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Original research

Advantages and difficulties of ultrasound analysis to determine the fertile period in bitches

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Abstract. Definition the fertile period is one of the most important factors contributing to fertilization. Therefore, this analysis is important to solve the problem of infertility. The methods that available today do not provide the expected result, because the vast majority of unsuccessful mating result are related to the incorrect determination of the ovulation period. Therefore, it is important to assess the condition of the ovaries, and the prospect is ultrasound examination, which is an effective method of monitoring the reproductive organs. Ultrasound analysis is a non-invasive and effective method to establish the fertile period in bitches which makes it possible to track changes in the ovaries, detect follicles, their number, and determine the true (morphological) signs of ovulation. Clinical-visual and hormonal studies which are based on the indirect determination of the optimal period of female insemination have the advantages in compare with vaginal cytology. Particularly, the use of ultrasound in real time allows to visualize the morphological changes of the ovaries and uterus – the irregular shape of the ovaries with large (0.6-1.2 cm) anechoic structures of a rounded or oval shape with a thin capsule, thickening of the endometrium (up to 0.5-0.8 cm) with a hypoechoic structure of the uterine horns. However, in our research we encountered certain difficulties, which turned out to be the same as other authors. Echography of the ovaries can seem a bit complicated, because the follicles that persist in them have a picture similar to that before and after ovulation. Some ovulated follicles do not always completely collapse (fall) during ovulation and are gradually replaced by luteal tissue while maintaining an echogenic picture on the days of the next ovulation. Non-ovulated follicles can complicate sonography interpretation. To diagnose ovulation, an ultrasound analysis should be performed twice a day, which is inconvenient for the owners. But if the ultrasound test is carried out less often than aforementioned number, there is a risk to miss the moment of ovulation, because the follicles before and after ovulation are very similar. An even more significant problem is the location of the ovaries in bitches – costovertebral angle surrounded by fat and an ovarian sac, which can create technical difficulties. In addition, this diagnostic approach may be more difficult in fat or very large dogs, as well as in dogs with thick skin (Shar Pei, Newfoundland).

Keywords: female dog; ultrasound analysis; optimal insemination time.

Переваги та складнощі за використання сонографії для визначення фертильного періоду у сук

Анотація. Визначення фертильного періоду, є одним з найважливіших факторів, що сприяють запліднення, і отже, має важливе значення у вирішенні проблеми неплідності (малоплідності). Існуючі на сьогодні методи не забезпечують очікуваного результату, бо переважна більшість нерезультативних в'язок пов'язані з неправильною визначенням періоду овуляції. Тому важливе значення має оцінка стану яєчників, а перспективу – ультразвукове дослідження, яке є ефективним методом контролю органів репродуктивної системи. Сонографічне дослідження є неінвазивним і ефективним методом визначення фертильного періоду у сук, що дає можливість відстежити зміни у яєчниках, виявити фолікули, їх кількість і визначати істині (морфологічні) ознаки овуляції. Порівняно з вагінальною цитологією, клініко-візуальним та гормональним дослідженнями, які ґрунтуються на опосередкованому визначенні оптимального часу осіменіння самок, використання ехографії в режимі реального часу дозволяє візуалізувати морфологічні зміни яєчників та матки – неправильна форма яєчників з великими (0,6-1,2 см) анехогенними структурами округлої чи овальної форми з тонкою капсулою, потовщення ендометрію (до 0,5-0,8 см) з гіпоехогенною структурою рогів матки. Однак, в наших дослідженнях ми зіштовхнулися і з певними труднощами, які виявилися тотожними з іншими авторами. Так, ехографія яєчників може видатися дещо складною, бо персистуючі в них фолікули мають картину, схожу з такою до і після овуляції. Деякі овульовані фолікули не завжди повністю колабуються (спадають) під час овуляції і поступово заміщаються лютеїновою тканиною, зберігаючи при цьому ехогенну картину в дні наступної овуляції. Можуть ускладнити інтерпретацію сонографії і неовульовані фолікули. За діагностики овуляції ультразвукове дослідження необхідно проводити двічі на день, що незручно для власників. Але якщо проводити дослідження рідше, можна пропустити момент

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овуляції, адже фолікули до та після овуляції дуже схожі. Ще більш значною проблемою є розташування яєчників у реберно-хребетному куті у сук, оточених жиром та яєчниковою сумкою, що може створити технічні труднощі. Крім того, дослідження може видатися складнішим у вгодованих собак дуже великого розміру або у тих, які мають товсту шкіру (шарпей, ньюфаундленд).

