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Cat pregnancy
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Caesarian section

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Dear Readers!

The meaning of the existence of each species is its extension; that is why the reproductive period is so crucial in veterinarians' practice. We support the females for pregnancy, deliver difficult births as well as perform the cesarean sections. We are happy with all healthy puppies and support the bitches during lactation. Like other species, we care for stud dogs and help support their condition and health.

Many factors can influence animal fertility, pregnancy physiology, and healthy births. The most important is to feed a complete and balanced diet, support stud dogs with optimal vitamin E and Se levels, reduce the risk of bacterial or viral infection, etc.

Unfortunately, we cannot avoid pathologies in reproduction – e.g. mammary gland cancer, polycystic ovarian disease, etc., as well as, e.g. phantom pregnancy in bitches.

Enjoy reading!



Anna Rutkowska
Editor-in chief



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TABLE OF CONTENTS 8/2023

In expert's opinion:

- 4 **Canine brucellosis**
Tadeusz Frymus
- 6 **Caesarian section**
Tomasz Kurusiewicz
- 8 **Cat pregnancy in clinical practice**
Andrzej Max
- 11 **Application of ultrasound fetometrics in predicting the date of delivery in bitches and kittens**
Piotr A. Socha
- 14 **Mammary gland tumours in bitches**
Ilona Kaszak
- 18 **Polycystic ovarian syndrome in domestic caviés**
Małgorzata Ponikowska
- 20 **Progesterone - not just a canine hormone.**
Indications and options for testing progesterone levels in cats
Dariusz Jagódka
- 23 **Stud dog – basic algorithms of assessing breeding value**
Anna Domosławska

Straight-off-the-shelf experience:

- 26 **The impact of vitamin E and selenium supplementation on semen quality in dogs with reduced fertility**
Rafał Kościeszka
- 28 **Phantom pregnancy in female dogs – is it a negligible issue?**
Małgorzata Rutkowska-Szulczyk

Vet Pharmacy:

- 31 **SemeVet**
- 31 **ProlactiNO**
- 31 **VetoMune**
- 31 **VetAminex**

Canine brucellosis

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Brucella canis in dogs causes chronic infections often with a latent course which is manifested mostly by infertility in males and females and less often by other than reproductive organs. This bacteria can also infect humans while cats are considered to be immune to this infection.

Aetiology

Almost the unique cause of canine brucellosis is the endemic bacterium *B. canis* which is the subject of this article.

Occasionally, dogs may be infected with *B. suis* (from pigs, hares, rodents, wild ruminants), which cannot be excluded in Poland, especially in hunting dogs. Outside Poland, in regions where these germs are still present, dogs may also be infected with *B. abortus* (from cattle) or *B. melitensis* (from sheep and goats).

Occurrence and significance of canine brucellosis

B. canis is probably present worldwide, although it is not detected in every place. In the USA, where the infection is notifiable in many states, the bacterium is considered a major cause of economic losses in dog breeding centres. They are mainly associated with reproductive losses, euthanasia of infected animals, and especially due to the necessary repeated testing of all dogs in a brucellosis outbreaks which in large kennels means considerable costs. In the 1970s, random studies in the US suggested that about 6-9% of stray dogs were seropositive. Recently in Oklahoma, anti-*B. canis* antibodies were found in 14% of dogs tested.

In Europe this pathogen is a less significant problem. However, the disease may be

underdiagnosed due to its non-specific and often latent nature, a difficult and expensive laboratory diagnostics and reduced attentiveness of breeders and clinicians to the disease. This is even more likely because case reports of canine brucellosis, although their number is limited, have been published in many European countries. This also refers to the former USSR, which is worrying given the fact that Polish breeders used to import dogs from those regions.

The first case of canine brucellosis in Poland was described in 1995 by Kopczewski et

al. (1) Another notable event took place in Sweden. It was a miscarriage due to brucellosis in a bitch which was imported pregnant from Poland in 2012. Finally, several years ago, an outbreak of brucellosis was reported in one of the Warsaw dog kennels (Czopowicz M. – information provided orally). Among the samples sent from Poland between 2011 and 2016 to the IDEXX laboratory for canine brucellosis testing, PCR and serological tests were positive in 6.7% (29/432) and 3 (6/164) of them, respectively (2). So undoubtedly this infection exists in our country as well.

