
Vaginal smear, progesterone levels, and ultrasound examination of the ovaries as methods of determining the moment of ovulation in bitches comparative study

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Abstract

Researches in the scientific literature reveal that the study of vaginal cytology and interpretation of progesterone values do not represent certain methods to determine the ovulation time. 6 different breed have been investigated in this study (Labrador Retriever, Tibetan Mastiff, Bichon Maltese, West Highlander White Terrier and the Bucovina Shepherd). All es were monitored in terms of cyto-vaginal smear, the P4 level and ultrasound examination to determine the ovulation moment. The rapid disappearance of the follicular antrum cavity, correspondent to ovulation, was detected only with two. Although ultrasound changes during the estrous cycle were well-studied, the exact ovulation moment cannot be predicted accurately. To optimize the results of determining the ovulation moment it is recommended to collate the ultrasound examination with at least one of the other two methods of investigation.

Key words: ovulation time, bitch , ultrasound

Introduction

Bitches are mono-estrous animals with a single reproductive cycle. Because ovulation occurs once or twice a year and due to the very high variety among individuals within the same breed and among breeds, it is very difficult to set a very accurate method for ovulation timing. Imagining and developing a non-invasive method of monitoring follicular development and ovulation is necessary both for research and for clinical practice. Development of some high-performance ultrasound apparatus has led to substantial progress in this area lately.

Until now, the ovulation moment has been set by collating the two methods of medical investigation- determining the progesterone level and studying the vaginal cytology . The study of literature concerning the determination of the ovulation moment by determining the progesterone level reveals vastly different data. Thus, progesterone values ranging from 4 to 10 ng/ml are set through clinical studies. In this study we wanted to obtain information about the effectiveness and coordination of the three methods of determining the ovulation moment.

Materials and methods

Six different breeds have been monitored in this study (Labrador Retriever, Tibetan Mastiff, Bichon Maltese, West Highlander White Terrier and the Bucovina Shepherd). Taking into account the very large differences among individuals of the same breed and among dog breeds, regarding the ovulation moment opposite the sexual cycle, pet owners were advised to bring the females for monitoring the estrous phase of the sexual cycle when the blood leak diminished , both chromatically and quantitatively. Thus, the owners were not called according to a fixed interval from the sexual cycle onset.

An attempt was made to investigate the vaginal cytology, the progesterone levels and ultrasound appearance of the ovaries in the females. To perform the cytovaginal smear, I needed a rod of wood to which a small amount of cotton wool was attached by rotating the rod. This improvised item was introduced into the vaginal vestibule on a nearly vertical direction from the bottom up, after a previous distance of vulvar lips performed by the same vet technician with the

opposite hand. After penetration into the vaginal vestibule the rod is placed in horizontal position in order to set its tip into the anterior vagina.

According to the literature there are differences concerning the interpretation based on cytovaginal smear in es between the vaginal vestibule or posterior vagina and anterior vagina. It is recommended to avoid the clitoris fossa which contains fragments of keratinized cells which can be confused with the superficial epithelial cells obtained from the during the estrus period. Once the rod inside the vagina the vet tehnician makes movements of rotation in the opposite direction to the direction of rotating the cotton wool. One should be aware of this fact because there is a risk of cotton wool to get detached from the rod. The sample taken was displayed on a glass blade by rolling over the wool part of the rod. The smearl dyeing was made using the Diff Quick method. The examination of cytovaginal smear was done by help of the Leica microscope owned by CLC Horia Cernescu.

Preparing the animal for the blood sampling began with shaving the middle area of the forearm and antisepting it with betadine solution 4%. The blood samples for determining the progesterone level in blood was done by stinging a needle attached to a syringe into the vena cefalica antebrahiala. A quantity of 1- 2.5 ml of blood was taken.

The blood taken was transferred into a vacuum container which does not contain anticoagulant substances . The container was labelled with the owner and pet IDs. The sample thus obtained was sent, as soon as possible, at Bioclinica laboratory. The determination of the progesterone level was done by means of the chemiluminiscence method, using the Siemens Advia Centaur device. The value obtained was expressed in mmoles/l and nanograms per milliliter.

Preparing the female for the ovaries ultrasound examination consisted in trimming or shaving the flank area , covering the transverse processes of the lumbar vertebrae 3 and 4. The female will be positioned in lateral or dorso-lateral decubitus, either left or right depending on the ovary to be viewed. There are some situations in which, due to the presence of certain portions of the intestine in the proximity of the ovary, viewing it is more difficult. In such situations, the females can be examined in upright position. It is worth mentioning that the animal being shaved is absolutely mandatory in order to have good quality images, so it should not be left to the discretion of the owner. Many owners refuse to have the animal shaved, due to aesthetic reasons. The echography of the ovaries should be performed after a 12-hour water diet to have an empty intestin.