Ключові слова: самки собак; ультразвукове дослідження; оптимальний час осіменіння.

Introduction

Determining the optimal time for mating or insemination is one of the most important factors contributing to fertilization (Tsutsui, 1988; Berezovs'kyj & Harenko, 2017; Skliarov et al., 2022). This, in turn, is important for solving the problem of infertility and low fertility (Hewitt & England, 2000; England, 2002; Pardo-Carmona et al., 2011; Labib et al., 2018). Current methods do not provide the expected result (Goodman, 2001; Derkach, 2011; Hahn et al., 2017; Labib et al., 2018; Plemjashov & Plahova, 2018). The main reason for this is that the methods proposed for detecting the fertile period are based on the indirect determination of ovulation which is the main criterion for successful insemination. After all, fertilization is possible only when a sperm and an ovum meet, which can remain viable only for a certain (short) time. That is too early as well as later with respect to ovulation, the entry of sperm to the place of fertilization significantly reduces the effectiveness of their contact. The anovulatory cycles are a sign of conception failure, i.e. infertility or low fertility (Koshevoj et al., 2008; Berezovs'kyj & Harenko, 2017; Skliarov, 2022).

Due to the fact that the majority of inconclusive relationships are associated with the incorrect determination of the ovulation period, the evaluation of the condition of the ovaries is important in the diagnosis of the optimal time of bitch insemination (Radokhlib & Krajewski, 2014; Holumbiovska & Stefanyk, 2018). Ultrasound examination is a perspective and an effective method of monitoring the reproductive system organs (Kähn & Volkmann, 2004; Mantziaras & Luvoni, 2020; Vlasenko et al., 2021).

Therefore, the purpose of our study was to assess the advantages and difficulties of using ultrasound to determine the fertile period in bitches.

Materials and methods

The experiments were carried out in the UniVet clinical diagnostic center of the Faculty of Veterinary Medicine of the Dnipro State Agrarian and Economic University and the veterinary clinic "Veterinary Space Discovery" of the city of Dnipro.

The bitches (n=24) of different breeds and stages of the reproductive cycle were included in the study. Ultrasound analysis was performed after appropriate preliminary preparation of the animal (fixation, treatment of the dorsal third of the lateral abdominal wall with ethyl alcohol, application of ultrasonographic gel) with Sonoace 6000 and GE Vivid devices equipped with 7.5-15.0 MHz sensors.

The reference point for the uterus was the urinary bladder and for the ovaries was the caudal poles of the kidneys of the corresponding side. The cervix was located dorsal to the bladder, the probe was advanced cranially to visualize the body of the uterus and its horns, and their morphological parameters were determined.

Results

The development of follicles in the ovary during the stages of the reproductive cycle were observed using ultrasound. At first, they are small structures that located in the surface layer of the cortex. In the center of the follicle there is a small ovum (oocyte of the first order) which surrounded by one layer of flattened follicular cells. These follicles are called primary (primordial). Then the primordial follicles and the ovum in them increase in size. Follicular cells turn into cubic, then cylindrical, multiply intensively and surround the ovum in several layers, forming a transparent shell. Such follicles are called secondary (growing) follicles. Proliferating follicular cells secrete a liquid that merging, squeezes the cells and resulted in the small cavity forming between them. This cavity, with the growth of follicles is increasingly stretched by follicular fluid. A mature (tertiary) follicle, or Graaf follicle, consists of a connective membrane (theca) and a multilayered epithelium (granular layer). Inside the Graafian follicle is a large cavity filled with follicular fluid and an egg-bearing tubercle located on the inner lateral wall of the follicle (Berezovs'kyj & Harenko, 2017; Songsasen et al., 2017).

Follicles usually reach their maximum development one day after the release of luteinizing hormone (LH), i.e. just before ovulation. According to the data of histological and laparoscopic studies, in most cases, ovulation occurs within 36-48 hours after the peak of LH concentration, although some follicles ovulate only after 96 hours. At the moment of ovulation, the follicles collapse, and free anechoic fluid appears around the ovary. After ovulation, corpora lutea are formed. In most cases, follicles do not ovulate all at once, but in sequence (Reynaud et al., 2012; Fedotov et al., 2014; Davydenko, 2017).

