Determining the optimal time of mating in bitches: particularities¹

Determinação do tempo ideal para cobrição de cadelas: particularidades

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Abstract

Timing the day of ovulation in the bitch is important, especially when using frozen semen. Clinical signs (vulval oedema, aspect of vulval discharge) or clinical techniques (vaginal cytology, vaginal endoscopy) used to detect ovulation in the bitch are not accurate enough. Hormonal assays are therefore commonly used by veterinarians to assess ovulation in the bitch. LH assays are difficult to perform and expensive. The estimation of the day of the pre-ovulatory LH-peak using progesterone assays maylead to some errors, as some bitches show a premature slight rise of blood progesterone several days before ovulation. However, it has been recently demonstrated that the progesterone plasma level at the time of ovulation, whatever the breed, is fairly constant. Therefore progesterone assays appear as a fairly reliable technique to assess ovulation in the bitch. At the Alfort Veterinary College (France), we investigated the use of ovarian ultrasonography to detect ovulation in the bitch. The oestrus period was followed using ultrasound and hormonal assays in several bitches belonging to 36 different breeds. The day of ovulation was clearly detected in 91.7% of the bitches (44/48). In most bitches the ovulation process appeared to be completed within 24 hours. No significant difference in ovulation time was seen between the left and the right ovary. Compared with progesterone assays, ovulation detection using ultrasound increased the accuracy of diction of the ovulation in 10.1% of the bitches, in which the delay between ovulation detected by ultrasonography and ovulation estimated by progesterone levels was more than 24 hours. Furthermore, one daily ovarian scanning was sufficient to perform a correct time of ovulation. The ultrasound features of the canine ovaries just before ovulation, during the ovulation process, and just after ovulation, are also described in this article.

Keywords: ovulation, ultrasonography, progesterone

Introduction

Timing the day of ovulation as accurately as possible is considered by most authors as one of the most important factor in order to determine when to inseminate bitches. This is especially important when using frozen semen, due to the relatively short survival of frozen/thawed semen in the female genital tract after artificial insemination. In this respect, many different techniques and plans for breeding have been tested by veterinarians over the past 20 years. Very recently, in accordance with what has been done in humans or in large animals, ovarian ultrasound examination in the bitch has been tested by some authors as a tool to diagnose ovulation in bitches.

Classical techniques used to detect the ovulation timing in the bitch

Ovulation timing in the bitch is not always an easy matter. Veterinarians have to remember that the oocytes of the bitch are ovulated two days after the LH peak in an immature state, and they need to mature at least 48 hours before being able to be fertilised. Recently, it has been demonstrated that the canine oocytes cannot be penetrated by sperm when they are still immature (Reynaud *et al.*, 2005). Therefore, even if it is essential for a veterinarian to be as accurate as possible to detect the day of ovulation, this day is reached at least 48 hours before the day of fertilisation.

When performing a natural mating or using Artificial Insemination with fresh semen, it is not essential to be very accurate, as the "fertile period", i.e. the time during which a mating or insemination can result in pregnancy, can sometimes be as long as 5 days before ovulation until 5 days after ovulation, especially if the semen of the sire is of good quality, therefore remaining alive and able to fertilise oocytes for a long period of time in the genital tract of the bitch after deposition (England and Concannon, 2002). However, when inseminating a bitch with frozen or chilled semen, which is supposed to have a shorter life "in vivo", it is recommended to perform it at the **optimal time of fertilisation which occurs between 2 to 4 days after ovulation**, when the oocytes are fully mature and have not underwent degeneration.

None of the **clinical assessments**, like the vulval oedema, the quantity and aspect of the vulval discharge (more or less haemorragic), the Amantea sign (turning the tail aside when the veterinarian touches the

perineal region) or the acceptance to be mounted by the male, are precise enough to detect the occurrence and the day of ovulation (England and Concannon, 2002).