Disease spreading

The only reservoir of *B. canis* remains the dog. Infection spreads quickly in highly concentrated kennels and among stray animals. The bacteria is excreted by an infected animal which may also be clinically healthy often only periodically (during bacteraemia) with all body fluids. Amniotic fluid and vaginal discharge excreted after miscarriage or during the heat, semen and urine are also important. The bacterium infects another animal through mucous membranes of the digestive, genital or respiratory tracts. Infection is therefore spread through mating, eating aborted fetuses or amniotic fluid, inside the uterus and also through contaminated objects or aerosol. Brucellosis is usually introduced into kennels with a clinically healthy but infected female or male dog which is purchased. The long incubation period and latent symptoms or asymptomatic infection symptoms facilitate it. The cases which were described in recent years in Europe were often associated with import of animals from the former Soviet Union as well as Spain and Germany.

Pathogenesis

Experimental infections show that approximately half of infected dogs do not develop pronounced disease symptoms. The only (or almost unique) symptom is bacteraemia, often periodical, which may last for

1-5 years. During bacteraemia such dogs excrete bacteria and infect other individuals.

The remaining half, after a long incubation period, slowly develop chronic inflammation with necrotic foci and granulomas in reproductive organs and less frequently in other areas of the body. Although anti-*B. canis* antibodies appear in serum within 2-4 weeks, cellular immunity is probably much more important in eradicating this intracellular germ.

Clinical symptoms

In bitches, placentitis is the predominant cause of fertility problems (Figure 1) - embryo resorptions, abortions (usually late, around day 45-59 of gestation) or stillborn puppies. Weak offspring may also die soon after birth. It is when the autopsy shows signs of generalized bacterial infection (enlarged spleen and lymph nodes, necrotic foci in the liver, lungs and other organs, cloudy fluid in the serous cavities, haemorrhagic lesions). However, infected but healthy puppies can also be born and they develop normally and only after reaching sexual activity by bitches, the disease may be activated and lead to abortion or to development of a permanent carrier status. In males, brucellosis may result in infertility due to epididymitis, and more often atrophy than testiculitis and therefore asymmetry of the scrotal contents. It is frequently accompanied by purulent inflammation of the scrotal skin secondary to its licking. Sometimes it is the only reason why owners bring their dogs to vets. In brucellosis prostatic hyperplasia is often unnoticeable to the owner.

As mentioned above, very rarely there are changes in other than the reproductive organs. They usually include discopondylitis, sometimes recurrent iritis, arthritis other than of spinal joints, enlargement of the spleen, liver, abscesses, glomerulonephritis or other disorders.

Bacteraemia is sometimes accompanied by mild deterioration of the general condition (fever, apathy, poor appetite), as well as enlargement of the lymph nodes. However, even upon consultation, it is very difficult to link these unspecific symptoms to brucellosis, because the course of many other diseases may be similar.



Fig. 1. Two dead fetuses, one of them mummified

Diagnostics

Considering unspecific symptoms, the suspicion of brucellosis always requires laboratory confirmation. Serological tests are often performed. Various methods can be used, but unfortunately their specificity and sensitivity are questionable. Therefore they result in many false results, especially false positives. Thus, every positive serology result needs to be confirmed. According to the principle of so-called 'paired sera test', an increase in the *B. canis* agglutinating antibody titre (after two tests at 2-4 week interval) will be more indicative of brucellosis than a single test. However, in chronic infections – which is the case in brucellosis – an increase in serum titres may no longer be apparent, and some animals may even be serologically negative at times. Therefore, when suspecting this disease (e.g. after abortion), negative serological results must also be confirmed, preferably by bacteriological examination, which is considered the 'gold standard' method. The best material for microbial culture are the organs of aborted fetuses, placenta, pathological vaginal or preputial discharge, abnormal looking semen or testicles with lesions after castration. However, the problem is that in clinically healthy dogs or dogs with unspecific symptoms (e.g. when we want to verify a positive serology result), the aforementioned material is unavailable. In these dogs only blood may be used for microbiological culture. Here, however, a negative result does not rule out brucellosis, since bacteraemia occurs only periodically. Commercial laboratories also offer PCR tests for canine brucellosis. Unfortunately, their sensitivity is unknown and may vary. It depends on the reaction primer, but also on prior antibiotic use, infection stage and other factors. Four primers were recently compared on nearly 600 sick and healthy dogs and their diagnostic sensitivities/specificities were 46%/96%, 70%/94%, 39%/97%, and 23%/100%,

