Relationship between Duration from Proestrus until Predicted Ovulation and Changes of Progesterone Concentration during this Time on Canine Litter Size

Indrė Mečionytė[1], Sigita Kerzienė[1], Lina Anskienė[1], Renata Japertienė[1], Giedrius Palubinskas[1]
[1]Department of Animal Breeding, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

Keywords: *canine, estrus, progesterone, litter size, POD.*

Abstract. *The aim of this research is to predict the number of puppies that may be born by duration of period from proestrus until POD and the change of P4 concentration during this time of medium size canines. The study was conducted when the oestrus cycles of clinically healthy medium size primiparous and multiparous canine females (n = 47) at 2–7 years old were observed. Canine females were observed from first proestrus signs (FPS) till litter was born. Blood samples were collected on day 5 from the onset of discharge from the vulva until the day when the progesterone (P4) concentration rate changed till 4 ng/mL. If P4 concentration was < 1 ng/mL on the day of test making, the test was repeated after 3 days; if it was 1–3 ng/mL, the test was repeated after 2 days; and if it was > 3 ng/mL, the test was repeated every day until the predicted ovulation day (POD). Mating was performed 2 times with medium size breed clinically healthy 2–8–year–old canine males, which already had offspring. The number of alive and stillbirth puppies in a litter were counted at a time when they were born. All females were divided into 3 groups by the litter size: small, medium, and large. The mean POD for females in this study was 11.36 ± 2.17 day. It was found that the fastest increase of P4 concentrations was in the medium ($y = 2.1092x + 1.097$, $R^2 = 0.9833$) and in the large ($y = 1.9792x + 1.2844$, $R^2 = 0.9999$) litter groups and the largest litters were born when P4 reached 4–8 ng/mL on days 9–14 day from FPS ($P < 0.05$). It was also observed that there was no correlation between progesterone concentration during POD and the number of puppies born ($y = -0.0522x + 6.2322$, $R^2 = 0.0233$).*

Introduction

A canine female can bring limited numbers of litters. However, it is expected that the female of a high breeding value will produce as many offspring as possible who will continue to maintain the breed at a high level or even contribute to the improvement of the breed. Therefore, in order to improve these aspirations, it is required to get as many offspring of such a female as possible in its litters (Schrack et al., 2017).

There is a variety of research that determines how one or another trait (mating time, season of the year, female age, body weight, etc.) affects the size of a litter (Gaytán et al., 2019; Arlt, 2018; Schrack et al., 2017; Wigham et al., 2017). One of the factors determining the size of the litter is the phenomenon of ovulation (Lee at al., 2005). However, there are several ways to detect ovulation. The progesterone (P4) assay is the most commonly used method to predict the ovulation day (POD) for successful mating (Hollinshead, Hanlon, 2017). P4 concentration starts to rise (basal P4 concentration <1 ng/ml (Kustritz et al., 2012) in the phase of the oestrus cycle called proestrus before LH surge (P4 concentration is 1.5–2 ng/mL during the LH peak (Kustritz et al., 2012)), which is associated with preovulatory follicular luteinization. POD occurs about 2–3 days after LH peak when P4 is from 4 ng/mL till 8 ng/mL (Hollinshead and Hanlon, 2019; Kustritz et al., 2012; Lee et al., 2005). There is a lack of information about how a rise speed of P4 from the first proestrus signs (FPS) until POD can affect a litter size according to an average rise of P4 of each day until POD (Hollinshead and Hanlon, 2019) and according to when POD occurs. A P4 rise is faster when there are more ovulating follicles (Knox et al., 2003) and some authors believe that the same may be true with canine females (Stornelli et al., 2020). Consequently, it is possible to link this trait with a canine litter size.

The size of the breed may affect the reproductive characteristics of females. One of the factors on which the number of puppies to be born depends is the size of the breed (Borgea et al., 2011). For example, according to researchers (Hollinshead and Hanlon, 2017; Borgea et al., 2011), an average litter size is 5.74 puppies per litter for medium size breeds. P4 levels may also vary slightly depending on the size of the breed. For example, in Kutzler et al.'s (2003) research, P4 was 1.79–5.00 at LH0–LH3 for medium size breeds.