After applying the gel on the freshly shaved area, an attempt was made to visualize the kidney caudal pole. This represents a landmark in the case of the examination of the ovaries because they are close to the kidney. The ovary is positioned caudo-laterally towards the distal portion of the kidney. An anatomical peculiarity of female genital apparatus, namely being positioned more cranially to the kidney compared to the left kidney. Thus, the right ovary is positioned more cranially compared to the left one. The echography of the ovaries should start with the left ovary, since it is easier to locate. It should be also taken into account the fact that the ovaries are located superficially, very close under the skin. The ultrasound examination was done using a My Lab 50 Esaote, Betford Hills, NY, USA apparatus equipped with a 3-9 MHz frequency convex probe set to mode B, property of CLC Horia Cernescu..

Results and discussions

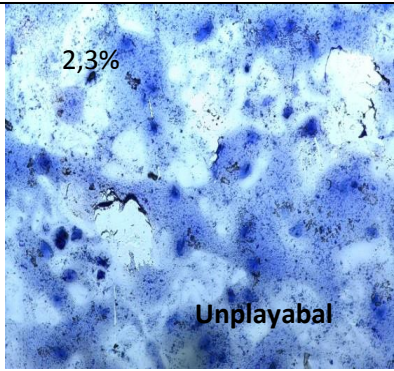
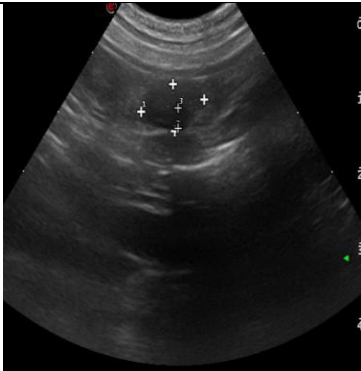
Please note that the study has been negatively impacted by a number of factors such as: the reduced availability of owners to be present at the clinic following the shedule established, the anxiety of the bitches that caused tachypnea and the tissues, and the hollow/cavitary organs containing gas.

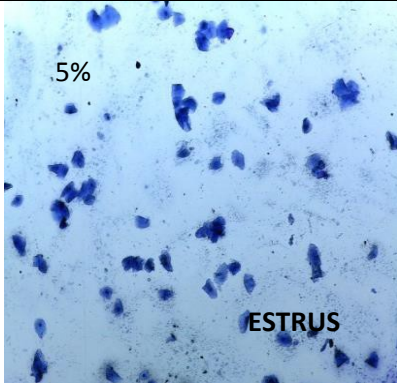
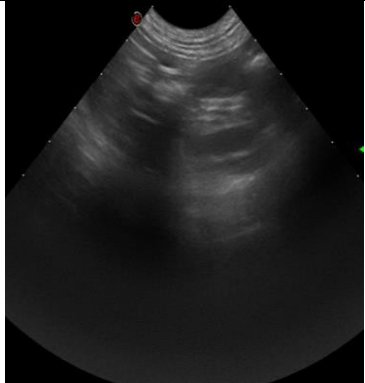
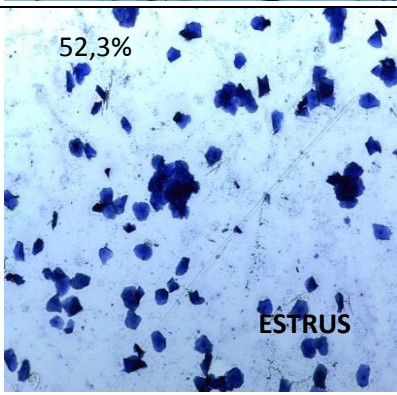
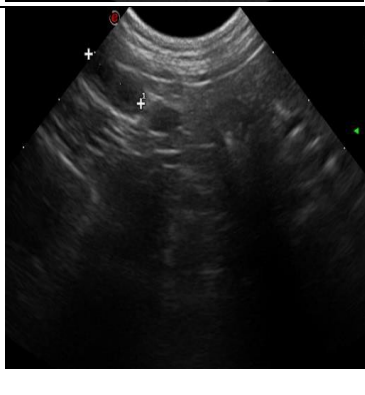
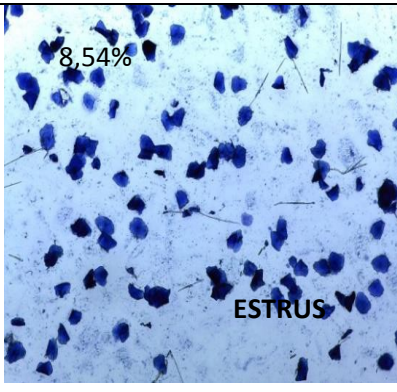
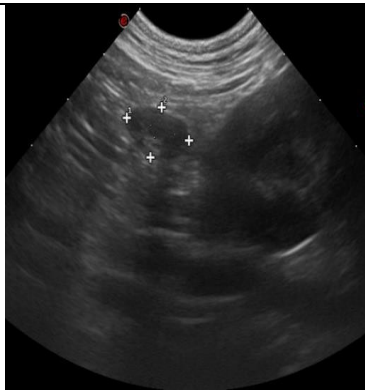
The Bucovina Shepherd beatch was investigated twice in a span of two days. At the first medical investigation the progesterone level was 1.9 ng/ml and the image of the cyto-vaginal smead was impossible to be interpreted due to the abundant presence of the estrus mucus. The second investigation showed that the superficial cells with no nucleus of all keratenized cells cheratinizate were around 72%. According to data in the scientific literature , at the percentage of cells without nucleus of 72 per cent, the female is supposed to be on the everge of ovulation, but the progesterone level of 2.5 ng/ml does not indicate this fact. The data in the literature show a very large range (4-10ng/ml) of the progesterone levels at the time of ovulation. Related to the percentage of cells with no nucleus, ENGLAND (3) consider that it is supposed to be 75, at the ovulation moment. This demonstrates that the percentage of the keratenized cells in the cyto-vaginal smear used to determine the moment of ovulation is not a good method.

