Turkish Society of Veterinary Gynecology
Congress of 9th National & 3rd International

2002
TÜRK VETERİNER JİNEKOLOJİ DERNEĞİ

TÜRK VETERİNER JİNEKOLOJİ DERNEĞİ

IX.ULUSAL & III.ULUSLARARASI *Kongresi*

24-27 MART 2022

Susesi Otel - ANTALYA

KONGRE BİLDİRİ ÖZETLERİ KİTABI

TÜRK VETERİNER JİNEKOLOJİ DERNEĞİ
IX. ULUSAL & III. ULUSLARARASI KONGRESİ

24 - 27 Mart 2022

Susesi Luxury Resort Otel – Antalya

TURKISH SOCIETY OF VETERINARY GYNECOLOGY
CONGRESS OF 9th NATIONAL & 3rd INTERNATIONAL

24 – 27 March 2022

Susesi Luxury Resort Otel – Antalya

Management of Reproduction in Dogs

Rita PAYAN-CARREIRA
CHRC - Comprehensive Health Research Centre & Dept. of Veterinary Medicine, ECT, Universidade de Évora [Pole at Mitra], Évora, Portugal

Abstract
Dogs are a unique domestic species regarding their reproductive function. In particular, the large variability in main events related to the canine estrous cycle raises a challenge when managing the reproductive function, especially in females. In this presentation we will revise the physiologic basis of the canine reproduction and will discuss some key-points to guarantee the reproductive success in female dogs.

Introduction

Dogs are a peculiar species in what respects to their reproductive function. Dogs are monoestric, meaning they have a complete estrous cycle at a time, separated from the following by a mandatory anestrus. Furthermore, the sexual cycle in is remarkably long compared with farm species, its duration modulated by the breed and genetic predispositions. The length of their proestrus/estrus phase is variable between bitches, as it is the length of anestrus. Consequently, the interestrous intervals (IEI) may vary between 4 and 12 months, even though the mean IEI length is around six to seven months. This sole fact contributes to the difficulties in predicting estrus and the fertility window when we have no access to the previous reproductive records, the records are incomplete, or the owner is unaware of the species-peculiarities. Aggravating this difficulty, we should also account that dogs ovulate an immature oocyte, needing to finalize its development in the oviducts, the moment of ovulation from the onset of proestrus is variable even in the same individual, and canine spermatozoa have a long lifespan in the female genital tract, being viable up to 9 days.

Albeit a higher frequency of estrus has been reported during the winter and summer, dogs are not seasonal whether they are free-living or privately owned. Another reproductive specificity is that dogs present long-lasting corpora lutea, and thus the pregnancy and non-pregnant diestrus stages are of similar duration. Additional reproductive characteristics respect being polytocous and a spontaneous ovulators.

Because of these characteristics, reproductive function management in female dogs is critical to maximize fertility at breeding or avoid unwanted pregnancies in intact females. Intend breeding must be thoughtfully planned to increase the outcome and cope with the dog owner's expectations. Also, each animal is individual (not much of a tailored procedure will cover all the situations and may even change according to the breeding method to use (mounting vs. insemination; fresh or chilled vs. frozen semen). Therefore, in the following sections, we will discuss the available approaches for managing canine breeding.

The canine estrous cycle

It is currently accepted that canine estrous cycle encompasses 4 stages: proestrus, estrus (together representing the follicular phase, the shorter phase of the canine cycle), diestrus (or luteal phase, albeit the designation may be controversial) and anestrus (Figure 1).

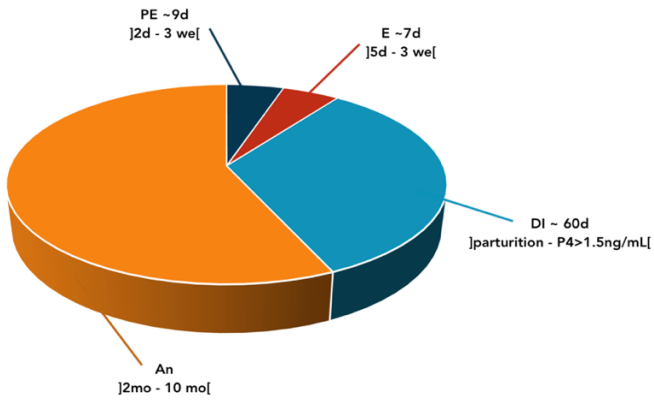

Figure 1. Representation of the canine estrous cycle.