According to our research, it has been established that in the stage of proestrus, the uterine horns are tubular organs, the lumen of which is not visualized, the endometrium is normal echogenic. The wall thickness is 0.3-0.5 cm, the endometrium – 0.2-0.3 mm (Fig. 1). Ovaries have an oval shape, normal echogenic inhomogeneous with hypoechoic multiple round formations (0.1-0.3 cm) structure, size 0.8-1.8 cm.

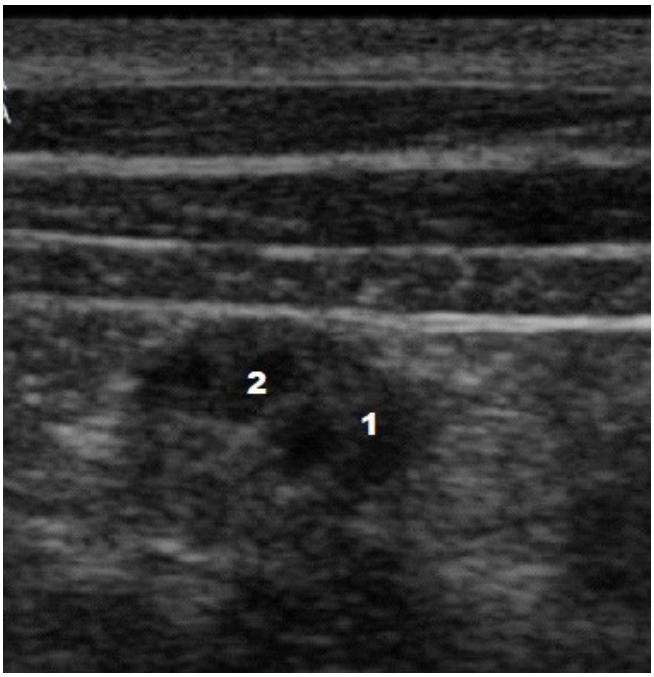

Fig. 1. Sonogram of an ovary in the stage of proestrus:
1 – parenchyma of the ovary, 2 – follicle.

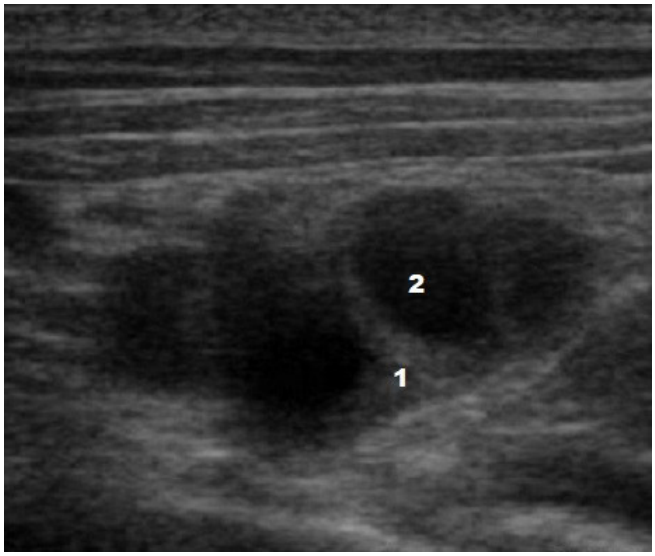

Fig. 2. Sonogram of the ovary in the stage of estrus:
1 – parenchyma of the ovary, 2 – follicle.

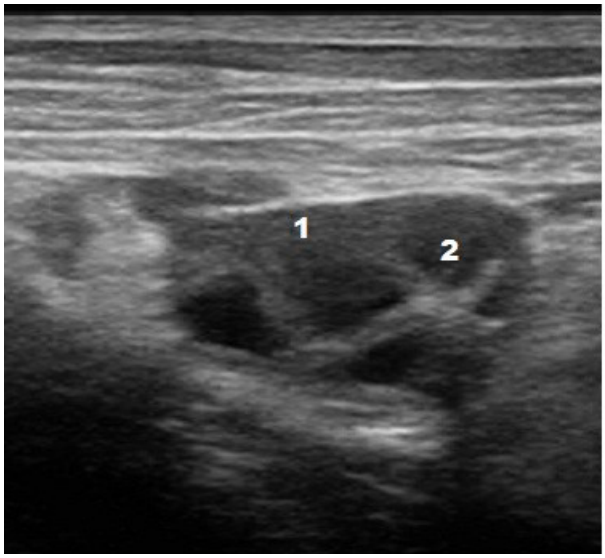

Fig. 3. Sonogram of the ovary in the stage of metestrus:
1 – ovarian parenchyma, 2 – corpus luteum.