Concerning the Labrador Retriever beatch, the oestrus cycle showed a percentage of 16 cells with no nucleus. The ovary follicles had an even appearence, with thickened walls, feature specific to the follicules before ovulation. Thus, in this situation, the two medical investigation collate.

The Tibetan Mastiff female was investigated according to the cyto-vaginal smear, the progesterone level and through the ultrasound method. In this particular situation, there is no discrepancy, meaning that the ultrasound results, the percentage of the superficial cells with no nucleus and the progesterone levels showed that the famale did not ovulate. The sonograph pointed that on the ovary monitoredthere are 6 follicles.

Tabel 1. The connection among the oestrus cycle, the P4 level and the ultrasound results in a West Highlander White Terrier bitch

Data	The cyto-vaginal smear/The percentage of the superficial no nucleus cells	The ultrasound appearance of the ovaries	aloare nivel P ₄
10.11.2015			1,1 ng/ml

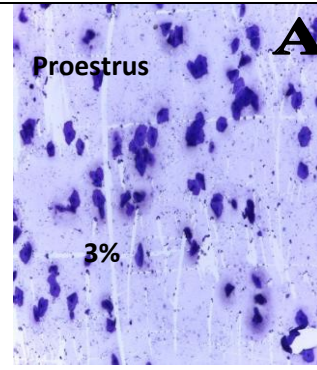
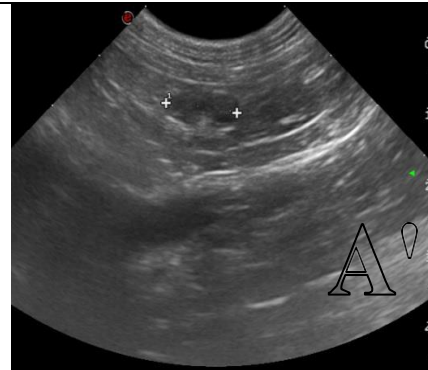
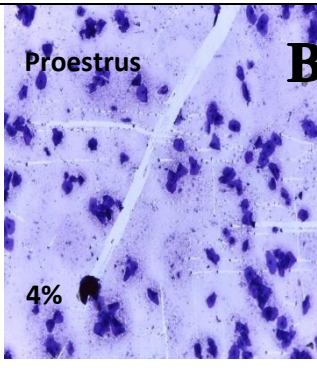
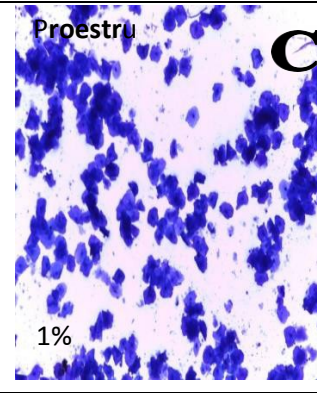
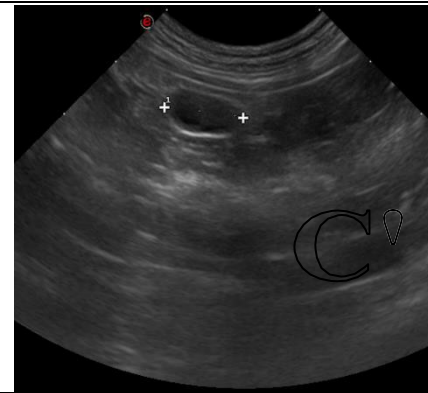
3.11.2015			0,73 ng/ml
7.11.2015			2,59 ng/ml
9.11.2015			6,4ng/ml