Age at puberty, and hence at the first estrous cycle varies with the breed size and conformation. It occurs between 4 to 14 months in most breeds; generally, we could accept that puberty occurs when the female reached between 75 and 80% of the adult body weight. It is possible that a minimal BCS or fat deposition will affect the age at puberty, alike the positive social cues, but in dogs these factors remain unclear.

Proestrus (PE) length averages 9 days (ranging from 2-3d to 3 we) and in most females it is recognized by the increased volume of the vulva, the increasing serosanguinous vaginal discharge, and the increased attractiveness to males. These clinical signs are determined by the increasing levels of estrogens produced by developing follicles (which rises from initial low values of 5–10 pg/mL to the concentrations of 45–120 pg/mL before ovulation). However, the intensity of the clinical signs can vary between females, with some females showing little vaginal discharge or lower ability to attract males, which makes more difficult to the owner the detection of heat.

At the end of PE, the estrogens (E2) levels decrease, in parallel with the rise in progesterone (P4) levels, which are associated with the luteinization of mural granulosa cells. This P4 increase drives the LH pre-ovulatory surge, which occurs with progesterone levels around 2ng/mL. The LH surge occurs early in estrus (E), and lasts for 36h (ranging from 24-72h). The length of estrus stage is around 7-8 days, but it is largely variable and may last up to 3 weeks in some female dogs. The moment of ovulation in relation to the onset of heat is not constant but related to the moment of the LH surge.

A spontaneous ovulation occurs 48–72h post-LH surge, consecutive to the sharp rise in P4 that follows the LH surge (P4 reaches 5 to 6 ng/mL at ovulation) and shifts the ratio E2/P4 toward a progesterone dominance. The decreased E2 levels drives a reduction in the reproductive tract edema and the intensity of the vaginal discharge, while the increased P4 dominance triggers estrus behavior (“standing heat” and lordosis). Nevertheless, one should remember that mounting is mainly a social behavior in dogs, so free ranging female dogs, with loose social hierarchies, may accept the mounting of males earlier in the cycle, even before the occurrence of LH surge. Therefore, the acceptance of the mounting is not good evidence for a successful breeding.

Estrus ends with the end of the “standing heat” when a dramatic shift in the E2/P4 ratio occurs. The diestrus is established with the P4 domination and lasts for close to 60d; or when P4 levels persistently remains below 1-2 ng/mL. Pregnant and non-pregnant bitches present a similar P4 pattern; they only diverge just prior to parturition, when in pregnant females a sharper decline in P4 levels is observed. In the first half of diestrus, the canine corpora lutea are relatively independent of gonadotrophins secretion, but after day 25-27, the CL depends on the gonadotrophin secretions, which is determined by PRL (the main luteotropic factor).

Following diestrus, the reproductive tissues enter a rather “silent” period – the anestrus. Anestrus is the stage that shows the highest duration of all; its length can range from close to 60 days, if the female shows a interestrous interval (IEI) of 4 month until close to 10 months, in those presenting a IEI of 12 months.

The ovarian activity is suspended during the anestrus, excepting for the last third of the stage, when the cyclic follicular recruitment takes place, responding to small bursts of FSH and LH secretion. Progesterone levels are basal (< 1ng/mL). A slight increase in estrogens levels has been reported in the last third of anestrus, albeit without notorious biologic effects.

Even though no major changes (behavioral or morphological) have been associated with anestrus, this is an important stage for the endometrial regeneration between estrous cycles. It has been shown that shortening of anestrus during cycle manipulation may be detrimental for the female´s fertility.

