

The Influence of Manner of Sustenance on Luteinizing Hormone Concentration in Dogs

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Abstract: The evaluation of the status of luteinizing hormone (LH) in the peripheral blood of dogs plays an important role in precise estrus detection. The measurement of the luteinizing hormone concentration in the peripheral blood is a reliable and exact method used for determining the optimal time for mating. The peak of luteinizing hormone (LH) is a key endocrinological occurrence in the estrus cycle that starts (provokes) ovulation and thereby determines the fertile period. Dogs (females) typically have two estrus cycles per year, in spring and autumn. The elevated seasonal activity during February-May might be induced, among other things, by the environmental factors that stimulate the hypothalamic–hypophyseal–ovarian axis. There are a number of evidences testifying to the fact that adenohypophysis secretion is affected by external or exteroceptive stimuli through the nervous system. This study included a total of 30 dogs (females) in the region of Tuzla whose blood samples were collected in spring for evaluation of the luteinizing hormone (LH) concentration. The dogs (females) were divided into three groups depending on the manner of sustenance: a) group A (dogs living exclusively in a home environment); b) group B (dogs living in a shelter environment); c) group C (stray dogs). LH concentration ranged from 0.312 ng/ml to 0.871 ng/ml during spring. No statistically significant differences were found in LH concentrations between groups A and B ($p=0.1202$), groups A and C during spring ($p=0.5444$), nor between group B and group C dogs during spring ($p=0.2888$). Based on the evaluated luteinizing hormone concentration, our study suggests that spring is the time of the year when all (female) dogs, irrespective of the manner of sustenance, become reproductively active. This study showed the (female) dog reproductive behaviour to be caused by internal and external factors such as photoperiod, temperature, ambient conditions, nourishment, the change of seasons, etc. These results play an important role

in estimating estrus in (female) dogs and observing all relevant factors that are crucial to maintaining the reproductive potential of (female) dogs.

Keywords: luteinizing hormone, dogs, spring

Introduction

The evaluation of the status of luteinizing hormone (LH) in the peripheral blood of dogs plays an important role in precise estrus detection. The measurement of the luteinizing hormone concentration in the peripheral blood is a reliable and exact method used for determining the optimal time for mating (Mutevelic *et al.* 2003). The peak of the luteinizing hormone (LH) is a key endocrinological occurrence in the estrus cycle that starts (provokes) ovulation and thereby determines the fertile period (Goodman 1992). Van Haaften *et al.* (1989) as well as Correa (2002) point out that the optimal time for mating can be best determined by tracking LH and progesterone levels. The sexual cycle of a (female) dog is divided into four stages: proestrus, estrus, diestrus and anestrus (Concannon *et al.* 1987, Holst and Phemister 1971, Olson *et al.* 1983, Shille *et al.* 1989). Each of these stages is characterized by an exact concentration of sexual hormones. The onset of estrus coincides with the sudden occurrence of LH hypophysis secretion (Concannon *et al.* 1975). Feldman and Nelsons (1987) indicate that the onset of the sexual cycle in a (female) dog is a result of a complex interaction between the environment and general health condition, ovarian condition, uterine condition, animal age and other unidentified factors.

The luteinizing hormone is a glycoprotein composed of two attached non-covalent subunits: general α -subunits and hormone-specific β -subunits. As opposed to other hormones of the frontal lobe of hypophysis (adenohypophysis), FSH and LH are secreted from the same type of hypophysis cells, basophilic gonadotropic cells (Jablonka – Shariff and Boine 2004), but are deposited in separate granules (Baenzing and Green 1988). The primary stimulator of LH synthesis and secretion is the hypothalamic decapeptide – gonadotropin-releasing hormone (GnRH) (Clayton 1989). Gonadotropins influence the ovaries by stimulating the secretion of the hormones as a response to FSH and LH. The factors affecting the secretion of gonadotropins are considered to be acting through GnRH secretion. The studies conducted by Karsch and Wayne (1988) and Lincoln *et al.* (1989) proved that olfaction, tactile, hearing, visual and psychogenic signals can accelerate the onset of the ripus season in seasonal animals or increase the level of LH in other species by increasing GnRH secretion. LH stimulates ovulation, induces the creation and secretion of estrogenic hormones from the theca interna cells, the function of the yellow body and stimulates the secretion of progesterone. LH has been proved to be pulsatilely secreted in (female) dogs (Concannon *et al.* 1986, Shille *et al.* 1989, Concannon 1993). The increase in pulsation is often associated with very early proestrus and might be considered a stimulus for the follicular wave development (Reimers *et al.*, 1978).