During the estrus stage, the endometrium thickens to 0.5-0.8 cm, the lumen of the uterine horns becomes visible and has a hypoechoic structure. Ovaries have irregular shape, have large (0.6-1.2 cm) anechoic structures (follicles) of rounded or oval shape with a thin capsule (Fig. 2).

In the metestrus stage, the endometrium looks normoechoic, 0.3-0.5 cm thick. The uterine cavity is mostly hypoechoic, 0.5-1.5 cm. Ovaries are mostly oval, hypoechoic inclusions of an irregular shape (sickle-shaped, spindle-shaped, etc.) structure with a normoechoic capsule thickened to 0.1-0.2 cm are observed (Fig. 3).

In the stage of anestrus, the uterus looks like a tubular organ without a lumen, the thickness of the endometrium is 1-3 mm, the ovaries are oval or round, homogeneous and normoechoic without local changes (Fig. 4).

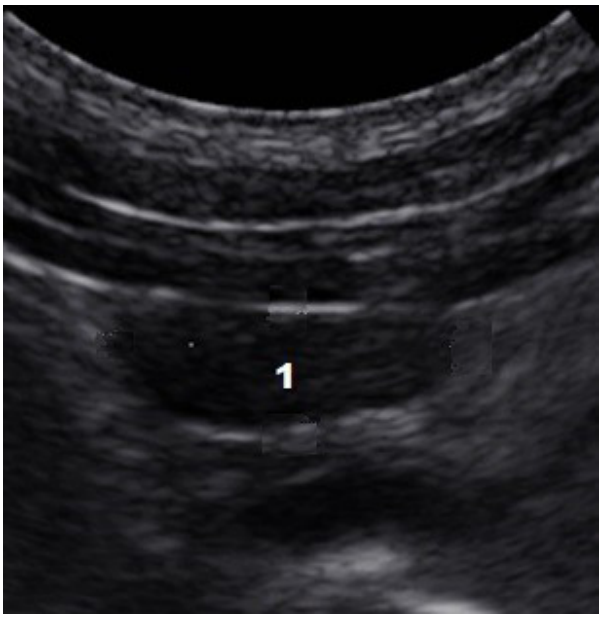

Fig. 4. Sonogram of the ovary in the stage of anestrus:
1 – ovarian parenchyma

In our research, we encountered certain difficulties with the use of ultrasound to determine the fertile period in bitches, which turned out to be identical to those of other authors. Thus, in order to determine ovulation, an ultrasound examination must be performed twice a day, which is inconvenient for the owners. But, if you conduct research less often, you can miss the moment of ovulation, because the follicles before and after ovulation are very similar (Plemjashov & Plahova, 2018). However, other authors (Levy & Fontbonne, 2007) decide that one daily ovarian scan was sufficient to determine the correct time of ovulation. Thus, this is rather an inconvenience, but a more significant problem is the location of the ovaries in the costovertebral angle in bitche, surrounded by fat and an ovarian sac, which creates technical difficulties. In addition, unlike other species of animals, the interpretation of the ultrasound picture of ovaries in domestic dogs is more difficult. Thus, a study on 48 different breeds of dogs carried out during the estrus using ultrasound and quantification of hormones (progesterone and LH) showed that 91.7% of individuals can accurately identify the day of ovulation using ovarian ultrasound (Lévy & Fontbonne, 2007). At the same time, in 4 bitches classified as large breeds, the ultrasound picture of the ovaries during ovulation was less accurate. In addition, the study may appear more difficult in fattened dogs of very large size or those with thicker skin (Shar Pei, Newfoundland) (Person & Bjuff, 2016).

