HORMONAL PROFILE OF IRAQI BITCHES DURING VARIOUS PHYSIOLOGICAL STAGES.

Nazih Wayes Zaid

Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Baghdad, Iraq.

(Received 10 May 2017, Accepted 28 May 2017)

Keywords: Dogs, Post-partum, Bromocriptine.

ABSTRACT

The study was conducted to investigate the success of inducted 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 luteam was dependent on both LH and prolactin secretion (1). Prolactin increase progesterone secretion several-fold more during pregnancy than non pregnant period due to stimulating of luteal function (9). Several studies were conducted in Iraq to evaluate the induction of estrus in dogs using gonadotropins hormones (10; 11, 12). This study is the first of its kind to use Dopamine agonist for inducting 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 matted with male and become 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 was collected from all

female dogs once a week from the cephalic vein. The serum separated from blood by centrifuge. The FSH, LH, estrogen and prolactin hormone were assayed using Gamma counter canine kits (Biomedical). The data was assessed by using one-way ANOVA, followed by LSD 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 induction of 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			
			FSH (ng/ml)	LH (ng/L)	Estrogen (ng/ml)	Prolactin (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
		1st month of pregnancy For all animals	9.3±1.9 bc	54±19 cd	52.8±16.4 abc	235.85±37.72 ab
		2nd 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		Treatment period (four weeks) For all animals	13.7±5.5 abc	54±19 cd	64.5±9.4 abc	267±16 a
	Non-responsive	1st month after treatment for non-responsive animals	5.0±0.5 c	32±16 d	41.6±8.1 abc	157±25 abc
		2nd month after treatment for non-responsive animals	3.0±0.1 c	32±16 d	33.5±1.3 c	104±16 c
	Responsive to treatment	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
		1st month of pregnancy For responsive animals	9.9±2.9 bc	50±16 cd	63.0±18.6 abc	204±63 abc
		2nd month of pregnancy For responsive animals	11.7±2.9 abc	79±16 ab	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**

- Numbers represent mean ± standard error of mean.
- 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) showed 100% proestrus signs with 100% success of conceiving, and after induction of estrus by using Bromocriptine 80% revealed fertile estrus with 80% conceives. This result is in agreement with the findings of (14; 15; 16; 17; 18; 19, 20) related to the same drug. Results also appeared to fit with (10) results, and more than the responsive percentage of (11, 12) who used gonadotropins hormonal drug in Iraqi dogs. Dopamine agonists increase basal (FSH) secretion, inducing ovarian follicular development; Or, perhaps they sensitive 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). Our observations suggesting 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). Estrus induction and ovulation has been used to shorten the bitch anoestrus period, which is mostly long (4-8 months) (1, 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, 23). Dopamine agonists disadvantage is prolongation of the duration of the induced estrus which last for more than 40 days (23, 24). Cabergoline applications were used to induce estrus cycle in bitches with pseudo pregnancy 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 mammalians (25).

Table (2) clearly indicates that FSH and LH have a shape curve during proestrus, estrus, pregnancy months and post-partum period. This finding is in agreement with previous study of (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 anestrous 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 might 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 inhibit 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 anestrous, whereas LH is 10-100 folds higher than anestrous concentrations (1). Likewise, our result agrees in part with his findings.

The estrogen and prolactin hormones showed 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 previous study (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 behavior 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, 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 reveal 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 luteam during cycle or pregnancy is maintained by that two hormones, so they must be stable to protect the survival of Corpus luteam.

Canine corpus luteam can synthesize 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 المراحل الفسلجية المختلفة

نزيه ويس زيد
فرع الجراحة والتوليد، كلية الطب البيطري-جامعة بغداد، العراق.

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 التابعه لكلية الطب البيطري-جامعة بغداد. تم تسفيد الكلاب وتم متابعتها حتى أصبحت حوامل. 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 عينة الدم مرة واحدة أسبوعياً من الوريد الوداجي بواسطة أنابيب خاصة ثم تم فصل مصل الدم بواسطة جهاز الطرد المركزي. 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 بالارتفاع خلال طور قبل الشبق والشبق ثم انخفضا 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) مقارنةً بفترة انعدام الشبق.

أظهرت النتائج أنه لهرموني المحفز لنمو الجريبات واللوتيني تناسق في مستوياتهما بدأً بارتفاعهما خلال ما قبل الشبق والشبق ومن ثم انخفاضه 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.