The aim of this research is to predict the number of puppies that may be born by duration of period from proestrus until POD and the change of P4 concentration during this time of medium size canines.

Correspondence to Indrė Mečionytė, Department of Animal Breeding, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania. E mail: indre.mecionyte@lsmuni.lt

Materials and methods

The research was conducted in compliance with the Law of Veterinary Medicine of the Republic of Lithuania (New wording from 1 July 2011: No. XI–1189, 30 November 2010 No. 148–7563 (20.10.2012), the Law on Animal Welfare and Protection of the Republic of Lithuania (No. XI–2271, 03.10.2012, Official Gazette 2012), and Keeping, care and usage requirements for animals used for scientific and educational purposes, approved by The State Food and Veterinary Service by official letter (No. B1–866, 31.10.2012).

Oestrus cycles of clinically healthy medium size primiparous and *multiparous* canine females (n = 47) at 2–7 years old were observed. Canine females were observed from FPS (vulva swelling and discharge from it) until a litter was born. Blood samples were collected on day 5 from FPS (onset of discharge from vulva) until the day when the P4 concentration rate changed up to 4 ng/mL. If the P4 concentration was < 1 ng/mL on the day of test making, the test was repeated after 3 days; if it was 1–3 ng/mL, the test was repeated after 2 days; and if it was > 3 ng/mL, the test was repeated every day until POD. Blood samples were taken from the cephalic vein in the morning and P4 concentration was determined by a electrochemiluminescence (ECL) Cobas e 411 analyzer (Hitachi High–Technologies Corporation, JAPAN).

The oestrus cycles of clinically healthy medium size primiparous and *multiparous purebred (vizslas, setter, Labradors)* canine females (n = 47) at 2–7 years old were observed. Canine females were observed from FPS (vulva swelling and discharge from it) until a litter was born in dog kennels in Lithuania and Poland. Blood samples were collected on day 5 from the cephalic vein from FPS (onset of discharge from vulva) until the day when the P4 concentration rate changed up to 4 ng/mL. Blood samples of females were collected to 5–mL tubes without additional reagents in the mornings and P4 concentration was determined by a electrochemiluminescence (ECL) Cobas e 411 analyzer (Hitachi High–Technologies Corporation, JAPAN). If P4 concentration was < 1 ng/mL on the day of test making, the test was repeated after 3 days; if it was 1–3 ng/mL, the test was repeated after 2 days; and if it was > 3 ng/mL, the test was repeated every day until POD.

Mating was performed 2 times with medium size breed clinically healthy 2–8–year–old canine males, which already had offspring in an environment familiar to the male (in its home). For the first time, it was done on day 2, and for the second time, it was done on day 3 after P4 reached 4–8 ng/mL.

The number of alive and stillbirth puppies in a litter were counted at a time when they were born. Thus, all females were divided into 3 groups by the litter size:

- small group – ⩽ 4 puppies in a litter (⩽ 25% of an average litter size) (n = 13);
- medium group – 5–7 puppies (n = 19);
- large group – ⩾ 8 puppies in a litter (⩾ 25% of an average litter size) (n = 15).

All data were collected into Microsoft Excel program and were processed using the statistical package SPSS 25.0 (SPSSInc., Chicago, IL, USA). A comparison between variables was calculated by the chi–square test (χ^2) (between POD days' groups: 5–8, 9–14 and from 15); one–way ANOVA test was used to define the differences among the means of investigated groups; the statistical significance of differences between groups was assessed by the post–hoc test criterion, LSD (between POD days' groups: 5–8, 9–14 and from 15; also between puppies' number in different POD groups). The results were considered statistically significant under $P \leqslant 0.05$. Linear regression was used to model the relationship between two variables (time in the peri–ovulatory period and litter size; P4 at POD and litter size) by fitting a linear equation to observed data.

