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Original article

Progesterone level does not distinguish the different course of canine ovulation determined by ultrasonography

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Abstract

The aim of this study was to compare progesterone concentrations in bitches during ovulation with a different course of follicular collapse as determined by ultrasonography. The study was conducted on 36 animals of different breeds, from 2 to 7 years old, and from 2 to 44 kg of body weight. Ultrasound examinations of ovaries were conducted in the peri-ovulatory period until ovulation was detected. Based on USG scans the animals were divided into two groups: group A with fast follicular collapse within 1 day, and group B with gradual follicular collapse within 2-3 days. Progesterone measurements were performed in both groups on the day when the beginning of ovulation was diagnosed by USG. Follicular collapse in group A was observed in 27.8% of animals (n=10) and in group B in 72.2% (n=26). There were no statistical differences ($p < 0.05$) in progesterone concentrations between groups A and B (6.68 ± 0.92 ng/ml and 6.81 ± 0.57 ng/ml respectively). From 31 bred bitches (natural mating or insemination) pregnancy was confirmed in 29 females (93.5%). These results provide information about the sufficient stability of progesterone concentration during ovulation regardless of the follicular collapse speed. Ultrasonography during the peri-ovulatory period combined with progesterone measurements allows progress in ovulation management.

Key words: follicular collapse, ovaries, canine, breeding management

Introduction

Dog breeding has gained increasing interest in recent decades. Successful dog reproduction is based predominantly on the determination of optimal insemination or mating time, which is related to ovulation. To overcome the main drawbacks of these currently applied methods there is a practical need for exact ovulation detection in the bitch. Over the past few years transabdominal ovarian ultrasonography

has been developed as a non-invasive method for this purpose (Boyd et al. 1993, England et al. 1993, Bocci et al. 2006, Fontbonne et al. 2006, Levy et al. 2007). However, in contrast to other species, ovarian ultrasonography in the bitch is difficult, because of the relatively small size of the ovarian structures, as well as their atypical performance in the preovulatory period. It has been shown that about 70-80% of follicles ovulate approximately at 24-72 hours after the LH peak (Concannon 2000, Goodman 2002, Fontbonne et al. 2006, Reynaud et al. 2006, Concannon 2009).

In the bitch, breeding time management has been demonstrated as a crucial event for spermatozoa transport within the female genital tract and successful fertilization (Goodman 2002, Fontbonne et al. 2006, Levy et al. 2007, England et al. 2009). In contrast, it has been shown that mistimed breedings are the most frequent cause of conception failure. To evaluate ovulation time in the bitch, several methods, such as cytology, progesterone measurements and vaginostomy have been used; however, to a great extent these methods are laboratory dependent and involve problems of subjectivity and variability (Goodman 2002, Fontbonne et al. 2006, Levy et al. 2007). Ovulation in 60-70% of bitches is a process lasting on average 48 hours and the follicles do not collapse rapidly after ovulation – they lose their shape gradually and are still filled with fluid. In ultrasound imaging some follicles in an intra-ovary position persist as hypoechogenic structures (England et al. 1989, England et al. 1993, Fontbonne et al. 2006). Young hemorrhagic corpora lutea then look similar to peri-ovulatory follicles. The remaining 30-40% of bitches ovulate similarly to females of other species, with a typical collapse of follicles immediately after ovulation (Fontbonne et al. 2006, Levy et al. 2007). In the opinion of England et al. (1993) detection of ovulation with ultrasound has limited practical value; however, the recent understanding of follicular dynamics and its documentation with ultrasound will improve use of this method in the future.

Due to the described ovulatory characteristic it has been suggested that ultrasound examination should be supported by determination of progesterone level in peripheral blood serum, since the diagnostic value of this hormone is well established (Fontbonne et al. 2006, Levy et al. 2007). Progesterone concentration is secreted sufficiently constantly at the time of ovulation to be a useful diagnostic tool for its detection (Concannon 2000, Fontbonne et al. 2006, Levy et al. 2007, England et al. 2009). This hormone rises slowly in proestrus from basal (0.2-0.4 ng/ml) to pre-LH surge values (0.6-1.0 ng/ml), and then rises rapidly around LH surge and pre-ovulatory luteinization of follicles (1-2 ng/ml) in order to achieve a concentration of 6 ng/ml during ovulation. In dogs, dominant follicles show morphological evidence of luteinization which causes this mid-proestrus rise of progesterone and which leads to initiation of oocyte maturation and ovulation (Concannon 2009). There is a lack of data about progesterone concentration in bitches with rapid and prolonged follicular collapse determined by ultrasonography.