A sporadic increase in LH secretion occurs during anestrus in (female) dogs as well as in other animals (Bon Durant *et al.* 1981). These sudden transient increases in plasma LH lead to two main, short but intensive secreting episodes. One LH maximum immediately precedes the onset of proestrus, whereas the other

precedes or coincides with the onset of estrus and the ensuing ovulation. They form a delicate mechanism of adjustment that is necessary during the process of coordinating follicular development with mating, ovulation and fertilization (Feldman and Nelson 1987).

Dogs (females) typically have two estrus cycles per year, in spring and autumn. The elevated seasonal activity during February-May might be induced, among other things, by the environmental factors that stimulate the hypothalamic–hypophyseal–ovarian axis (Feldman and Nelson 1987). There are many evidences for external or exteroceptive stimulus affecting the adenohypophysis secretion through the nervous system (Jovanović 1986).

The aim of this study is to evaluate the concentration of luteinising hormone as well as the effect of spring season on ovary.

Material and Methods

This study included a total of 30 (female) dogs in the region of Tuzla, whose blood samples were collected in spring for evaluation of the luteinizing hormone (LH) concentration. The dogs were at different stages of estrus cycle as determined by the luteinising hormone concentration. The (female) dogs were divided into three groups depending on the manner of sustenance: a) group A (dogs living exclusively in a home environment); b) group B (dogs living in a shelter environment); c) group C (stray dogs). The laboratory evaluation of the serum concentration of luteinizing hormone was performed at the Public Health Institution, University Clinical Centre Tuzla, Internal Medicine Clinic, Department of Nuclear Medicine. Blood was taken by means of a vein tap (*v. brachiocephalica antebrachii*, *v. saphaena*), the random sampling method, at the Veterinary Station in Tuzla. The 5ml sample coagulated 30 minutes after blood collection. The blood sample was centrifuged at 3000 revolutions for 10 minutes to obtain the serum which was kept at -20°C prior to being processed. The serums were heated at +4°C, 24 hours before processing. Upon reaching the room temperature, the serums were homogenized and processed using the Delfia method.

The Arcus Quickstat statistical processing programme was used to statistically analyze the obtained values. These were subjected to standard statistical methods of the descriptive statistics for mean value, standard deviations and the median. The statistical significance of differences was established at the 5 % level.

Results and Discussion

The overall complexity and the current importance of endocrinological studies, given the specific role of the ovary in the neuroendocrine system, as well as the significant impact of different factors, especially climate, in terms of the effect on estrus occurrence and detection, inspired this study on luteinizing hormone concentration, as well as the effect of external factors on ovary activity.

LH levels were determined during the spring season in (female) dogs from groups A, B, C. They are given in Figure 1.