respectively (3). In cases of aborted fetuses, histopathological examination may be of some help.

Thus, laboratory confirmation of canine brucellosis remains a major challenge. In all cases, apart from isolation of *B. canis*, many tests, often inconclusive and expensive, have to be performed to confirm or exclude it. And this may be one of the reasons for the disease to be underdiagnosed.

Management of infected kennel

Once a case of brucellosis has been identified in a breeding until it is free of infection, it is advisable to suspend mating and especially refrain from participation of dogs in shows and other gatherings, as they may excrete the germ (e.g., in urine). Therefore, disinfection is also important (e.g., quaternary ammonium compounds, chlorine and iodine compounds, ethanol, formalin). It is also important to inform owners that *B. canis* can be transmitted to humans. Therefore, rubber gloves should be used, especially for gynaecological and andrological manipulations, even when dealing with clinically healthy individuals. All other dogs should be tested serologically for this infection. As described above, negative result of serology is rarely false and such animals, if clinically healthy, may be considered to be noninfected. In contrast, positive serology results are often false and the status of such dogs should be verified with other methods.

There is no consensus when it comes to management of infected dogs (they may be in a quite a good clinical state or completely healthy and fertile). Some have suggested neutering for clinically healthy dogs to eliminate mating and abortion – the main routes of infection. However, neutering will not eliminate the infection and dogs may continue to spread germs for several years and run the risk for the health of other dogs and people. Others suggested treatment with antibiotics which are reported to be somewhat effective. Treatment with doxycycline in combination with streptomycin or gentamicin was recommended. There are also reports of quinolones being effective. Although antibiotics may reduce bacteraemia and anti-*B. canis* antibody titres and improve overall clinical status (even normal puppies may be born), it is not certain that the infection will be completely eradicated. As mentioned, it may persist even in seronegative dogs. Therefore, in order to consider that the dog has not only suppressed the disease but to completely eliminate the infection, multiple bacteriological blood tests with negative results must be performed. Since this entire treatment and testing procedure is time consuming in some countries (e.g., Sweden) due to the risk it poses for human health.

Prevention

There is no vaccination against canine brucellosis. The basis of its prevention in breeding should be the quarantine (minimum one month) before introducing a new dog and examining him for brucellosis twice in this time at an interval of one month. Only non-infected animals should be used for mating. All aborted fetuses should be examined bacteriologically which is fundamental for early detection of this latent infection.

Human infection caused by *Brucella canis*

Humans can be infected with *B. canis* by dogs, including clinically healthy dogs. Immunocompromised children and adults are particularly at risk. Some consider it an (undiagnosed) occupational disease of the staff of small animal clinics and other professionals working with dogs (4,5), but there is no evidence for this. This germ is considered clearly less pathogenic to humans than other *Brucella* species and the course of this chronic infection is also often latent in humans. It has been speculated that this infection is often unrecognized or even forgotten in humans, especially since the diagnostic difficulties in human medicine are the same as in dogs (5). Recently in the USA, among 306 people professionally handling dogs (breeders, kennel and shelter workers, small animal clinic staff, dog handlers) 11 subjects (3.6%) were seropositive for *B. canis* and two of them had symptoms that could be attributed to this infection (6). They include mainly fever (especially of unknown origin), and non-specific symptoms such as headache, sweats, chills, weakness, nausea, weight loss, muscle pain. However, more serious disorders such as enlargement of the spleen, hepatitis, endocarditis, osteomyelitis and others are also known. It is believed that the body often eliminates this infection on its own and an early recognized process responds well to treatment.