Discussion

There are a number of methods for determining the optimal time of insemination in bitches (Hayer et al., 1993; Hewitt & England, 2000; Niżanski, 2004; Lévy & Fontbonne, 2007; Homych et al., 2010; Derkach et al., 2011; Bordjugov et al., 2013; Plemjashov & Plahova, 2018; Skliarov et al., 2021), which, however, have certain limitations regarding practical implementation related to economic feasibility, technical complexity, practical ability, objectivity, and informativeness. The most informative and objective is ultrasound examination – the only method that allows determining the true (morphological) signs of ovulation (Boyd et al., 1993; England, 2002; England et al., 2009; Fedotov et al., 2014; Kopylovich & Bogdanova, 2018).

Thus, according to Barbosa et al. (2013) the ovaries on the day of ovulation had a volume of 0.64 ± 0.06 cm^3, and follicle cavities

of 0.46 ± 0.01 cm³. In the studies of Lévy & Fontbonne (2007), the day of ovulation was clearly defined in 91.7% of bitches (44/48). In most bitches, the ovulation process is completed within 24 hours. There was no significant difference in ovulation time between the left and right ovaries. Compared to progesterone testing, ultrasound detection of ovulation has increased the accuracy of ovulation detection in 10.1% of bitches in which the delay between ultrasound-detected ovulation and progesterone-estimated ovulation was greater than 24 hours.

Eker & Salmanoğlu (2006) have identified follicles as anechoic spherical structures. The number of follicles on the 6th day of proestrus was 3 into the both left and right ovary. The average follicle size was 0.67 ± 0.06 cm in the right and 0.48 ± 0.02 cm in the left ovary, during the follicular phase. Apparent ovulation was characterized by rapid disappearance of the anechoic antrum in both ovaries within 24 hours. This finding was consistent with progressive obliteration of the anechoic area and was characteristic of postovulatory corpora lutea in both ovaries. Corpora lutea looked like structures with an anechoic lumen of 3.5-4 mm and thick walls. In conclusion, the ultrasound data associated with cyclic changes in the ovaries were consistent with the female's hormonal and vaginal cytological data.

Fedotov and coauthors reported that the state of the ovaries during the proestrus stage can be monitored using ultrasound (Fedotov et al. 2014). Ovarian follicles are visible as rounded hypoechoic or anechoic structures. As both the follicles develop and the size of the ovaries increases accordingly, their identification by ultrasound becomes much easier. At the beginning of proestrus, anechoic follicles filled with a small amount of fluid can be found inside the ovarian stroma. Further, during proestrus, the follicles increase in a volume, and their outlines become less spherical. After ovulation, follicles cease to be anechoic and become a hypoechoic structure (probably early corpus luteum). However, these structures are short-lived and soon become anechoic again with hypoechoic walls about 2 mm thick. The authors conclude that the ultrasound method of research is one of the most accurate methods of determining ovulation in bitches provided daily research is carried out (Fedotov et al., 2014).

Person & Bjuff (2016) reported that in the anestrus phase, the ovary is small in size and oval in shape. Its picture has a homogeneous character, and echogenicity is similar to that of the cortical layer of the kidney. In the proestrus phase, the ovaries, despite their small size, are relatively easy to detect by ultrasound (the ovary does not exceed 1.5 cm in Border Collie bitches weighing 20 kg). Several small anechoic follicles are visualized, surrounded by a thin echogenic wall at least 1.0 mm thick. The size of the follicles varies from 4.0 mm to 5.0 mm in the estrus phase, due to the accumulation of a large amount of fluid in the follicles, the size of the ovaries increases. The ovary takes on a morula form picture. The wall of the follicles becomes thicker (over 1.0 mm) and more echogenic in parallel with the process of ovulatory luteinization. According to the size of the dog in the follicles, it varies between 6.0 and 9.0 mm. In preovulatory follicles, the wall is very thick and clearly exceeds 1 mm, and the size exceeds 6.0 mm. Yellow hemorrhagic formations quickly appear on the ovary after ovulation. Their picture can be very similar to preovulatory follicles. Therefore, it is very important to carry out ultrasound of two ovaries every day and register the picture and before conducting a new examination, it must be reviewed again.

In the ovary, the day after ovulation, is noted the complete disappearance of the follicle cavity (follicular collapse). Several hypoechoic intraovarian structures are also visualized, as occurs in approximately 50% of dogs. In the ovary at the diestrus stage, a large number of follicles disappear after ovulation. Attention should be paid to the presence of yellow corpuscles that appear and clearly evolve. After ovulation, fluid does not accumulate inside the ovary, and its wall does not take on a pathological shape (Person & Bjuff, 2016).