Results

The increase of P4 concentration from FPS until POD varied depending on a litter size. The fastest increase of P4 concentrations was observed in a medium litter group and in a large litter group. The range between P4 rise was 32.32%–34.1% comparing the small litter group with the medium and the large group (Fig. 1).

The comparison of the concentration of P4 during POD and the number of puppies in the litter showed that the number of puppies in the litter did not depend on the concentration of P4 during POD. The most common P4 concentrations during POD were 4–5 ng/mL and 6–7 ng/mL, less frequently 5–6 ng/mL, 7–8 ng/mL, (Fig. 2).

POD of canine females was 11.36 ± 2.17 days from FPS used in this study. The highest number of females reached P4 concentration (specific for POD) on days 9–14. A smaller part (19.15%) reached POD on days 5–8 days and from day 15 from FPS ($\chi^2 = 45.957$, df = 2, $P < 0.001$). It was found that POD was related to a litter size. Canine females that ovulated from day 15 from FPS produced 40.22% less puppies than females that ovulated on days 9–14 ($P < 0.05$) and 33.8% less than females that ovulated on day 5–8 from FPS ($P > 0.05$), (Fig. 3).

Discussion

The rise of P4 can vary in each oestrus cycle of a canine female. According to our data, P4 rises from FPS until POD more slowly for females which produce small litters. Hollinshead and Hanlon (2019) found that slow rise of P4 concentration on this period had a negative effect on the litter size but this was statistically significant only when insemination was made with frozen semen. Their first theory for these results was that there are less ovulating follicles when the P4 curve rises slowly in oestrus. Their

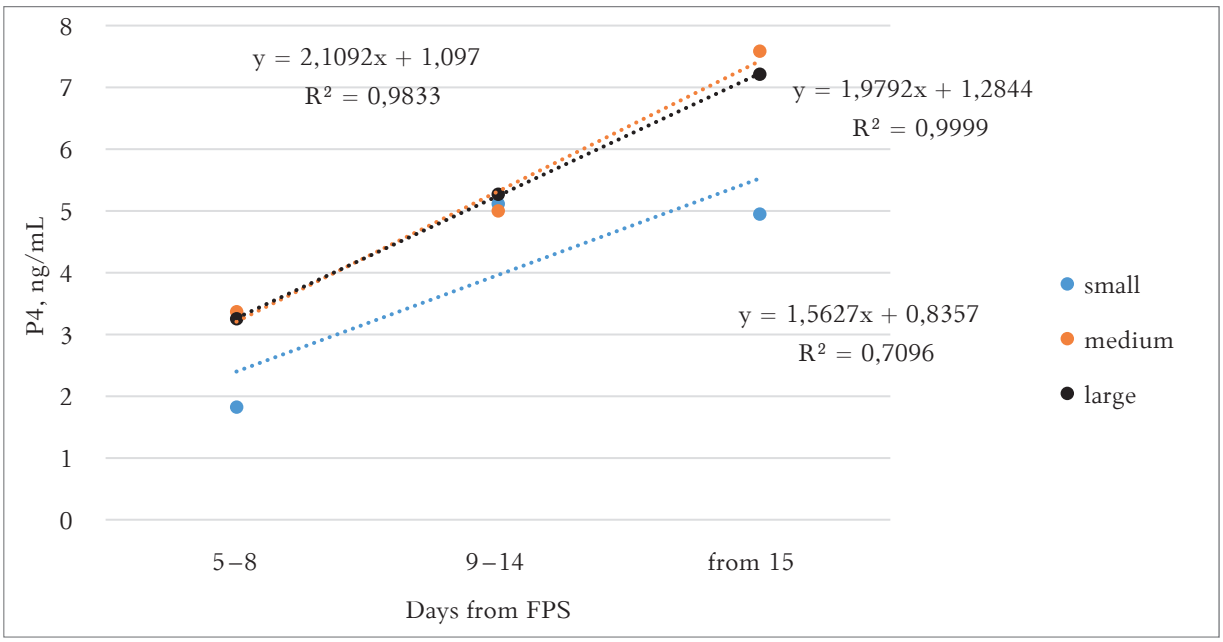

Fig. 1. Dependence of the litter size on progesterone level changes from the first proestrus signs until the predicted ovulation day