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Caesarian section

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A caesarean section is a surgical method of terminating labor or an ongoing pregnancy in order to exclude or reduce the risk of life-threatening or perinatal complications for the pregnant mother or fetuses.

Considering the present knowledge and diagnostic possibilities, we may very precisely assess the obstetric status and the condition of both fetuses and the mother. The measured parameters allow us to choose the course of action as an indication to carry out the surgery in a scheduled (preventative) or urgent mode.

Indications

Indications for a cesarean section are: abnormal positioning of the foetus during an ongoing labour, cephalopelvic disproportion (disproportion between the foetus and the size of the birth canal occurring mainly in miniature breeds, brachycephalic breeds and in case of singleton pregnancies), premature birth, cervical dystocia, noticeable foetal bradycardia, abnormal pregnancy development and pathologies directly affecting maternal health, uterine horn torsion or rupture, birth canal obstruction and lack of positive maternal response to pharmacological treatment.

In my practice, elective caesarean section is performed in average 40-50% of cases of labour termination.

The advantage is the reduction of complication rate associated with a prolonged labour. These may include electrolyte disturbances, shock or haemorrhage, which increase the risk of maternal death or decrease the survival rate of the offspring. In addition, thanks to scheduling a caesarean section, our staff gains time needed to conduct tests and prepare the animal for surgery.

Appointing the date of caesarean section

In order to determine the optimal due date, we use several methods that allow us to safely plan and with determine the right date. The most commonly applied methods include:

- **measurement of LH, P4 (progesterone) hormones level:**

Duration of the gestation period in dogs is 65 ± 1 days counting from the pre-ovulatory release of LH or 63 ± 1 days from the onset of ovulation. The expected date of delivery may be determined by measuring serum progesterone level which should be less than 2 ng/ml within 24 hours before delivery.

- **cytological examination of a vaginal smear which is performed to determine the mating date:**

By determining the dioestrus phase based on cytological examination of vaginal smear, we can determine the probable date of delivery.

- **measurement of internal temperature:**

Due to the decrease in progesterone level the internal temperature decreases by 1°C about 24-36h before the expected delivery. Due to the low sensitivity of this method and the fact that approximately 30-40% of parturient bitches do not show this type of change, this method should not be considered the most reliable.

- **imaging diagnostics:**

Foetal biometry (foetometrics)

Measurement of selected foetal structures and the application of mathematical formulas enable fairly precise determination of the duration of pregnancy and thus the time of expected delivery. Foetal biometry based on ultrasound examination is the most accurate method for determining the date necessary to schedule a caesarean section, as well as for specification of a due date which is important in case of lack of knowledge of a mating date. One of the most commonly used measurements is ICC (inner chorionic cavity diameter) which may be performed from the first days after implantation to about 25-27 days before delivery.

- DBP= mm-68,68/1,53
- DBP= mm-82,13/1,80
- DBP= mm-105,1/2,5
- DBP= mm-88,1/1,9

Upon the ultrasound examination, we measure more than one gestational ampulla from both horns of the uterus. Another measurement is BP (biparietal diameter) which is performed <25 days before birth.

- DBP= mm-25,11/0,67
- DBP= mm-29,18/0,70
- DBP= mm-30/0,8
- DBP= mm-23,39/0,47

It should be kept in mind that factors such as breed and litter size also affect duration of the gestation period.

- **pregnancy monitoring:**

Ultrasound: A follow-up ultrasound examination provides information on development and potential foetal pathologies which may be crucial in deciding how and when to terminate pregnancy. Radiological examination: Diagnostic imaging, X-ray is quite an effective method of obtaining information regarding the stage of development, proportion and size of the litter. The examination can be performed after the 45th day of gestation.

When planning a caesarean section, the most important thing to remember is to perform it as close as possible to the expected delivery date in order to minimize the risk of neonates death caused by insufficient development of the respiratory system.