REFERENCES

1. Concannon, P.W.; Castracane, V.D.; Temple, M. and Montanez, A. (2009). Ovarian function in dogs and other carnivores. *Anim Reprod*, 6 (1): 172-193.
2. Concannon, W. (2011). Reproductive cycles of the domestic bitch. *Anim Reprod Sci*, 124 (3-4): 200-210.
3. Sirivaidyapong, S. (2011). Control of Oestrus and Ovulation in Dog and Cat: An Update. *Thai J Vet Med Suppl*, 41: 65-68.
4. Ajitkumar, G.; Praseeda, R.; Rajankutty, K. and Alex, P.C. (2010). Comparative efficacy of high vs. low dose cabergoline treatment regimen in inducing fertile oestrus in anoestrous dogs. *J Anim Vet Adv*, 9 (12): 1735-1738.

5. Shinde, S.; Krishnappa, B.; Kumar, B.; Perumal, P.; Gupta, H.K. and Srivastava, S.K. (2014). Induction of Fertile Estrus in Bitch (Pug) With Cabergolin-A Case Report. *Intern J Vet Sci*, 3 (1): 1-3.
6. Aiello, S.E. and Moses, M.A. (2016). The Merck Veterinary Manual. 11th ed. USA: Wiley Company.
7. Tsutsui, T.; Takahashi, F.; Hori, T.; Kawakami, E. and Concannon, P.W. (2009). Prolonged duration of fertility of dog ova. *Reprod Domes Anim*, 44 (2): 230-233.
8. Linde-Forsberg, C. (2007). Biology of reproduction of the dog and modern reproductive technology. **In:** Ruvinsky, A. and Sampson, J.; editors. The Genetics of the Dog. USA: CABI Publishing. pp: 401-432.
9. Weinbauer, G.F.; Niehoff, M.; Niehaus, M.; Srivastav, S.; Fuchs, A.; Van Esch, E. and Cline, J.M. (2008). Physiology and Endocrinology of the Ovarian Cycle in Macaques. *Toxicol Path*, 36 (7): 7-23.
10. Al-Hamedawi, T.M. (2013). Induction of Fertile Estrus in Bitches using Equine Chorionic Gonadotropine (eCG) and Human Chorionic Gonadotropine (hCG). *Iraqi J Vet Med*, 37 (1): 102–105.
11. Ibrahim, N.S. and Nawaf, S.A.A.H. (2015). Induction of estrus with (PMSG and hCG) in Iraqi bitches. *Indian J Res*, 4 (6): 334-335.

12. Nawaf, S.A.A.H. (2015). Induction of fertile estrus and progesterone assay in bitch in Iraq. Master Science Thesis-Veterinary Medicine College-University of Baghdad.

13. Al-Mohammed, N.T.; Al-Rawi, K.M.; Younis, M.A. and Al-Morani, W.K. (1986). Principles of Statistics. Book House for Printing and Publishing, Al-Mosel University.

14. Zoldag, L.; Fekete, S.; Csaky, I. and Bersenyi, A. (2001). Fertile estrus induced in bitches by bromocrytpine, a dopamine agonist: a clinical trial. *Theriogeno*, 55: 1657–1666.

15. Gobello, C.; Castex, G. and Corrada, Y. (2002). Use of cabergoline to treat primary and secondary anoestrus in dogs. *J Amer Vet Med Assoc*, 220: 1653-1654.

16. Rota, A.; Mollo, A.; Marinelli, L.; Gabai, G. and Vincenti, L. (2003). Evaluation of cabergoline and buserelin efficacy for oestrous induction in the bitch. *Reprod Domes Anim*, 38: 440-443.

17. Gobello, C.; Castex, G.; Sota, R.D.L. and Corrada, Y. (2004). Shortening of interostrous intervals with cabergoline in bitches: A clinical trials. *J Amer Anim Hosp Asso*, 40: 115-119.

18. Cirit, U.; Bacinoglu, S.; Tas, M. and Alkan, S. (2007). Use of a decreased dose of cabergoline to treat secondary anoestrus in bitches. *Bull Vet Inst Pulawy*, 51: 43-46.

19. Spattini, G.; Borghi, V.; Thuroczy, J.; Balogh, L.; Scaramuzzi, R.J. and De Rensis, F. (2007). Follicular development and plasma concentrations of LH and prolactin in anestrous female dogs treated with the dopamine agonist cabergoline. *Theriogeno*, 68: 826–833.

20. Ajikumar, G.; Praseeda, R.; Rajankutty, K. and Alex, P.C. (2010). Comparative efficacy of high vs. low dose carbergoline treatment regimen in inducing fertile oestrus in anoestrousdogs. *J Anim Vet Adv*, 9 (12): 1735-1738.