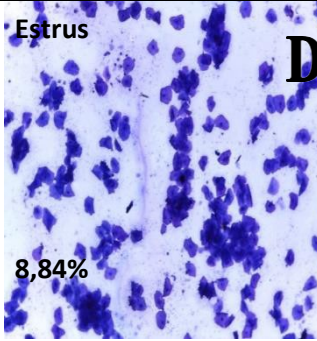
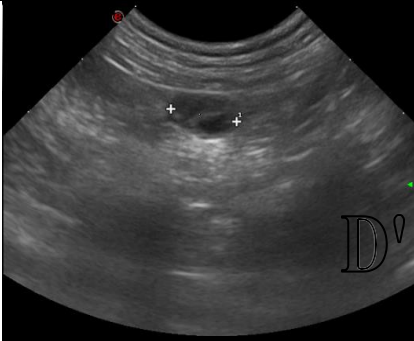
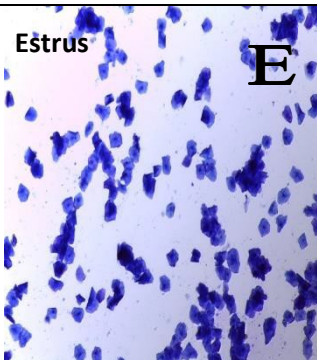
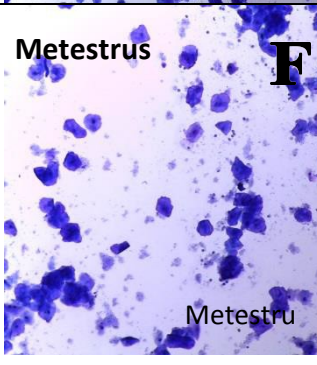
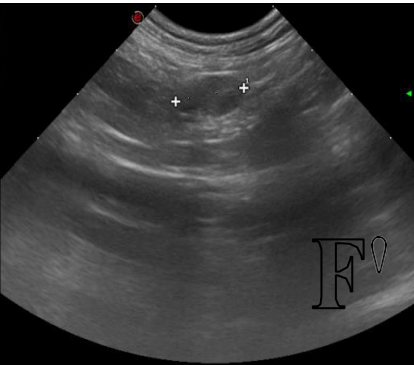
The West Highlander White Terrier female was investigated, using all the three methods, 4 times, every 2-4 days, according to its evolution concerning the sexual cycle. Even if the first cyto-vaginal smear was impossible to interpret (Table 1), speaking from the cytology point, the female had an ascending evolution regarding the cells without a nucleus. The same trend was found in the progesterone level. The beach was at a basic/basal level (values between 0.73 ng/ml and ng/ml) during the first three days of the investigation. On the last day, it was noticed that the progesterone value of 4ng/ml (value which most studies consider is an indicator for ovulation) collated neither with theultrasound image of the ovaries, nor with the vaginal cytology, . We would have expected that at a progesterone value of 6,4 ng/ml, the procentage of the cells with no nucleus to be 75%, (ENGLAND and LEVY) (2, 3) and the follicles to be well-defined.. Ultrasound follicles appearance was, however, one specific to

the preovulation follicles.(Table 1). This reveals at least

a fact, namely, at a progesterone value of 6/ml, 4ng , the female did not ovulate. We consider that, out of the three methods of investigation, the ovaries ultrasound examination brings the most precious information about the time of ovulation. The data in the scientific literature on progesterone level at which ovulation occurs, are vastly different ranging from 4-10 ng/ml (5).