Fig 1. Ultrasound

Preparation for a caesarean section procedure

Preparation, anaesthesia and surgical technique of caesarean section: In health-threatening situation or other indications such as uterine overload / numerous foetuses/ we may face the decision to terminate the pregnancy earlier by performing a scheduled caesarean section. Administration of dexamethasone about 24 h before surgery improves the foetal survival thanks to accelerated production of surfactant in the alveoli /56-58 day of gestation/. In case of elevated P4 levels, the single administration of aglepristone 24 h before the scheduled caesarean sections (15 mg/kg b.w.) accelerates the elimination of progesterone block and creation of the conditions similar to physiological labour. During preparation for the procedure, it is important to remember to carry out appropriate additional examinations to determine the condition of the mother, to perform an ultrasound and evaluate the maturity of foetuses / correlated with previous data / the date of ovulation and mating / to confirm the correctness of the due date.

Prior to anaesthesia, initial oxygen therapy is recommended to improve the condition of puppies exposed to inhalation anaesthetics. We also should provide intravenous access and intubate the patient. The anaesthetic protocol should be tailored to the current situation of the parturient mother and the condition of the foetuses. We try to shorten the surgery duration to limit the respiratory depression of the pups as a result of their exposure to inhalation anaesthetics which contributes to higher neonatal

survival and maternal condition after delivery. All the equipment and personnel required for induction and maintenance of anaesthesia, perioperative management, surgery, and neonatal resuscitation should be prepared and available before induction of anaesthesia. Puppies should be provided with optimal conditions (temperature, humidity) immediately after delivery, resuscitated, dried, and examined. They should be fed up to a maximum of 3-4 h after delivery. In case of insufficient milk production, oxytocin or metoclopramide may be used.



Fig 2. Litter of puppies

Conclusions

In order to properly schedule, prepare and perform a caesarean section, it is required to choose the optimum date. Monitoring of the pregnancy enables the choice of the method of delivery/pregnancy termination. Experience in obstetrics, taking care of details and choice of anaesthesia is also important.

The choice of the method of delivery is now a common topic of debate among veterinarians and breeders. These decisions are influenced by many factors such as possibility of perinatal complications, the general condition of the patient, condition of the fetuses, experience and emotional sensitivity of the caregiver, and value of the mother and litter. Given the invasiveness of caesarean section, it is important to consider whether natural labour is preferable, particularly in patients without clear indications for surgery. The health of the mother and the newborn puppies should be a priority when deciding on the method of delivery. Thus, experienced personnel should decide on the optimal method of delivery.

References available at the editorial office.

Cat pregnancy in clinical practice

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According to 2018 data from the European Pet Food Industry Federation (FEDIAF), cats are one of the most popular pets in Europe. Their number in the European Union alone exceeds 74 million, while the total number in all European countries including Russia is about 103 million. In Poland, the population of cats is estimated at about 6 million. These animals are therefore an important group of patients in veterinary clinics. Veterinary surgeons take care of breeding cats, pedigree cats, as well as stray or shelter cats. One of the areas of interest for cat owners is reproduction, which is subject to regulations regarding mammals in general, but different in details from regulations concerning other domestic animals. Reproductive cycle varies animals varies according to taxonomic background, but also environmental conditions and individual characteristics.

Pregnancy is a topic of special interest. On the one hand it is a desirable stage of reproduction and on the other hand - in some groups of animals - as an unwanted and troublesome situation. Given characteristics of various species, it is possible to evaluate specific parameters, and recognize pathological conditions as well as to take required steps, which especially applies to pregnancy.

Beginning of the gestation period and its duration

In obstetrical practice, one of the key issues is the length of gestation period and the expected date of delivery. To establish the actual length of pregnancy, it is important to determine its beginning. Assuming zygote formation as the beginning of pregnancy, the fertilization ability of male and female gametes should be considered in relation to the mating/insemination moment. While in case of spermatozoa this ability is achieved by the process of capacitation following a similar pattern in many different mammalian species, there are significant differences in case of oocytes. In dogs (and foxes as well) oocytes are ovulated as immature gametes in prophase I of meiotic division. In contrast, queens - like most female mammals - release oocytes at metaphase II stage of meiotic division capable of immediate fertilisation.. The problem, however, is a provoked ovulation in this species in response to copulatory stimuli which stimulate, via the hypothalamus and gonadotropin-releasing hormones, the release of LH from the pituitary gland. It has been found that in order for the concentration of this

gonadotropin to reach a level guaranteeing ovulation the accumulation of these stimuli (several acts of copulation) in a fairly short time (several hours) is necessary. In case of biotechnical procedures such as artificial insemination or when the male is not very active, ovulation is induced pharmacologically with GnRH or hCG analogues around the moment of semen deposition.