As described by England et al. (2009) coauthors the follicles are identified during late anestrus (between days 100 and 60 before the preovulatory LH surge), and there appeared to be a population shift from small follicles (1-3 mm in diameter) to large follicles (>4 mm in diameter) about 2 days before the LH surge. The corpora lutea could be reliably identified, although most of them had cavities. In a narrow range of the studied clinical population, trends were observed between age and reproductive capacity. In a study by Kim et al. (2009), ovarian size increased from 76.8 ± 7.5 mm² on day 12 (day 0 = day of ovulation) to 114.4 ± 5.5 mm² on day 8, and there was no significant difference between the two ovaries. Ovaries were recognized by their proximity to the caudal pole of the kidney and were moderately echogenic, oval in shape with a smooth outline. Follicle size increased from 8.1 ± 4.5 mm² on day 12 to 114.4 ± 5.5 mm² on day 0, and there was no significant difference between both ovaries. The number of follicles increased from 2.8 ± 0.7 on day 12 to 1.1 ± 0.1 on day 0, and there was no significant difference between both ovaries. Follicles were small, anechoic, fluid-filled structures at the beginning of proestrus, becoming more enlarged and indistinguishable from each follicle at the end of proestrus. The size of the corpora lutea increased from 19.3 ± 2.1 mm² on day 0 to 26.4 ± 8.1 mm² on day 8, and there was no significant difference between the two ovaries. The number of corpora lutea increased from 1.4 ± 0.6 on day 0 to 2.9 ± 0.4 on day 38, and there was no significant difference between the two ovaries. The corpora lutea had a small anechoic cavity and a thin hyperechoic wall at the beginning of diestrus, became more hyperechoic and enlarged homogeneous structures.

Ultrasound analysis of the ovaries seems somewhat complicated, because the follicles that persist in them have a picture similar to that before and after ovulation. Some ruptured follicles do not always completely collapse during ovulation and are gradually replaced by yellow tissue, while maintaining an echogenic pattern in the days of the next ovulation. In addition, ultrasound examination of ovulation in dogs is associated with such specific difficulties as the small size of follicles, their large number and the unusual course of ovulation in bitches (England & Allen, 1989, Levy et al., 2007). The follicles that have not ovulated can complicate the interpretation of the ultrasound picture (Boyd et al., 1993; Fontbonne & Malandain, 2006; Levy et al., 2007). In this regard, it is recommended to perform at least two ultrasound analyses per day in order to finally accurately determine ovulation (Person & Bjuff, 2016).

Silva et al. (1996) reported that the ovaries appeared as anechoic structures in ultrasound picture approximately 5 days before the predicted LH peak and gradually increased in a volume. The greatest changes were observed between days 2 and 4 after the LH peak: echogenicity varied widely from one animal to another and from one day to the next, ranging from complete anechoic to mixed hypo- and hyper-echogenicity. Then, starting on the 6th day, the ovaries always appeared as hypoechoic structures similar to corpora lutea. During the laparoscopy, small follicles were detected 10 days before the LH peak. They slowly increased in size to become large protruding follicles around the day of the LH peak (day 0). On the 1st day, corpora lutea were observed for the first time and were present in all animals on the 5th day. During this period, preceding day 5, both corpora lutea and follicles were clearly visible on the surface of some ovaries. The authors note that neither ultrasound nor laparoscopy allowed to accurately determine the time of ovulation. Indeed, follicular collapse was never observed, but changes in the echogenicity and appearance of the ovaries observed by laparoscopy suggested that ovulation occurred between days 2 and 4, when the progesterone concentration was equal 12.6 ± 6.2 and 32.1 ± 10.9 nmol/l respectively.