Table 2. Connection among estrous cycle, the P4 level and the ovaries ultrasound appearance in a Bichon Maltese bitch

Data	The cyto-vaginal smear/The procentage of the superficial no nucleus cells	The ultrasound appearance of the ovaries	P4 value ng/ml
3.06.2016	 <p>Proestrus A 3%</p>	 <p>A</p>	-
5.06.2016	 <p>Proestrus B 4%</p>	-	-
6.06. 2016	 <p>Proestrus C 1%</p>	 <p>C</p>	2,52

8.06.2016	<p>Estrus</p>  <p>8,84%</p> <p>D</p>	 <p>D'</p>	2,32
10.06.2016	<p>Estrus</p>  <p>E</p>		5,39 g/ml
3.06.2016	<p>Metestrus</p>  <p>Metestru</p> <p>F</p>	 <p>F'</p>	20,24 ng/ml

The Bichon female was investigated, using all the three methods, six times, every 1-3 days, according to its evolution concerning the sexual cycle

Speaking from the cytology point, the female had an ascending evolution regarding the cells without a nucleus. From one medical exam to another, the percentage of the cells with no progressive nucleus grew. According to Table 2, when the cells with no nucleus were 23,68%, the progesterone level was 5,39 ng/ml, very close to ovulation time.. On the penultimate day of the investigation the female was examined only hormonally and cytologically, because we had no access at the ultrasound apparatus at the weekend. On the last day of the examination, the female had a smear specific to the metestrus period beginning, and the P4 level was 20,24 ng/ml.

The data in the scientific literature show that considering the percentage of cells with no nucleus out of all keratinized cells in order to determine the ovulation time, involves great difficulty and it is subjected to errors of interpretation. This fact is due to the cyto-vaginal smear being uneven. Thus, the percentage of keratinized cells with no nucleus differs from one image to another.

The evolution of the progesterone levels was normal relative to the follicular development and cytology. Thus, the female had a basal/ basic P4 level until about 3 days before ovulation. About 2 days after ovulation the P4 level was 20.24 ng/ml. The P4 value in a certain period of time after ovulation differs greatly from one bitch to another and it might be due to the number of ovarian follicles and to the number of corpus luteum. Thus, a larger number of corpus luteum will cause a higher and sudden progesterone level.

In terms of the ultrasound results, the ovarian follicles had an ecogenous appearance to the last medical examination. Since the last examination, a change in the ovaries appearance could be noticed- they turned hypo-ecogenous. Also, the ovaries form evolved from a round shape at the beginning of the monitoring (A', C') to elliptical form (D') at the end of it.

We noticed that the females belonging to large breeds and the obese ones, the examination using the echography/ultrasound apparatus is performed with greater difficulty, situation reported also in the scientific literature (5). The easiest ultrasound examination can be done during the follicular sexual cycle. (proestrus and the estrus). During the proestrus cycle, the ultrasound appearance of the ovaries show regular and ecogenous shape, having 6-9 mm, with very thin follicular wall. The follicles grow progressively from the early follicular phase until its end. With the approaching of ovulation time, the preovulation follicle thickens its wall up to 1mm. According to the information in the literature a preovulating follicle can change its shape becoming flat, situation that the current study did register. At the ovulation time the follicular cavity disappears (follicular collapse).

There is information in the literature that in half of the cases, after ovulation, some hypo-ecogenous structures are still found inside the ovary (8). These structures are different from the preovulation follicles being smaller and irregularly shaped (9).

It is rather difficult to assess the number of follicles according to the literature (5). These researchers notice that 45 % of the follicles do not ovulate. These round ecogenous structures can be seen up to three days after ovulation. This situation may mislead an inexperienced examiner. Another structure that may mislead the vet is the liquid present between the ovary and ovarian bursa, situation that can occur in 40% of cases (1, 4). A day after the ovulation, there is a blood accumulation in the follicular cavity that leads to the appearance of the hemorrhagic corpus luteum. They have a structure similar to the preovulation. It is therefore very important that the ovaries examination to take place daily in order to show the exact ovulation moment.

According to LEVY and FONTBONNE (5), detecting the ovulation moment by ultrasound investigation is just 10 percent more accurate compared to the progesterone level determination. Setting the ovulation moment is imperiously needed especially if the artificial insemination is done with chilled or frozen semen, or if the animal is susceptible of infertility (6).

The bitch ovaries are difficult, and sometimes impossible to be examined through ultrasonography, because they are small, difficult to be differentiated from the surrounding tissues and are often obscured by the intestinal gas (3). The rapid disappearance of the follicular cavity, correspondent to the ovulation, was detected only in two bitches. Although ultrasound changes during the estrous cycle were well-studied, the exact moment of ovulation cannot be predicted (6).

Conclusions

- Examining the ovaries in the follicular phase of the sexual cycle by ultrasound device is easier in the situation of a small or medium –sized female than a large one.
- The ultrasound examination is the most certain method out of the three ones described.
- Even if the study faced some non-ideal situations, we can conclude that the ovaries ultrasound examination is a valuable method to predict the ovulation moment in bitches.

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- To optimize the results of determining the ovulation moment it is recommended to collate the ultrasound examination with at least one of the other two methods of investigation.

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