It is assumed that conception takes place on the next day after successful mating and that is when pregnancy starts in cats. The average gestation period lasts 63-65 days, although, according to literature, it may vary between 52 and 74 days from the first or last mating (13). The average litter size in cats is 4, but it depends on the breed. Occasionally, pregnancies with very numerous foetuses may occur, as happened in one queen with 18 foetuses which were found during ovariohysterectomy (Beaver, 1973; cited in 13).

Diagnosis of pregnancy

Clinical examination and diagnostic imaging are primarily used to diagnose pregnancy. Hormonal tests are also available. Compared with dogs, pregnancy can be diagnosed relatively early in cats. During clinical examination (preferably fasting), spherical dilatation of the uterine horns (Fig. 1) can be palpated where the embryos are surrounded by membranes (Fig. 2). Gestational sacs (ampullae) may be palpated from day 15 of pregnancy, but at this early stage the diagnosis is not entirely reliable, especially when the result seems to be negative. The uncertain result of the early examination may be verified later (around



Fig. 1. Spherical dilatation of the uterine horns



Fig. 2. Embryo surrounded by foetal membranes

day 25), when the gestational sacs reach a diameter of about 2.5 cm (8), but not after the day 30 when they become elongated and lose their characteristic shape. Abdominal palpation is performed with one or both hands, avoiding the knee folds (Fig. 3). This examination is reliable and easy in most cases.

Ultrasound is another option. It provides the possibility to try to diagnose the pregnancy earlier, namely from day 17 with a 5-7.5 MHz transducer, and with the use of a 10 MHz probe even from day 10, when the internal diameter of the gestational sac is about 3.5 mm and reaches 10 mm after another 10 days. The whitish embryo can be seen from about day 14 of pregnancy, but a clear C-shaped image of the embryo is revealed around day 18 (20). Early diagnosis of pregnancy is particularly indicated in cats which are prone (based on previous reproductive failure) to embryo death. It may, to some extent, help to distinguish this pathology from failure to conceive. In addition to ruling out or confirming pregnancy, ultrasound is helpful in assessing pregnancy development, foetal viability, and condition of the placenta (14, 17). It can also indicate features of embryo/foetal death, such as the



Fig. 3. Abdominal palpation

appearance of echogenic inclusions (turbidity) in amniotic fluid that is normally non-echogenic (Figure 4).

Clinical examination at a later stage allows to palpate the foetuses (palpation through abdomen wall) in the last 2 weeks of gestation. At this time X-ray might also be helpful and show mineralized skeleton of foetuses.

Another diagnostic option is the detection of relaxin, a hormone produced by the placenta in cats. Qualitative (cassette) immunochromatographic tests, also called Rapid Immunomigration Tests (RIM), have been developed for the detection of relaxin in serum or plasma. The hormone is sometimes detectable as early as on day 20, but the sensitivity (ability to detect pregnant animals) and specificity (ability to detect non-pregnant animals) reaches 100% from about day 29 (4). In practice it is recommended to use the test from day 26 after mating. In queens that for various reasons (e.g. significant restlessness, susceptibility to stress, aggressiveness) cannot be subjected to clinical examination, ultrasound or blood sampling, a urine relaxin test may be applicable. However, it is advisable to dilute it with protein-phosphate buffer (7) or serum from a non-pregnant cat. Testing of such a sample can be considered reliable from day 28 of gestation, although in some cases positive results have been obtained as early as day 21 after mating (6).