Furthermore, it is well known that there is no reliability on a predetermined ovulation day, and consequently, a predetermined mating date. Some bitches may ovulate as early as day 5 of the heat period, and others as late as day 30. In the same bitch, it has been shown that significant variations of the day of ovulation may occur among successive heat periods in around 44% of the cases (Badinand and Fontbonne, 1993).

In these conditions, it is highly recommended to use complementary clinical tests to accurately time ovulation. **Vaginal cytology** cannot be used to detect ovulation prospectively. At the end of the heat period, the "onset of vaginal metestrus", when there is a sudden increase in intermediate cells and parabasal cells, occurs around 5 days after ovulation (Holst and Phemister, 1974). But, it only helps to detect ovulation retrospectively.

Vaginal endoscopy is performed by some authors to determine the "fertile period", but once again, with this method, which also requires to rely on an expensive equipment, it is impossible to be accurate in timing the exact day of ovulation.

Hormonal assays are therefore commonly used by veterinarians for this purpose. **LH assays** are ideal in theory, but timing the LH peak requires at least two blood samples per day every day, and, in most countries, no commercial assays for canine LH are available. Researchers willing to perform LH assays therefore have to rely on expensive and time consuming radio-immunoassay tests.

It has been suggested to estimate the day of the preovulatory LH peak using **progesterone assays**. England and Concannon (2002) have suggested that the plasma progesterone exceeds 2.0 ng/mL (6.5 nmol/L) at the time of the LH surge or on the following day. Some unpublished personal data lead us to think, however, that, at least in some breeds (german shepherd...), only assaying this early pre-ovulatory increase in plasma progesterone may not be accurate enough to accurately estimate the day of ovulation.



It seems wiser to us to continue assaying progesterone until it reaches a value considered to indicate with certainty that ovulation has occurred. According to Arbeiter (1993), a reliable identification of mating time in bitches requires monitoring of rising progesterone concentrations up to at least 32.0 nmol/L (11 ng/ml). Recently, it has in fact be demonstrated that the progesterone plasma level at the time of ovulation, whatever the breed, is fairly constant (Marseloo *et al.*, 2004). Therefore, progesterone assays appear as one of the most reliable technique to assess ovulation in the bitch.

In France, in order to reduce the number of progesterone assays during the heat period in a bitch, and therefore reduce the price of the detection of ovulation, veterinarians often combine vaginal cytology and progesterone tests, in the following scheme.

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Ovulation detection using ovarian ultrasonography

The last technique to determine ovulation in the bitch, which is also the newest, is ovulation detection under ovarian ultrasound scanning. Unfortunately, it is in accordance to all authors that, in the bitch, the ultrasound images of the ovaries around ovulation are more difficult to analyse than in other species. Previous studies have shown that the ovarian follicles just before and just after ovulation look very similar (England *et al.*, 2003), some follicles don't collapse at the time of ovulation (Hayer *et al.*, 1993, Yeager and Concannon, 1996) and, furthermore, non-ovulated follicles often remain after ovulation (Wallace *et al.*, 1992). Considering these difficulties, recommendations have been made by people who have studied this technique. For example, at least two daily examinations are recommended by some authors in order to determinate ovulation with accuracy (England and Yeager, 1993). However, even when following a very precise protocol and frequent examinations, ovulation could only be diagnosed in 15.4 % (2/13) and 54.5 % (6/11) bitches (Hayer *et al.*, 1993, Hase *et al.*, 2000). This lead some people to think that, in the bitch, the accuracy of the detection of ovulation was difficult to obtain using ultrasound.

But, at the beginning of the 21st century, due to the fact that new ultrasound machines with high performances are readily available in veterinary clinics, it may be useful to reconsider the interests of the followup of oestrus using ultrasonography. For this purpose, a series of experiments were performed at the Alfort Veterinary College (Paris, France) in order to see wether ovarian ultrasound examination by ultrasound could be a reliable and precise enough method to determinate ovulation in bitches.