The window of fertility

The window of fertility refers to the interval of time when the female dog can successfully mate and achieve pregnancy. As said previously, the canine heat (comprising the proestrus and estrus stage) presents large individual variations between bitches. Also, it is not easy to detect the fertility window in the female dog based on her behavior or the clinical signs. Additionally, because of the species particularities, the window of fertility does show different width according to the type of breeding to be used (mating vs. insemination; fresh vs. chilled vs. frozen semen). The inability to correctly identify the window of fertility has been responsible by 50 to 80% of the bitch infertility in natural service.

Summarizing important physiologic events that affect the length of the window of fertility:

- Primary oocytes are ovulated 48 to 60h post-LH surge (day 2 to 3 post-LH)
- Oocytes must mature in distal oviducts for 96 to 108h (day 4 to 5)
- Oocytes remain viable for 24 to 48h post-ovulation in most females (days 5-6), albeit this period in some bitches (we only never know which ones…) can be extended up to 120-144h (day 9-10).
- The canine spermatozoa have a long lifespan, if used in natural services (~9 days), but its viability decreases after processing (fresh semen ~5 to 7d; chilled semen ~4 days; frozen semen~ 12-24h)

As with any other planned event, we must consider some key issues:

- To trace a strategy to deal with any female in heat to identify the window of fertility
- To be aware of the method that will be used for breeding, as it will delimit the fertile period we must track
- If artificial insemination is to be used, to be aware of the type of semen to be used and how will the delivery occur.

It will be convenient to perform a thorough clinical exam to the female a short time before the intended breeding, or in the early days of proestrus. This is a crucial step whether it respects a nulliparous female or one that whelped a few months ago. Besides, in some situations it will be necessary to perform additional testing (e.g., for *Brucella canis* or the canine herpesvirus, particularly if using the natural mating), including genetic screenings according to the breed club. Collect the most important data from her previous reproductive history, existing treatments that could interfere with fertility, and current diet. In the physical exam, screen the BCS, the respiratory, cardiovascular, endocrine, and musculo-skeletal systems. Also check the skin to discard fungal or parasitic diseases needing correction before breeding the female.

For the exam of the reproductive tract, it is important to evaluate the vulva and explore the vagina, if possible, using endoscopy. While with a vaginoscope or digital exploration, only the most caudal part of the vagina (the vestibule) can be explored, the use of a vaginoscope allows inspection of the vaginal vault, and test the existence of constrictures, existing septum or masses that would hinder the reproduction and may request correction before breeding.

Ultrasonography is recommended to discard ovarian or uterine abnormalities, particularly in older females, that may suffer from subclinical cystic endometrial hyperplasia or ovarian cysts.

Females declared as infertile raise a challenge to the practitioner and may request a uterine biopsy or the collection of material for cytology or microbiological culture.

What about "that" strategy…

In dogs, to delimit the fertile window we need to monitor the heat evolution to detect the LH surge or the ovulation day (crucial if using frozen semen). No single method exists; therefore, several methods can be combined. Those most frequently used in the clinics include the vaginal cytology combined with the determination of P4 blood levels. According to the needs we can also include the ultrasonography to precise the moment of ovulation (when using frozen semen), or alternative methods such as the vaginoscopy or the vaginal resistivity, albeit with variable efficiency.

- Monitoring the rising estrogen levels

The vaginal cytology is a simple and cheap method to collect information on the estrogen impregnation of the tissues. Upon estrogen stimulation, the vaginal epithelium thickens because of cell proliferation. Cells in more superficial layers are poorly nourished and die; they are easily exfoliated. Accompanying the rise in estrogens, vaginal epithelial cells (round cells; intermediate cells) become larger, the ratio nucleus:cytoplasma decreases, cells limits fold and assume an irregular shape and the nucleus exhibits signs of pyknosis or disappears (superficial, cornified or keratinized cells). The presence of additional elements (neutrophils or erythrocytes) also changes from proestrus to estrus, helping in defining the window of fertility, or in the diagnosis of some clinical conditions. In diestrus, we observe a sudden transition toward the dominance of round cells in the cytology and the presence of neutrophils also hints to the existence of higher progesterone levels.