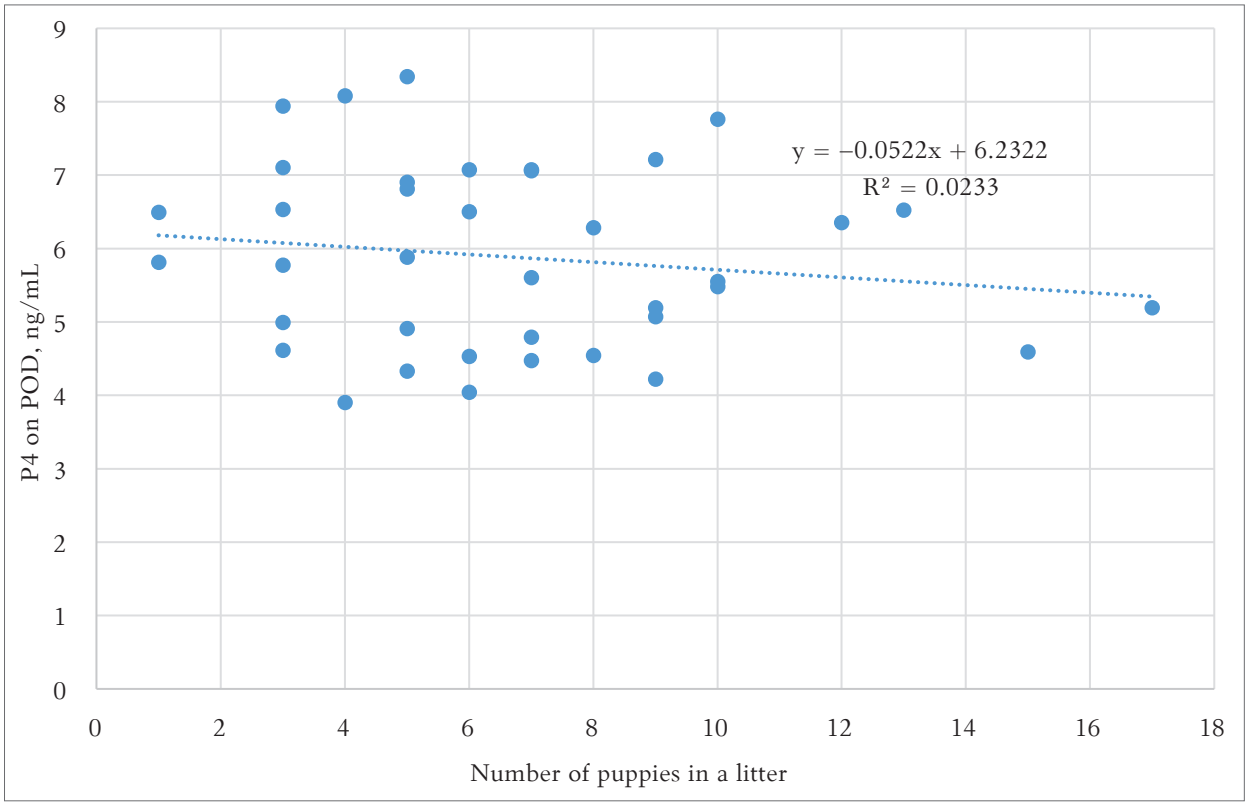

Fig. 2. Relationship between progesterone concentration during the predicted ovulation day and the number of puppies in a canine litter