Alltogether, **in this study**, the oestrus period was followed using ultrasound and hormonal assays (progesterone, LH) in several bitches belonging to 36 different breeds. In beagle and non-beagle bitches, aged between 9 months and 8 years, most of them belonging to private owners, an attempt to detect ovulation by ultrasound during their heats, in comparison with progesterone testing, was made. The day of ovulation was

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clearly detected in 91.7% (44/48) of the bitches. 4 bitches, however, showed imprecise ovarian aspects around ovulation. They all belonged to large breeds (german and belgian shepherds, labrador retriever, dogo argentino).

In most bitches, the ovulation process appeared to be completed within 24 hours. In 14/41 bitches, it was even completed in less than 12 hours. No significant difference in the ovulation time was seen between the left and the right ovary.

Furthermore, in these bitches, the progesterone level at the time of ovulation appeared to be fairly constant. It was 6.25 +/- 1.55 ng/mL (*Chemiluminescence assay, Progesterone II* ®, *Roche diagnostics, Germany*). In all the bitches, when considering arbitrarily that the ovulation occurred the day at which the progesterone plasma level reached 6 ng/mL, the ovarian ultrasound follow-up technique could increase the accuracy of the detection of ovulation in 10.1 % of the bitches (7/69), in which the delay between ovulation and the progesterone level reaching 5 ng/mL was more than 24 hours. Thus, in the bitch, the detection of ovulation using ovarian ultrasound appeared to be the most accurate clinical method. Furthermore, two daily examinations did not improve the detection of the day of ovulation. Therefore, one daily ovarian scanning appears to be sufficient to determinate a correct timing of ovulation.

Now, in practice, how can veterinarians use this very interesting technique. High frequency linear or curvi-linear probes are recommended. However, most of the images of the ovaries, even in small breeds, were done using 7.5 MHz probes. However, it was sometimes useful to confirm the slight changes in the ovarian aspects with higher frequencies, between 8 and 10 MHz.

Breeders owning expensive pure-bred bitches undergoing shows or competitions are often reluctant to use ovarian scanning for the detection of ovulation, because they fear the shaving of the hair in the abdominal region. According to our own experience, this appears unnecessary. Clipping the hair in long-haired breeds (afghans, golden retrievers...) or applying a large quantity of ultrasound gel on the region that has to be scanned, appears to be, most of the time, sufficient to get good images of the ovaries, that are in a very superficial position in the bitch.

In order to perform the ovarian ultrasound examination, we recommend to put the bitches in a "doggyrelax", laying in a dorso-lateral right or left position (see photo), in order to scan respectively the left and the right ovaries. Sometimes, however, due to the presence of intestines near the right ovary, that often interfere with the imaging of this ovary, it may be useful to make the bitch stand on the table.



Figures 3. Different positions of the bitch in order to scan the ovaries. The bitch can lie on a down-lateral left or right recumbency. Sometimes, the right ovary will be easier if the bitch is standing on the table.

The ovaries in the bitch are located in general in front of the 3rd or the 4th transverse lumbary apophysis. They can be found caudo-laterally from the distal part of the kidneys. The right kidney being more cranial than the left kidney, the right ovary is located more cranially than the left kidney.

The best method consists in beginning with the left ovary, which is easier to find for veterinarians who are not familiar with the ovarian ultrasonography. With the probe, the veterinarian has to detect the kidney, and then to scan the caudo-lateral region of the kidney, remembering that the ovary has a very superficial location under the skin. The ovarian cortex appears a little bit less echoic - "darker" - than the renal cortex.

The ovaries may be more difficult to find in giant breeds, or in obese bitches. Some breeds have a thick

skin that makes it difficult to get good images when performing all kind of abdominal ultrasonographies: sharpeis, chow-chows, newfoundlands ...

During the anoestrus period, the ovaries are not always easy to find. They have a small size, and appear a little bit heterogenous, especially in post-puberal bitches, where remnants of anterior corpora lutea can be visualised.