For a finer delimitation of the window of fertility, the vaginal cytology needs to be complemented with the determination of progesterone levels.

The vaginal endoscopy (or vaginoscopy) is also used to analyze the features of the vaginal vault (in terms of edema, color, and fluids), and the changes that reflect the estradiol rise. In PE the vaginal epithelium is pink; the edema drives the roundness of the vaginal folds, and the serous-hemorrhagic discharge within the recesses between adjacent folds. When the estrogen levels decrease, in parallel with the increase in P4, the vaginal folders gradually become wrinkled (or crenulated), the epithelium becomes whitish and a decrease in the fluid content is observed. In diestrus, the vaginal epithelium is flat, dry and it easily whitens and then turns reddish when touched with the tip of the endoscope.

The vaginoscopy is not a good indicator of ovulation (it only reflects the estrogen impregnation, like the vaginal cytology), and therefore it should also be complemented with progesterone determinations. Besides, like for the vaginal cytology, it requests multiple sequential observations, but contrary to the vaginal cytology, is less precise regarding the indications for the beginning of blood collection to surveil the progesterone determination.

Besides the combination with the vaginal cytology, other methods can be used to monitor the estrogen rise, albeit with different sensitivity or the efficiency. One example is the Draminski´s detector of ovulation, that measures the resistivity of the vaginal mucus to define the optimum moment for mating. The Draminsky device detects 69-75% of ovulations with a precision of 24h. Still, a maximal difference of about 60h exists between the actual moment of ovulation and the one detected by the device. If the difference may be negligible when using the natural mounting or the AI with fresh semen, it can compromise the fertility of the cycle when using frozen semen. The existence of vaginitis may hinder the Draminsky success in detecting the ovulation day.

To complement the detection of ovulation with this method, the determination of P4 levels in the blood remains a necessity to maximize the female´ fertility.

- Detecting the LH surge directly

In the practice we can use the determination of LH surge to estimate the occurrence of ovulation. The LH rises for 12-24h, before dropping for an additional 12 to 36h period. In total, the LH surge lasts for 1.5 to 2 days. The LH surge coincides with the change in the ratio estrogen:progesterone and is followed by the expression of the heat behavior (lordosis and mating acceptance is mediated by progesterone in dogs).

A semi-quantitative method is available in some countries to detect the LH surge. The kit sets a threshold at 1ng/mL to signal the presence of LH (a Yes/No kind of response). It will require a minimum of 2 blood samples per day, to be scheduled for when the superficial cells reach a frequency of 50 a 60%. However, in some cases, successive blood samplings will be needed to identify the LH rise, which compromise its routine use in clinical settings. Another handicap respects the fact that its use does not ensure the correct determination of the ovulation day, hence impairing its application in case of AI with frozen semen.

- The use of progesterone to estimate the day of LH surge and ovulation

The progesterone levels can be monitored to follow the occurrence of two crucial events in the canine estrus: the LH surge and the ovulation.

As the rise in P4 during the estrus shows variations even within the same female, it is not possible to estimate the female fertility based upon a single blood sample. Thus, multiple samplings will be need through time. One should be aware that we need "calibrate" the result from our in-clinics P4 equipment with a reference one. Often the machines have small differences that could impact the efficiency of the determination.
A usual question is when to start with the collection of blood samples for P4 determination. The vaginal cytology can be used as a guide: if the vaginal cytology collected at day 4 or 5 after the heat onset represents the beginning of PE, another cytology must be schedule in 3-4 days (but n blood collected for P4). And we keep adjusting the collection of cytological samples until the cytology shows ca. 80% of superficial cells. At this moment, we should start collecting blood for P4 determinations.
The peripheral levels of P4 should be interpreted to estimate the day of LH surge and of ovulation, as follows:

P4 < 1 ng/mL→ still no follicular luteinization started → wait for 3 - 4 days

P4 between 1 & 1,9 ng/mL → a few hours until the LH surge → reassess next day

P4 between 3 & 5 ng/mL → we are close to ovulation → reassess in 1 or 2 days

P4 between 5 & 10 ng/mL → ovulation → Schedule mating or IA according to the type of semen.