The aims of this study were: a) to compare progesterone concentration in bitches with various follicular performance in the peri-ovulatory period, and b) to

evaluate the clinical usefulness of ultrasonographic imaging supported by progesterone measurements for detection of ovulation in mated bitches.

Materials and Methods

In this study 36 bitches of different breeds aged 2-7 years and weighing from 2 to 44 kg were used. The examined animals were ordinary patients of the Department of Animal Reproduction with Clinic presented there for breeding management. To confirm the phase of oestrus cycle vaginostomy and vaginal cytology were performed as a routine examination. The first ultrasonographic examinations were performed on the 4th or 5th day of proestrus and were continued once daily until ovulation was diagnosed and the formation of corpora lutea was observed.

Ultrasonography of the ovaries was conducted on standing animals with an 8.0 MHz MyLab30Gold (Esoate) probe. The average period of examination was 15 minutes (10-20 minutes). The onset of ovulation was diagnosed when the cavities in the majority of the follicles, after their maximum diameter, disappeared fast within 1 day (rapid collapse – young corpora lutea with 1-2 mm thick walls) or they became slowly smaller, were filled with fluid, and lost their regular spherical shape over 2-3 days (prolonged collapse).

According to the ultrasound findings the bitches were assigned retrospectively to one of the two groups: group A – rapid follicular collapse within 1 day (n=10), group B – prolonged follicular disappearance lasting 2-3 days (n=26).

To confirm the ovulation time, progesterone levels in peripheral blood were additionally measured using the chemiluminescence method (Progesterone II[®], Roche diagnostic, Germany). A single blood sample (cephalic vein) from each dog was collected on the first day of ovulation detected by ultrasound and the average concentration of progesterone was compared between groups A and B.

To evaluate fertility, 31 experimental animals were presented for breeding. Natural mating (n=20) or insemination with fresh semen (n=11) was performed 2 days after ultrasonographic determination of ovulation. To detect pregnancy, ultrasound examination (MyLab30Gold, Esoate) on standing or lying animals was performed using a 6.5-8.0 MHz probe 23 days after breeding.

Statistical analyses

The data for progesterone levels were calculated for group A and B as means and standard deviation

Table 1. Progesterone concentrations in peripheral blood during ovulation with rapid (n=10) and prolonged follicular collapse (n=26).

	Group A	Group B
	Rapid follicular collapse (n=10)	Prolonged follicular collapse (n=26)
Range of progesterone concentration (ng/mL)	5.10 – 8.10	5.98 – 8.11
Mean \pm SD ($p < 0.05$)	6.68 \pm 0.92 ^a	6.81 \pm 0.57 ^b

a, b – no statistical difference between group A and group B ($p < 0.05$)

and were compared using Student's *t*-test. Results were considered significant at $p < 0.05$.

Results

In general, ultrasound observations of ovulation were associated with elevated progesterone levels with an average concentration of 6.78 ± 0.67 ng/ml. There was no difference ($p < 0.05$) in progesterone levels between bitches of group A with rapid follicular collapse (6.68 ± 0.92 ng/ml) compared to those in group B with persistent hypoechoic structures (6.81 ± 0.57 ng/ml) (Table 1).

In 26 of 36 cases (72.2%) one-two days after the beginning of ovulation was detected, some hypoechoic follicular structures were still observed, whereas in the remaining 10 bitches (27.8%) the follicles collapsed rapidly (Fig. 5). In the post-ovulatory period corpora lutea were observed as hyperechoic structures compared to non-echoic follicles (Figs. 3, 4).

In all bitches growing follicles were detected in the proestrus stage (Fig. 1). Their diameter was between