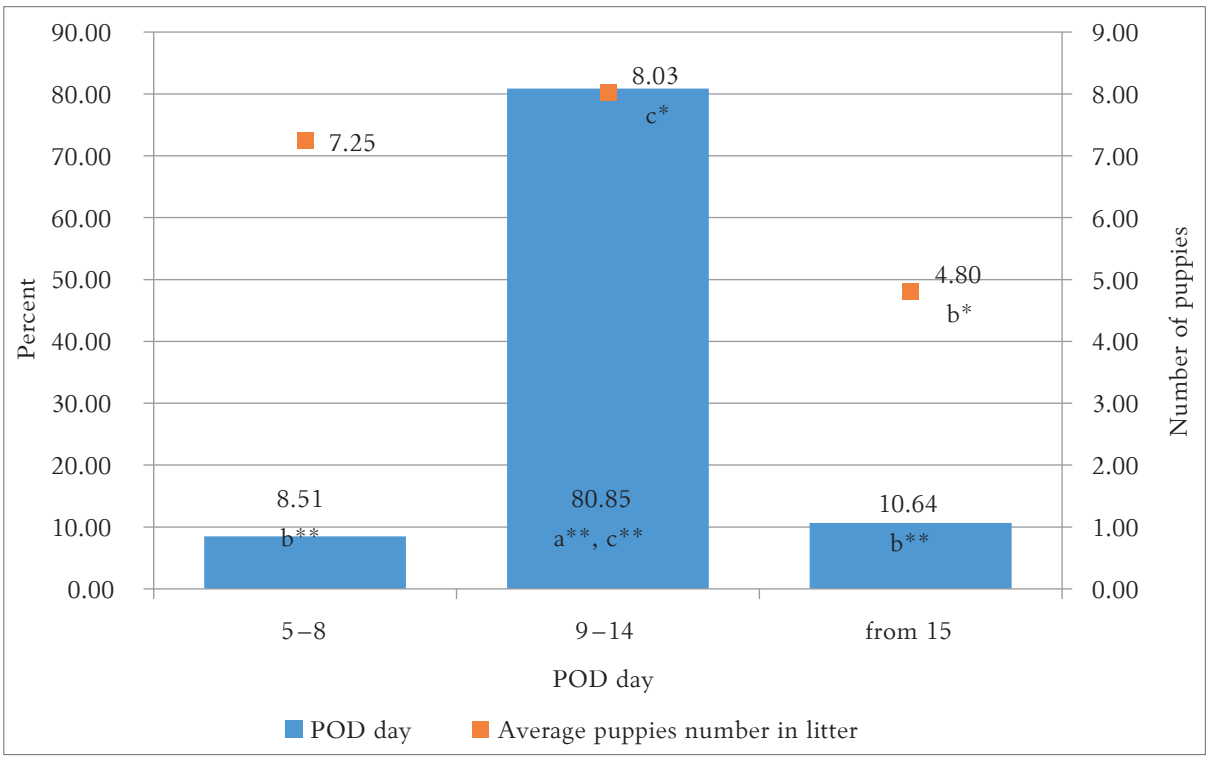

Fig. 3. The distribution of days from the first proestrus sign until the predicted ovulation day by progesterone concentration (4–8 ng/mL) and average litter size depending on POD.
Different letters a, b, and c indicate statistically significant differences between the groups (– $P < 0.05$; ** – $P < 0.01$).*

second theory was that follicles are ovulating later. This research was one of the first report about the dependence of the P4 concentration rate on the litter size. Our research suggests coming back to the first idea, and further research should focus on these ideas in order to get more accurate knowledge. It would be comprehensive to make P4 concentration and follicle count tests at the same time.

Some research studies have shown that ovulation could be predicted with a P4 test. P4 concentration at the POD is 4–8 ng/mL (Hollinshead and Hanlon, 2019; Rota et al., 2016; Lee et al., 2005). Other researchers have determined the effect of the breed size on P4 concentration at POD (Borgea et al., 2011), but there is no research available about the effect of P4 concentration at POD on the litter size. Thus, one of the objectives of our research was to find out how P4 concentration at POD affects the litter size. No statistically significant impact of this factor on the size of the litter in medium size canines was estimated ($P > 0.05$).