If we are using frozen semen, the female should be monitored with aid of the ultrasound to ascertain the moment of ovulation and better schedule the application of semen in the uterus using a transcervical approach, guided by vaginoscopy. This combined approach can improve up to10% the efficiency of the ovulation detection.

It is important to mention that fertility, in the female dog, respects not only the ability to obtain full term pregnancy, but also to achieve it with the standard number of puppies to her specific breed. A small number of puppies per pregnancy usual reflects either a mismatched window of fertility or a fertility problem in the female or the male.

Matching the window of fertility with the sperm deposition in the female

As mentioned before, sperm survival in the canine female genital tract is in general long (up to 9 days) in natural mating conditions, albeit it might be influenced by the age of the animal, the parity, and the general reproductive health of both the male and female partners. If AI is used, fresh and chilled semen may have rather similar lifespans (up to 5 days), depending largely on the extender used, while the lifespan of frozen semen is rather short, since the canine semen is predisposed to precocious capacitation during freezing/thawing procedures. Moreover, frozen semen is more sensitive to changes in que quality of the female genital tract, mainly in respect to pH (which is changed during inflammation), the ionic charge of the epithelium and the content in oxidative radicals.

So, the length of the fertility window varies mainly according to the type of semen to be used, being the shortest in case of AI with frozen semen (Figure 2). This is an important parameter in the clinical practice. Yet we may consider that if our goal is to maximize female fertility, our focus should be to work near ovulation time.

The canine artificial insemination has been growing in the past decades worldwide, either for sanitary reasons or the considerations on the female wellbeing when long distance voyages are needed.

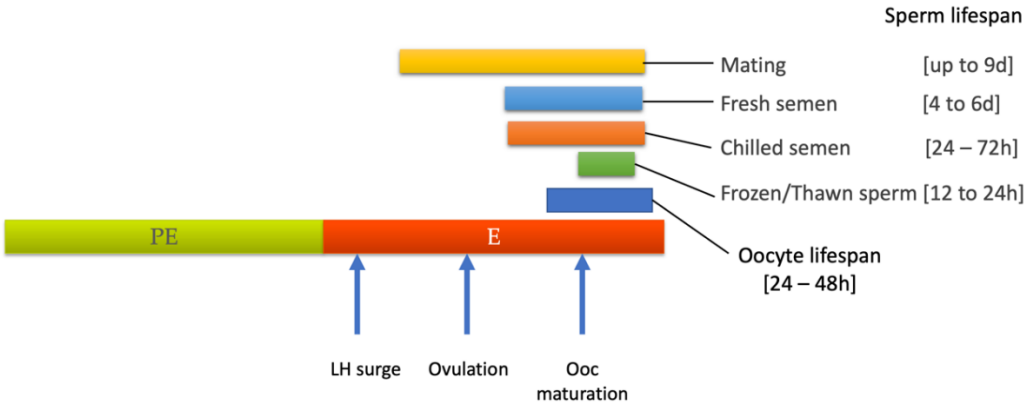

Figure 2. Representation of the type of semen in the length of the window of fertility in dogs.

Considering the sperm lifespan, to female dogs must be breed one or two days after ovulation, twice or trice (48h apart) or until receptivity disappears, when natural mating is used; two to three days after ovulation, twice, if fresh or fresh chilled semen are used; three to four days after ovulation, twice, in the case of using frozen semen.

Checking the outcome success

The diagnosis of pregnancy is a crucial step to ensure the breeding success. But when should we do it?