Tsuchida and coauthors observed ovarian follicles as contoured anechoic structures before ovulation (Tsuchida et al., 2022). Ovulation (follicular collapse) was determined by transabdominal ultrasound when the follicles became cloudy and the contours

were unclear. Ultrasound imaging allowed to determine the day of ovulation for 94.7% (178/188) follicles by the appearance of follicle collapse or corpus luteum. Ovulation occurred between LH 0 (the day of the LH surge) and LH 5, with 48.0%, 33.5% and 15.0% for LH 2, LH 3 and LH 1, respectively. The total number of ovulations on LH 2 and LH 3 was 81.5% (141/173) of the total number of ovulations in the 24 studied cycles. Ovulation occurred in 12 cycles on 2 days and on 3 days in 12 cycles. It was observed that 17 cycles (70.8%) with several days of ovulation showed the highest number of ovulations on LH 2. The mean follicle diameter 3 days before the LH surge was less than 5 mm and then exceeded 5 mm 2 days before the LH surge. The average follicle diameter at the time of ovulation (follicular collapse) was 6.1 ± 1.0 mm. On the day before ovulation, the average diameters of follicles that ovulated on LH 1, LH 2, and LH 3 were 5.0 ± 0.7 mm, 5.8 ± 1.2 mm, and 6.2 ± 1.3 mm, respectively. There was a significant difference in follicle diameter between LH1 and LH2, LH2 and LH3, and LH1 and LH3. This indicates that it is difficult to determine the day of ovulation based on the size of the follicle. This study showed that combining ultrasound with progesterone monitoring can monitor follicular development, ovulation, and ovarian luteinization. In addition, the time range of the ovulation process was clarified. These results may contribute to an accurate understanding of optimal mating times and improved breeding efficiency, including artificial insemination and embryo transfer for Labrador retrievers.

According to Otava and coauthors report, although ultrasound changes during the estrous cycle have been well studied, the exact moment of ovulation cannot be predicted with accuracy (Otava et al., 2017). In order to optimize the results of determining the moment of ovulation, it is recommended to compare the ultrasound examination with at least one of the other two research methods.

According to Bicudo and coauthors data, traditional ultrasonographic imaging made it possible to trace the development of follicles and/or collapse and formation of corpora lutea in 85.7% of observed follicles (Bicudo et al., 2010). Renton et al. (1992) compared the accuracy of progesterone and ultrasound in determining ovulation in bitches. The authors report that ultrasound was less accurate, as only four of 16 assessments were agreed, and another 6 were done within the same day. However, this is verified if only those bitches were included when they were probed with ultrasound analysis by the latest equipment application. Besides, 3 bitches just were confirmed of 11 matched and 6 of the remaining 8 were within one day of LH of predicted ovulation. It was concluded that currently, the measurement of progesterone in the plasma is a better indicator of ovulation than ultrasound.

In a study by Domoslawska et al. (2014) conducted in 26 out of 36 cases (72.2%), some hypoechoic follicular structures were still observed 1-2 days after the onset of ovulation, while in the remaining 10 bitches (27.8%) the follicles rapidly collapsed. In the postovulatory period, corpora lutea were observed as hyperechoic structures compared to non-echoic follicles. Growing follicles were detected in all bitches in the proestrus stage. At the first ultrasound, their diameter was between 0.8 and 3.0 mm. Their continuous development was observed in the following days. Just before ovulation, follicle size ranged from 4.0 mm to 7.0 mm depending on body size. In 95% of cases, we managed to determine the beginning of ovulation, while in 5% of bitches, such a diagnosis was complicated due to technical problems related to obesity or excitement of the studied dogs. The time of ovulation varied. On the other hand, 2 female Chihuahuas ovulated earlier, on the 5th day, and 3 other females (Alaskan Malamutes) ovulated on the 14th and 16th days.

It should be noted that the size of the follicles depends primarily on the size of the body, and, for example, in giant breeds they can reach up to 9 mm (Fontbonne & Malandain, 2006, Levy et al., 2007). In the literature, there is still a lack of accurate data on the morphological features of follicles in bitches of different sizes and breeds. Another problem is the anatomical location of the ovaries, which is associated with difficulties in visualization. The right ovary is located more cranially than the left ovary and is covered by the rib of the arch (England & Allen, 1989; Fontbonne & Malandain, 2006, Levy et al., 2007). In addition, due to the superficial location of the ovaries in the abdominal cavity, it is necessary to use probes with a high frequency (Boyd et al., 1993; England et al., 1993; Bocci et al., 2006, Fontbonne & Malandain, 2006, Levy et al., 2007). In this study, both ovaries are examined, which in some cases can last up to 20 minutes due to special difficulties with visualization of the right ovary. They were previously described by Fontbonne & Malandain (2006) in German Shepherds and by England & Allen (1989) in deep-chested breeds. In a study by Domoslawska et al. (2014), ultrasonography was also complicated in obese females. In 5% of bitches, detection of ovulation was extremely difficult, mainly due to technical problems. The authors believe that ultrasound of the ovaries in the periovulatory period in combination with progesterone measurement is an appropriate method for veterinary practice, which allows progress in ovulation control.