Another issue is the determination of the number of foetuses, which is important at the moment parturition. In case of multiple pregnancies, it is not always easy to determine the actual number of foetuses, neither in humans nor in animals. A spectacular example from human medicine concerns the delivery of nonuplets (a 25-year-old Mali woman who underwent a caesarean section in Morocco on May 4, 2021, at 30 weeks' gestation) when seven children were expected based on previous

examinations (media report). In cats, ultrasound and palpation may be used to count foetal ampullae. The more numerous the litter, the greater possibility of an error. Therefore, such assessment is not fully reliable. A higher accuracy (close to 100%) is offered by radiological examination performed after mineralization of foetal skeletons. Late radiographic examination, performed above the 55th day of gestation, is considered the most useful for this purpose (3).

Placenta characteristics

Feline placenta, as in other carnivores, is zonary and endotheliochorial. However, not all secretory functions are the same within this group of animals. In cats, as in dogs, placenta secretes relaxin which was mentioned before, prostaglandin F2 α and leptin; unlike in dogs, it also secretes progesterone (as an additional source of progesterone to corpus luteum) and estrogens (1, 15). The oestradiol level increases before parturition in queens.

Predicting the date of delivery

Despite the possibility of fairly accurate determination of the onset of pregnancy, from a practical point of view the moment of its termination, i.e. delivery, remains important. An interesting study was performed in cats of different breeds, aged 0.8-8.5 years, weight 3.1-7.4 kg, with a number of foetuses ranging from 1 to 7. The animals were in labour or in the period preceding it (9). Biometric parameters of the foetuses and progesterone level were measured. In particular, the values of biparietal diameter (BP), abdominal diameter (AD) and eye diameter (ED) were evaluated. BP had the highest correlation with the date of delivery but the accuracy of determining the due date with the precision of 1 day was only 27%, and with the precision of 2 days 53%. Kittens' head dimensions were related

to their body weight, which was inversely proportional to litter size. Differences were also observed between breeds and individual kittens in the same litter. Variability in fetometry can be partially avoided, as in dogs, by complying with breed standards which have been developed, for example, for Maine coon cats (16).

Progesterone during pregnancy in cats becomes detectable in the blood 2-3 days after gestation and peaks at 30-40 ng/ml after about 3 weeks and then gradually decreases. Unlike in dogs, in cats there is no rapid drop of progesterone below the threshold level before delivery. It is only afterwards that the hormone concentration decreases below 1 ng/ml (18). It is important to note that high blood progesterone levels also occur in cats that have ovulated but are not pregnant; the dioestrus phase, called pseudopregnancy in this case, is shorter than pregnancy and lasts 40-50 days. In the study mentioned above (9), the progesterone level on the day of delivery was 3.18 ± 1.68 ng/ml. Thus, neither fetal biometry nor progesterone level measurements allow to accurately predict the due date. Authors suggest using both of these tests together. Clinical observation of characteristic symptoms and the recognition of the upcoming or ongoing first stage of labour are also important.

Pregnancy loss

In cats, viral and bacterial infections as well as *Toxoplasma gondii* infections are important abortive factors (5). In case of viral diseases, the most important are species-specific pathogens namely herpesvirus (FHV-1), feline leukaemia (FeLV), panleukopenia (FPV), infectious peritonitis (FIPV), acquired immunodeficiency syndrome (FIV), and calicivirus (FCV). Embryo/foetal death can occur as a result of severe maternal illness, direct intrauterine infection with the pathogen (via the placenta) or as a consequence of placentitis and inflammatory cytokines (2). Pregnancy loss due to viral damage to the endometrium at placental attachment sites has also been suggested (19). Viruses may also be one of the causes of foetal death and mummification (11). Therefore it is required to provide proper diagnostics and immunoprophylaxis in catteries. In cases of a miscarriage, laboratory tests focused on finding the cause are indicated. In such a case, it is advisable to use internet information or to contact the chosen laboratory in order to establish the type of material to be tested (maternal and foetal samples), the time and method of its collection and the conditions of transport. It is advisable to donate the aborted foetus whole, but if the clinician dissects it to observe pathomorphological changes, the internal organs (lungs, liver, kidneys, heart), abdominal fluid and placenta, as well as samples from other macroscopically altered sites should be tested. However, de-

