له خلال فترة ما بعد الولادة في الحيوانات الطبيعية والمستحدثة الشبق، بينما كان للأستروجين والبرولاكتين دوراً مهماً خلال الفترات المذكورة.

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