The rapid "follicular collapse" that is normal in other species occurs only in a limited number of bitches. Levy & Fontbonne (2007) found this phenomenon in only 37% of females. This conclusion is consistent with our study, as we observed about a third of females with this type of ovulation. Thus, it can be assumed that only about one-third of female dogs ovulate in the same way as other species, while in the remaining bitches, ovulation is characterized by the appearance of persistent hypoechoic smaller structures of irregular shape.

Aires et al. (2021) performed a sonographic assessment of the female reproductive tract during the follicular phase of the estrous cycle using high-density (HD) ultrasound techniques. According to their data, the size of the ovaries increased with the progression of the stage of the follicular phase. After ovulation, fluid was found around the ovaries. Characteristic hyper echogenicity of adipose tissue around the ovaries was observed at all time points. There was a difference in the number of ovarian structures in each group of measurements at each assessment. There was a difference in the diameter of the largest ovarian structure and in the mean value of the wall thickness at all time points when there were assessments. The authors believe that the HD ultrasound technique provides excellent image resolution, allowing more accurate characterization of the female's reproductive structures and the changes that occur during the follicular phase of the estrous cycle.

The authors believe that contrast-enhanced ultrasonography (CEUS) is a new method that allows detection of the microcirculatory bed for both qualitative and quantitative assessment of tissue perfusion and can be a valuable tool for understanding the physiological changes in the ovaries of bitches, in particular during the intensive vascular changes during follicular phases of the estrous cycle and the formation of corpora lutea, in order to provide a better understanding of the physiological features during these periods improve reproductive management (Aires et al., 2022).

Bergeron notes that ultrasound monitoring of ovarian blood flow changes is ideal tool to estimate of periovulatory events in bitches (Bergeron, 2010). Despite the fact that determining the frequency of ovulation using ultrasound is imprecise, it is the only non-invasive method. Finally, quantitative changes in follicle/corpus luteum echotexture do not reflect subjectively described changes.

There were found out no significant changes in follicle/corpus luteum echotexture compared to days from the preovulatory LH peak (Bergeron et al., 2013). They observed a significant decrease in the number of follicles/corpus luteum between days -1 and 3, -1 and 4, and 0 and 3, relative to the preovulatory LH peak. The authors concluded that once-daily color Doppler ultrasonography

more accurately identified the preovulatory LH peak than B-mode ultrasound and allowed for the prospective assessment of the ovulation progress.

Conclusion

Ultrasound analysis is a non-invasive and effective method of determining the fertile period in bitches, which makes it possible to monitor changes in the ovaries, detect follicles, their number and determine the true (morphological) signs of ovulation. Compared with vaginal cytology, clinical-visual and hormonal studies, which are based on the indirect determination of the optimal time of insemination of females, the use of real-time sonography allows to visualize morphological changes of the ovaries and uterus – the irregular shape of the ovaries with large (0.6-1.2 cm) anechoic structures of rounded or oval shape with a thin capsule, thickening of the endometrium (up to 0.5-0.8 cm) with a hypoechoic structure of the uterine horns.

The results of our study evidence that certain difficulties to apply ultrasound analysis of the bitch fertile period estimation turned out to be the same as other authors. The ultrasound analysis of the ovaries can seem somewhat complicated because the follicles that persist in them have a picture similar to that before and after ovulation. Some ovulated follicles do not always completely collapse (fall) during ovulation and are gradually replaced by luteal tissue, while maintaining an echogenic pattern in the days of the next ovulation. In addition, follicles that did not ovulate can complicate the interpretation of the ultrasound picture. To determine ovulation, an ultrasound examination must be performed twice a day, which is inconvenient for the owners. But if you conduct research less often, you can miss the moment of ovulation, because the follicles before and after ovulation are very similar. At the same time, other authors believe that one daily scan of the ovaries is enough to establish the exact time of ovulation. An even more significant problem is the location of the ovaries in the costovertebral angle in bitches, surrounded by fat and the ovarian sac, which can create technical difficulties. In addition, the study may seem more difficult in fat dogs of very large size or in those with thick skin (Shar Pei, Newfoundland).

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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