Fig. 1. Rising follicles

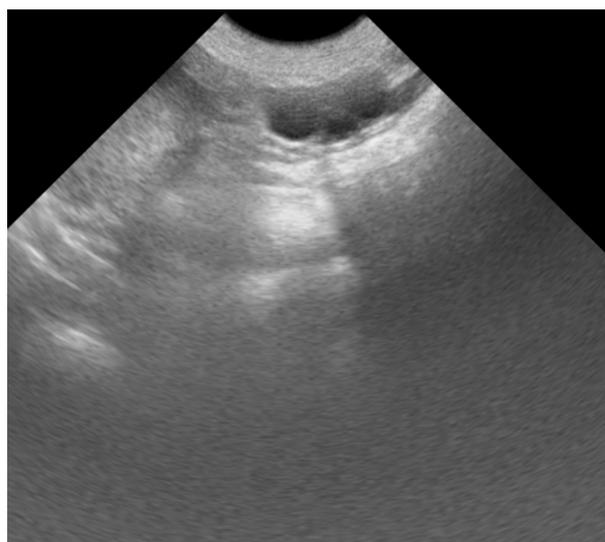


Fig. 2. Follicles one day before ovulation

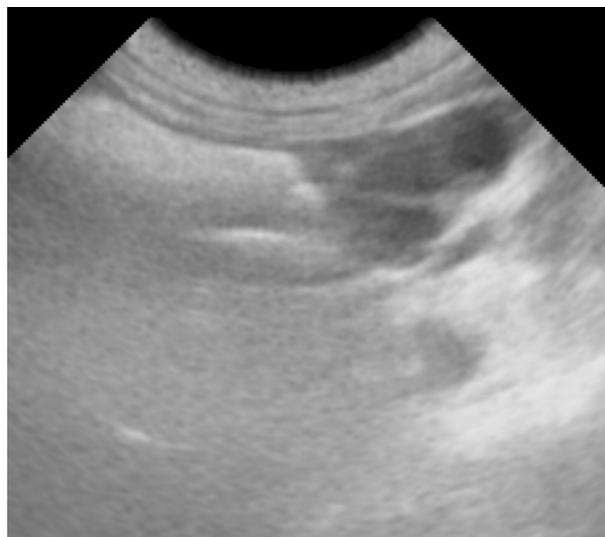


Fig. 3. Formation of *corpora lutea* after rapid follicular collapse.

0.8 and 3.0 mm during the first ultrasonographic examination. In the following days their continuous development was observed. Just before ovulation the

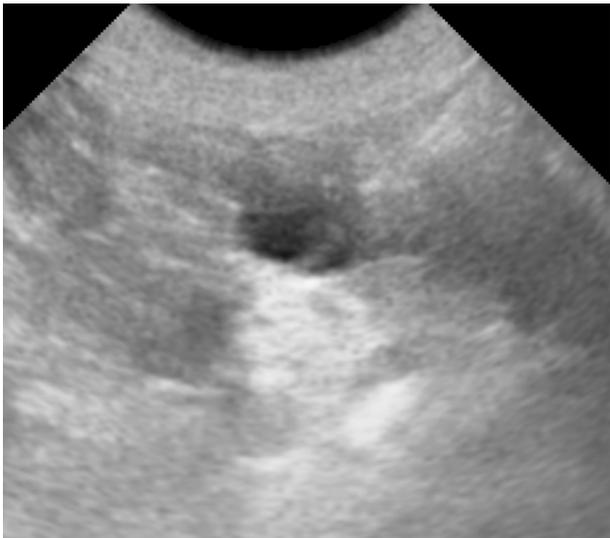


Fig. 4. Formation of *corpora lutea* one day after ovulation during prolonged follicular collapse

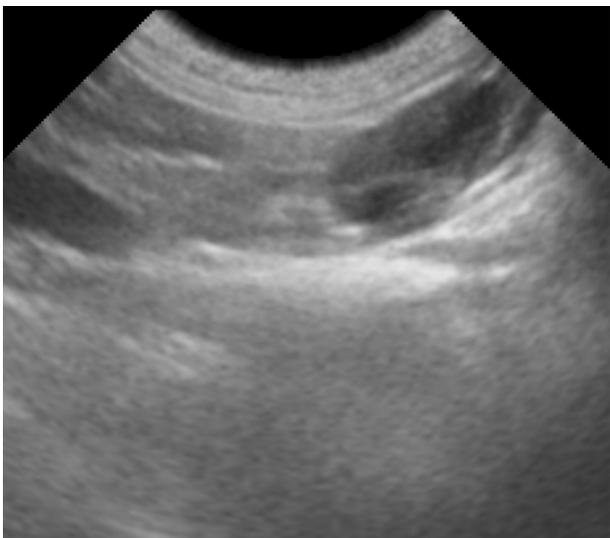


Fig. 5. Hypoechoic structures one day after ovulation

size of follicles ranged from 4.0 to 7.0 mm depending on the body size (Fig. 2). In 95% of cases we were able to determine the beginning of ovulation, whereas in 5% of bitches such a diagnosis was impeded due to technical problems related to obesity or excitation of the examined dogs.

The time of ovulation varied. In most cases ($n=31$) ovulation occurred between days 6 and 10 from the beginning of proestrus (on average 8.67 ± 1.27 days). In contrast, in 2 bitches of the Chihuahua breed ovulation appeared earlier, on the 5th day, whereas in 3 other females (Alaskan Malamutes) it appeared on the 14th ($n=1$) and 16th ($n=2$) day.