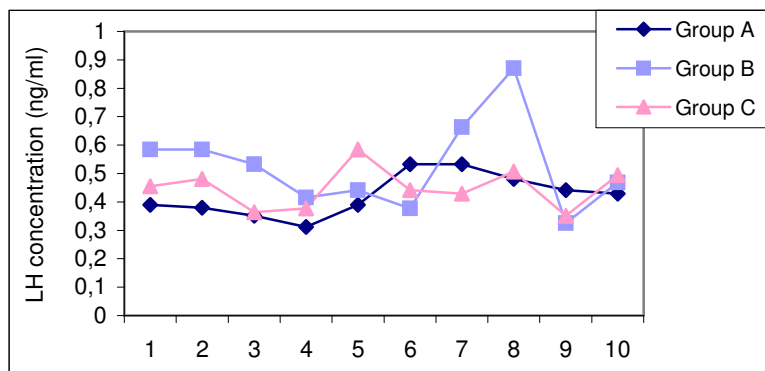


Fig. 1. The concentration LH in test groups

LH concentrations in the test groups ranged from 0.312 ng/ml – 0.871 ng/ml during spring. The (female) dogs living in a home environment had LH concentrations of 0.533ng/ml to 0.312ng/ml. As for the (female) dogs living in a shelter environment, the highest value was 0.871 ng/ml and the lowest – 0.325 ng/ml. The highest value of LH concentration in group C dogs during the spring season was 0.585 ng/ml, whereas the lowest value was 0.351ng/ml.

The Kolmogorov–Smirnov and Shapiro–Wilk tests did not reveal any statistically significant differences between the normal distribution and the distribution of LH concentration in group A ($p = 0.159$; $p = 0.390$), nor in group C ($p = 0.153$; $p = 0.306$), whereas a statistically significant difference was found between the normal distribution and the distribution of LH concentration in group B ($p = 0.021$; $p = 0.010$) (Table 1).

Tab. 1. Normality test for the LH A, B, C group

Variables	Normality test			
	Kolmogorov-Smirnov test		Shapiro-Wilk test	
	Statistic	Sig.	Statistic	Sig.
LH group A	0.1191	0.159	0.9657	0.390
LH group B	0.1515	0.021	0.9147	0.010
LH group C	0.1196	0.153	0.9609	0.306

A comparison of LH concentrations between group A and group B dogs ($p = 0.1202$) did not show any statistically significant differences, nor did a comparison between group A and group C (female) dogs during spring ($p = 0.5444$) (Table 2).

Tab. 2. P-values as determined by the Mann – Whitney tests

Hormone - season	group A – group B	group A - group C	group B - group C
LH (spring)	$p = 0.1202$	$p = 0.5444$	$p = 0.2888$

The Mann–Whitney test used in this study did not reveal any statistically significant differences in LH concentration between group B and group C during the spring season ($p=0.2888$).

An analysis of the results obtained to date, especially those of studies similar to the present one suggested significant variations in luteinizing hormone concentration in (female) dogs during different phases of the estrus cycle. Given the diversity of conditions and the manner of sustenance, the study was conducted in order to analyze all the relevant factors crucial to maintaining the reproductive potential of (female) dogs. According to Sandoval *et al.* (2005), the reproductive behaviour of mammals is directly caused by internal and external factors including photoperiod, temperature, ambient conditions, nourishment, and changes of seasons.

Olson *et al.* (1982) reported an increase in LH concentration in the serum prior to and at the onset of proestrus, which is in agreement with the results of our study. This underlines the findings of Hase *et al.* (2000) that the level of LH is over 10ng/ml around the LH wave, which is inconsistent with the present results showing the maximum LH value of 0.871ng/ml during early estrus. However, according to the above authors, ovulation can be predicted by determining LH concentration only if certain tests have been made several times a day, which provides an explanation for the low values of LH obtained in our study.

In terms of seasonality (entrance into the estrus cycle during certain seasons of the year), different results have been obtained by a number of authors. According to Gilbert (1998), (female) dogs are defined as being unseasoned monoestrical animals, meaning that estrus is displayed to a greater or lesser extent irrespective of the season of the year.

The evaluation of luteinizing hormone concentration in this study implied that the majority of (female) dogs became reproductively active during the spring season, regardless of sustenance conditions, which is in agreement with the studies by Christiea and Bella (1971) (cited by Feldman and Nelson 1987) who reported the occurrence of reproductive activities during the early months of the year (for example in February, March, April, i.e. during late winter/early spring). Feldman and Nelson believe that mating and giving birth to puppies can begin in any period of the year, but the subtle maximums occur in late winter/early spring and in autumn.