21. Kutzler, M.A. (2005). Induction and synchronization of estrus in dogs. *Theriogeno*, 64: 766–775.

22. Kutzler, M.A. (2007). Estrus induction and synchronization in canids and felids. *Theriogeno*, 68: 354-374.

23. Root Kustritz, M.V. (2012). Managing the reproductive cycle in the Bitch. *Vet Clinic of North America: Small Anim Pract*, 42: 423-437.

24. Wiebe, V.J. and Howard, J.P. (2009). Pharmacologic advances in canine and feline reproduction. *Top Comp Anim Med*, 24: 71-99.

25. Gunay, A.; Gunay, U. and Soylu, M.K. (2004). Cabergoline applications in early and late anoestrus periods on German Shepherd dogs. *Rev Med Vet*, 155 (11): 557-560.

26. Verstegen, J.; Onclin, K.; Silva, L. and Concannon, P. (1997). Termination of obligate anoestrus and induction of fertile ovarian cycles in dogs by administration of purified pig LH. *J Reprod Fertil*, 111: 35-40.

27. Shacham, S.; Harris, D.; Ben-Shlomo, H.; Cohen, I.; Bonfil, D.; Przedecki, F.; Lewy, H.; Ashkenazi, I.E.; Seger, R. and Naor, Z. (2001). Mechanism of GnRH receptor signaling on gonadotropin release and gene expression in pituitary gonadotrophs. *Vit Horm*, 63: 63-90.

28. Beijerink, N.J.; Kooistra, H.S.; Dieleman, S.J. and Okkens, A.C. (2004). Serotonin antagonist-induced lowering of prolactin secretion does not affect the pattern of pulsatile secretion of follicle-stimulating hormone and luteinizing hormone in the bitch. *Reprod*, 128: 181-188.

29. Kooistra, H.S. and Okkens, A.C. (2001). Secretion of prolactin and growth hormone in relation to ovarian activity in the dog. *Reprod Domes Anim*, 36: 115-119.

30. Concannon, P.; Tsutsui, T. and Shille, V. (2001). Embryo development, hormonal requirements and maternal responses during canine pregnancy. *J Reprod Fertil. Suppl*, 57: 159-169.

31. Galac, S.; Kooistra, H.S.; Butinar, J.; Bevers, M.M.; Dieleman, S.J.; Voorhout, G. and Okkens, A.C. (2000). Termination of mid-gestation pregnancy in bitches with aglepristone, a progesterone receptor antagonist. *Theriogeno*, 53: 941-950.

32. Lee, W.M.; Kooistra, H.S.; Mol, J.A.; Dieleman, S.J. and Schaefers-Okkens, A.C. (2006). Ovariectomy during the luteal phase influences secretion of prolactin, growth hormone, and insulin-like growth factor-I in the bitch. *Theriogeno*, 66: 484-490.

33. Rodas-Ruiza, J.; Tabares-Sernab, C.J. and Giraldo-Echeverria, C.A. (2015). Evaluation of prostaglandin F2α, estradiol benzoate and deslorelin acetate protocol for oestrus induction in bitches. *Arch Med Vet*, 47: 395-399.

34. McBride, M.W.; Aughey, E.; O'Shaughnessy, P.J. and Jeffcoate, I.A. (2001). Ovarian function and FSH receptor characteristics during canine anoestrus. *J Reprod Fertil*, 57: 3-10.

35. Kowalewski, M.P.; Michel, E.; Gram, A.; Boos, A.; Guscetti, F.; Hoffmann, B.; Aslan, S. and Reichler, I. (2011). Luteal and placental function in the bitch: spatio-temporal changes in prolactin receptor (PRLr) expression at dioestrus, pregnancy and normal and induced parturition. *Reprod Biol Endocrino*, 3 (9): 109.

36. Meij, B.P.; Kooistra, H.S. and Rijnberk, A. (2010). Hypothalamus-Pituitary System. **In:** Rijnberk, A. and Kooistra, H.S.; Editors. Clinical Endocrinology of Dogs and Cats. Germany: pp: 13-54.

37. Kooistra, H.S. and Okkens, A.C. (2002). Secretion of growth hormone and prolactin during progression of the luteal phase in healthy dogs: a review. *Molecul Cell Endocrino*, 197: 167-172.

38. Hoffmann, B.; Busges, F.; Engel, E.; Kowalewski, M.P. and Papa, P. (2004). Regulation of corpus luteum-function in the bitch. *Reprod Domes Anim*, 39: 232-240.

39. Onclin K, Verstegen JP, Concannon PW. Time-related changes in canine luteal regulation: in vivo effects of LH on progesterone and prolactin during pregnancy. *J Reprod Fert* 2000, 118 (2): 417-424.