A range of P4 rise from FPS until POD on the same POD day can be different. This time depends on proestrus and preovulatory duration. These two periods can vary (proestrus may be 9–10 days, and preovulatory may be 1–7 days) (Concannon, 2010). The average POD day is day 9 (Domoslawska et al., 2014). According to our data, it occurs on days 9–14. The size of the litter depends on when the POD occurs. The largest litters are born on days 9–14.

Conclusion

The litter size was affected by an increasing mode of P4 concentration from proestrus until POD and the day of POD ($P < 0.05$), but P4 concentration at POD did not affect the litter size ($P > 0.05$).

References:

1. Arlt S. Canine ovulation timing: A survey on methodology and an assessment on reliability of vaginal cytology. Reproduction in domestic animals. 2018, 53(3):53-62
2. Borgea K.S., Tønnessena R., Nødtvedta A., Indrebøa A. Litter size at birth in purebred dogs—A retrospective study of 224 breed. Theriogenology. 2011, 75:911-919.
3. Concannon P.W. Reproductive cycles of the domestic bitch. Animal Reproduction Science. 2010, 124(3-4):200-10.
4. Domoslawska A., Jurczak A., Janowski T. Progesterone level does not distinguish the different course of canine ovulation determined by ultrasonography. Polish Journal of Veterinary Sciences. 2014, 17 (2): 293-297.
5. Gaytán L., Rascón C.R., Angel-García O., Véliz F.G., Contreras V., Mellado M. Factors influencing English Bulldog bitch fertility after surgical uterine deposition of fresh semen. Theriogenology. 2019, 142:315-319.
6. Hollinshead F.K., Hanlon DW. Normal progesterone profiles during estrus in the bitch: A prospectiveanalysis of 1420 estrous cycles. Theriogenology. 2019, 125:37-42
7. Hollinshead F.K., Hanlon DW. Factors affecting the reproductive performance of bitches: A prospective cohort study involving 1203 inseminations with fresh and frozen semen. Theriogenology. 2017, 101:62-72.
8. Knox R.V., Vatzias G., Naber C.H., Zimmerman D.R. Plasma gonadotropins and ovarian hormones during the estrous cycle in high compared to low ovulation rate gilts. *Journal of Animal Science.* 2003, 81 (1): 249–260.

9. Kutzler M.A., Mohammed H.O., Lamb S.V., Meyers-Wallen V.N. Accuracy of canine parturition date prediction from the initial rise in preovulatory progesterone concentration. Theriogenology. 2003, 60 (6): 1187-1196.
10. Lee B.C., Kim M.K., Jang G., Oh H.J., Yuda F., Kim H.J. Dogs cloned from adult somatic cells. Nature. 2005, 436:641.
11. Kustritz M.V. R. Managing the reproductive cycle in the bitch. Vet Clin North Am Small Anim Pract. 2012, 42:423–37
12. Rota A., Vannozzi I., Marianelli S., Gavazza A., Lubas G. Laboratory and Clinical Evaluation of a Feia Method for Canine Serum Progesterone Assay. Reproduction in Domestic animals. 2016, 51(1):69-74.
13. Schrack J., Dolf G., Reichler I.M., Schelling C. Factors influencing litter size and puppy losses in the Entlebucher Mountain dog. Theriogenology. 2017, 95: 163-17.
14. Stornelli M.C., Praderio R.G., García M.F., Favre R.N., Mitacek M.C.G., Sota R.L., Stornelli M.A. Serum progesterone concentration, volume, and apoptosis of corpora lutea in early, middle and late diestrus in the bitch. Animal Reproduction Science. 2020, 221: 106651
15. Wigham E.E., Moxon R.S., England G.C.W, Wood J.L.N, Morters M.K. Seasonality in oestrus and litter size in an assistance dog breeding colony in the United Kingdom. Veterinary record. 2017, 181(14):371.

Received 9 June 2021
Accepted 8 July 2021