Sandoval *et al.* (2005) observed the occurrence of estrus over three-month periods throughout the year. As their results showed variations in mating between months, the authors suggested that dogs were seasonal monoestrical animals.

Conclusion

The obtained results suggest the following:

- out of the 30 test dogs, the lowest luteinizing hormone concentration of 0.312ng/ml was found in the dogs living exclusively in a home environment. The maximum LH concentration value was 0.871ng/ml, as determined in the dogs living in a shelter environment.

- the (female) dogs living in a home environment had the LH concentration of 0.533ng/ml to 0.312ng/ml. The highest and lowest LH concentrations in the shelter (female) dogs were 0.871ng/ml and 0.325ng/ml, respectively. The highest value of LH concentration in group C during the spring season was 0.585ng/ml and the lowest 0.351ng/ml.

- the Mann – Whitney tests did not reveal any statistically significant differences in LH concentration between group A and group B dogs during the spring season ($p=0.1202$).

- no statistically significant differences were found in luteinizing hormone concentration between group A and group C (female) dogs during the spring season ($p=0.5444$), nor between group B and group C dogs during spring ($p=0.2888$) and winter ($p=0.5189$).

- based on the luteinizing hormone concentration, this study suggested that spring is the time of the year when all (female) dogs, irrespective of the manner of sustenance, become reproductively active.

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UTJECAJ UVJETA DRŽANJA NA KONCENTRACIJU LUTEINIZIRAJUĆEG HORMONA KOD PASA

- originalni naučni rad -

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Rezime

Istraživanje nivoa koncentracija luteinizirajućeg hormona u perifernoj krvi pasa je bitno za preciznu detekciju estrusa. Mjerenje koncentracije luteinizirajućeg hormona u perifernoj krvi je pouzdana i precizna metoda za određivanje optimalnog vremena za parenje. Vrhunac luteinizirajućeg hormona ključan je endokrinološki događaj za pojavljivanje estrusnog ciklusa, koji započinje (izaziva) ovulaciju i time određuje fertilni period. Smatra se da pas (ženka) doživljava estrusni ciklus dva puta godišnje, u proljeće i u jesen. Pojačana sezonska aktivnost u periodu od februara do maja može biti izazvana, između ostalog i faktorima sredine koji stimuliraju hipotalamusno-hipofizno-ovarijsku osovinu. Postoji mnogo dokaza o tome da vanjski ili eksteroceptivni stimulansi preko nervnog sistema utiču na lučenje hormona adenohipofize. Našim istraživanjem smo obuhvatili ukupno 30 pasa (ženki) sa područja opštine Tuzla, kojima je u proljeće uzet uzorak krvi i izmjerena koncentracija luteinizirajućeg hormona (LH). Psi su po načinu ishrane bili podjeljeni u tri grupe: grupa A (psi koji su isključivo živjeli u kućnim uvjetima); grupa B (psi koji su živjeli u azilu); grupa C (psi litalice). Utvrđena razlika koncentracije luteinizirajućeg hormona varirala je od 0,312 ng/ml – 0, 871 ng/ml u proljeće. Upoređujući LH koncentraciju A i B grupe ($p=0,1202$), pasa iz grupa A i C tokom proljeća ($p=0,5444$), i nivo LH koncentracije grupa B i C tokom proljeća ($p=0,2888$) nismo utvrdili nikakvu statistički značajnu razliku. Našim istraživanjem smo zaključili, na temelju koncentracije luteinizirajućeg hormona, da je proljeće doba godine kada svi psi (ženke), bez obzira na uvjete držanja, postaju reproduktivno aktivni. Tako da smo utvrdili da je reproduktivno ponašanje kod pasa (ženki) prouzrokovano i unutarnjim i vanjskim faktorima kao što su fotoperiod, temperatura, uslovi ambijenta, ishrana, kao i promjena godišnjih doba i sl. Ovi rezultati imaju značajnu primjenu u procjeni estrusa kod pasa i opažanju svih relevantnih faktora koji su bitni za održavanje reproduktivnog potencijala pasa (ženki).