In pro-oestrous bitches, the shape of the ovaries is easier to see. The ovaries are often found in a more caudo-ventral position from the kidneys. They contain several small circular anechoic follicles, surrounded by a thin echoic wall, less than 1 mm in thickness. At the end of proestrus, during the pre-ovulatory period, the size of the ovaries increase and, due to the large amount of anechoic fluid within the follicles, they become really easy to visualise. At this stage, there is a pre-luteinisation of the follicles, secreting small amount of progesterone. Ultrasonographically, the follicular walls become thicker, around 1 mm in width. Depending on the size of breed, the maximum size of the pre-ovulatory follicles varies between 6 to 9 mm. These pre-ovulatory follicles are usually wide circular anechoic structures, however, when numerous within the same ovary, they sometimes appear flattened and packed together. Some unpublished data makes us think that it is difficult to accurately estimate the number of follicles using ultrasound. In another experiment, some bitches were laparotomised just after an ultrasound examination made at the pre-ovulatory period, and the number of follicles counted under ultrasound appeared generally underestimated.

At the time of ovulation, in some cases a complete disappearance of the follicular cavities ("follicular collapsus") can be visualised. In our experimental study, this follicular collapsus was seen in only 37.5% (18/48 bitches) of the cases. However, in 50% of the cases (28/48 bitches), some intra-ovarian hypoechoic structures persist. These intra-ovarian structures always show a very different aspect than the pre-ovulatory follicles, being smaller and irregular (not circular) in shape. Furthermore, in 45.9% of the cases (22/48 bitches), we found apparently non-ovulated follicles, that remain round and anechoic up to 3 days after ovulation was detected in the other follicles in the same ovary. This high percentage of non-ovulated follicles is in accordance with the work of Wallace *et al.* (1992) who found this phenomenon in 7/10 bitches. This can be very disturbing for a non-experimented veterinarian. The mistake not to be made is to wait for the totality of the follicles to ovulate to time ovulation, as it will not happen in a large number of bitches. Finally, in 39.6 % of the bitches (19/48), some liquid was visualised between the ovary and the ovarian bursa in the hours following ovulation. It is probably the intra-follicular fluid that accumulates for a while after the follicular opening at ovulation.

From one day after ovulation, blood accumulates in young haemorrhagic corpora lutea. Their appearance is sometimes very similar to pre-ovulatory follicles. Therefore, it is very important to make daily ovarian examinations at the pre-ovulatory period to detect ovulation. If no ultrasound is made on the day of ovulation, the veterinarians may be confused and wonder if the bitch is just before or just after the ovulation time.

In conclusion, ovulation can be accurately determined using ovarian ultrasonography in the bitch. However, compared with progesterone assays, this technique increases the accuracy of the determination in only around 10% of the cases. This is the reason why, in our daily clinical work, we now use this technique only when it is important to be as precise as possible in the detection of the day of ovulation. This is for example the case when an Artificial Insemination with frozen or chilled semen has to be done, or when we want to check the occurrence and the qualitative aspects of ovulation in bitches that are presented in consultation for infertility diagnosis.



Figures 4. Ultrasonographic appearance of the ovaries during the late proestrus (preovulatory) period in the bitch. Due to the large amount of anechoic fluid within the follicles, they become really easy to visualize; the follicular walk become thicker, around 1 mm in width. Depending on the size of breed, the size of the pre-ovulatory follicles varies between 6 to 9 mm.

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Ultrasonographic appearance of the ovaries of the bitch at the time of ovulation. A complete disappearance of the follicular cavities ('follicular collapses') can be visualized (photos a and b). However, in 50 % of the cases, some intra-ovarian hypochoic structures persist (photos c and d). Often non-ovulated round follicles remain in the same ovary (photo c). In around 40% of the cases, some liquid is visualized between the ovary and the ovarian bursa in the hours following ovulation (photo f).



Ultrasonographic appearance of the ovaries in the bitch just after ovulation. From one day after ovulation, blood accumulates in young haemorthagic corpora lutea, their appearance is sometimes very similar to pre-ovulatory follicles. Therefore, it is very important to make daily ovarian examinations at the pre-ovulatory period to detect ovulation.

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