Pregnancy was confirmed in 29 females, whereas one Beauceron and one Chihuahua did not become pregnant. The total pregnancy rate was 93.5%.

Discussion

A novel aspect of our study is the comparison of progesterone level in the periovulatory period in bitches with different performance of ovulation. We did not observe any significant difference in progesterone concentration between the two groups. This finding does not confirm the usefulness of progesterone measurements as an indicative parameter to distinguish different aspects of ovulation seen by ultrasonography. It seems that progesterone originating from the luteinized membrana granulosa cells is secreted in a similar pattern, independently of the developmental features of the ovulatory follicles observed in ultrasonographic imaging.

In general, progesterone level is accepted as a practicable tool for detection of ovulation in the bitch (Wallace et al. 1992, Bocci et al. 2006, Fontbonne et al. 2006, Levy et al. 2007). According to the fertility results from this study, as well as from everyday experience (unpublished data), information about the progesterone level is very helpful for management of canine ovulation. It seems that, especially if USG diagnosis of ovulation time is difficult, the progesterone level can support these cases due to its stable concentration at this point. The main features of this phenomenon are ovulation lasting 2-3 days, with the persistence of fluid in follicles and their irregular shape (Wallace et al. 1992, Boyd et al. 1993, Fontbonne et al. 2006, Levy et al. 2007, England et al. 2009). A fast “follicular collapsus”, which is normal for other species occurs only in a limited proportion of bitches. Levy and Fontbonne (2007) detected this phenomenon in only 37% of females. This finding is consistent with our study, since we observed 28% of bitches with this type of ovulation. Thus it could be assumed that only about one third of dogs ovulate in a comparable way to other species, whereas the remaining bitches have ovulation characterized by the appearance of persistent hypoechoic smaller structures with irregular shape.

Our study was also an attempt to detect ovulation time and the formation of corpora lutea by ultrasonographic examination in bitches of different breeds combined with measurements of progesterone with respect to their future fertility. The results of this study revealed that the applicability of such a method was high. This correct time of ovulation was confirmed by the very high pregnancy rate (94.5%) obtained in inseminated or mated bitches. Fontbonne et al. (2006) and Levy et al. (2007) achieved a comparable pregnancy rate of 92 percent. However, comparison of these results with the results of our study is difficult due to the different study population, equipment used and experience of the examiner. It seems

that the progesterone levels used in our study as a supportive tool in uncertain cases enhanced the accuracy of our diagnosis. We used bitches of different sized breeds, which reflects well the conditions of everyday veterinary practice.

Ultrasonographic diagnosis of canine ovulation is not an easy clinical procedure, and should be further refined (Fontbonne et al. 2006, Levy et al. 2007, England et al. 2009). To demonstrate the right time of ovulation repeated examination of the bitch is required. It has been reported that, especially in cases of prolonged follicular collapse, interpretation of ultrasonographic images is difficult (Boyd et al. 1993, Fontbonne et al. 2006, Levy et al. 2007). Moreover, ultrasound examination of canine ovulation is related to specific difficulties such as the small size of follicles, their high number and the unusual course of ovulation in the bitch (England et al. 1989, Levy et al. 2007). In our study preovulatory follicles were 4-7 mm in size and in many cases were located very close to each other. The size of follicles depends primarily on the body size, and, for example, in giant breeds can reach up to 9 mm (Fontbonne et al. 2006, Levy et al. 2007). In the literature there is still a lack of precise data concerning morphological features of follicles in bitches of different sizes and breeds. Another problem is the anatomical location of ovaries and associated with the imaging difficulty. The right ovary is located more cranially, compared to the left ovary, and is covered by an arch rib (England et al. 1989, Fontbonne et al. 2006, Levy et al. 2007). Furthermore, due to the superficial position of the ovaries in the abdominal cavity the use of probes with high frequency is necessary (Boyd et al. 1993, England et al. 1993, Bocci et al. 2006, Fontbonne et al. 2006, Levy et al. 2007). In this study the examination of both ovaries lasted in some cases up to 20 minutes due to the particular difficulties with imaging of the right ovary. Such difficulties were earlier described by Fontbonne et al. (2006) in the German Shepherd and by England et al. (1989) in breeds with deep chests. In our study, ultrasound examination was also impeded in obese females. In 5% of bitches detection of ovulation was extremely difficult mainly due to technical problems.

In conclusion, progesterone concentration does not distinguish the different course of canine ovula-

tion determined ultrasonographically. Ultrasonography of the ovaries around the periovulatory period combined with progesterone measurements is a relevant method for veterinary practice, allowing progress in ovulation management.

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