In the dog, embryos enter the uterus between day 8 to 10 post-fertilization and remain floating freely in the uterus until day 14 post- fertilization, when they stop at the site implantation will occur. Around day 15 post-fertilization implantation begins, and by days 16-17 the first signs of endometrial invasion are observed. Still, the placenta is only complete by day 30, when the implantation chambers are visible. Canine pregnancy lasts for 9 weeks. Nevertheless, as the oocyte fertilization can occur for 24 to 36h, the moment of fertilization is a difficult parameter to estimate. Therefore, the average length of the canine pregnancy can be estimated on the base of different events (table 1). Factors such as the litter number or the breed or size of the dog may affect the pregnancy length.

Table 1. Average pregnancy length in dogs.

Counted from	Pregnancy length
Mating	*57 a 72 d*
1st day cytologic diestrus	*57±3 d [range 54 – 60d]*
Ovulation	*63 ±1 d [range 62-64d]*
LH surge	*65 ±2 d [range 63-67d]*

In dogs, different methods are available to diagnosis pregnancy with different efficiency, but in the clinical practice the most currently used is the ultrasound. Contrasting to most farm species, a precocious diagnosis is difficult.

It is currently believed that progesterone measurements are not useful for pregnancy diagnosis in dogs, since they have long lasting corpora lutea and no significant differences exist between pregnant and non-pregnant bitches. In a very recent study, differences in the two groups (pregnant vs. non-pregnant) were only detected at the end of pregnancy, after day 52. Also, clinical signs associated with pregnancy are not precocious enough and may be inconspicuous to present an adequate specificity.

Transabdominal palpation between days 26 to 35 post-fertilization might be used, with a reported accuracy of 75 to 80%, but it is not easily performed in all animals. Still, it is recommended to confirm the negative or positive results later.

Implantation and placentation are immune-mediated events, and trigger some changes in inflammatory mediators, such as acute phase proteins (APP). They variations in blood, however, are not specific for pregnancy, and the measurement of APP has low specificity.

Relaxin, a hormone produced by the placenta, increases in the maternal blood from days 21-24 post-fertilization until the week preceding parturition. A Relaxin-based pregnancy test is available for dogs. Relaxin peaks between pregnancy days 30-35 and remains in high levels until parturition or abortion. Its sensitivity is not adequate to avoid the need for additional pregnancy detection, since its levels remain high for some time after pregnancy loss; therefore, negative results should be confirmed again later (some false negatives have been reported mainly between the pregnancy days 20 and 30 days). Also, the changes in relaxin secretion do not closely follow the death of the litter, originating false positives.

Nevertheless, due to these issues and the fact that the relaxin test is more suitable after the 4 weeks of pregnancy, it is not competitive compared to the ultrasound. The ultrasonography presents an identical gap for the first 3 weeks of pregnancy, but it further allows to estimate the fetal age from the fetus morphological development and to assess fetal vitality and well-being (particularly at the end of pregnancy) and detect some major malformations. Albeit the determination of the phase of pregnancy is not in focus here, it deserves mention that ultrasound is a useful tool to predict the approximate time for parturition in case the day of LH surge or day of ovulation were not identified during the precedent heat.

An adequate pregnancy monitoring will allow the practitioner to support the owner with the necessary information to assist the female owner during the parturition, and to decide on any intervention (namely during dystocia) it may be needed. In term pregnancy, the clinical signs of impending parturition together with the abdominal ultrasound and progesterone measurements will indicate the veterinarian that the puppies are mature.

The x-ray is not suitable for pregnancy diagnosis, since the fetal skeleton opacity will only be detectable after day 45; it is better suit after day 54, and it may be helpful to estimate the litter size and the relative dimension of the fetus and the maternal pelvis. However, it does not give any information on the fetal viability, even though it may hint at fetal death.

At the end of this presentation, it is important to stress that it is important to the success of the canine reproductive management to create clinical protocols to guarantee the maximum fertility for a particular pair female/semen. Albeit in here we discussed the generic approaches to identify and monitor the window of fertility, we should remember that each bitch is a unique case and the should adapt and revise our interventions according to the data we gather from clinical observations and evolve accordingly.

A full list of references can be obtained from the author. Kaynaklar listesi için lütfen yazar ile diyaloğa geçiniz. (rtpayan@uevora.pt or rtpayan@gmail.com)