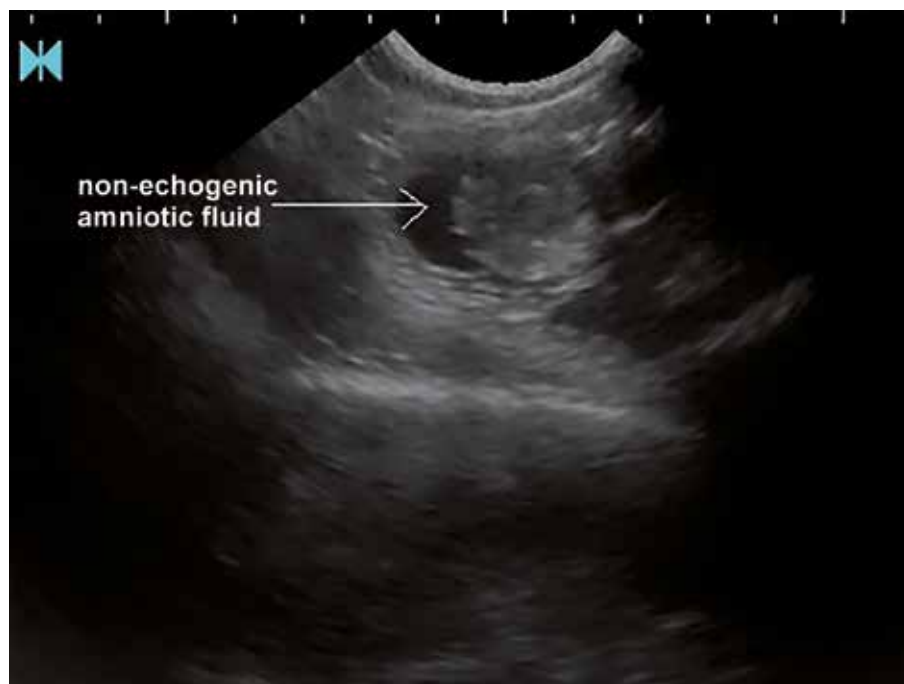


Fig. 4. Non-echogenic amniotic fluid

spite even extensive testing, the cause of a miscarriage may remain unidentified (10).

Among non-infectious causes of habitual abortions (usually at the same stage of pregnancy), hypoluteoidism should be considered. In case of its suspicion, repetitive measurements of blood progesterone levels are necessary to detect abnormal or excessive drop in progesterone levels during pregnancy. In the emergency situation, exogenous progesterone is used at a dose of 2.5 mg per animal (subcutaneously or intramuscularly) every 2-3 days until the 56th day of gestation. Alternatively, synthetic gestagens, e.g. altrenogest at a dose of 0.088 mg/kg (the same dosage as in case of dogs) can be administered orally. It should be discontinued though 2 days before the expected date of delivery.

It should also be remembered that certain drugs must be used with caution in pregnant queens because of their potential teratogenic and lethal effect. Some antibiotics (chloramphenicol, tetracyclines, gentamicin), metronidazole, griseofulvin, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, misoprostol, isotretinoin, as well as some antineoplastics and pho-

sphoorganic insecticides are known to have such effects. A risk-benefit ratio should always be considered before their application (12).

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Brucella canis Ab

Detects antibodies against *Brucella canis*
This is a test based on the immunochromatographic method (IC), used for the qualitative detection of antibodies against *Brucella canis* in whole blood, serum, or plasma of dogs.

Sensitivity: 90,3%.

Specificity: 99,9%



Application of ultrasound fetometrics in predicting the date of delivery in bitches and kittens

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Introduction

Ultrasound in canine and feline obstetrics is used primarily to diagnose physiologic and pathologic conditions of reproductive system, and in pregnancy to evaluate development and viability of embryos and fetuses, diagnose pathologic conditions, determine litter size and predict the date of delivery. In a bitch, actual pregnancy duration is dependent on endocrine factors that is: 65 ± 1 days from the LH peak (P4 $\uparrow$), 63 ± 1 days from ovulation, and 57 ± 1 days from the onset of dioestrus, although the apparent length of pregnancy depends on timing of mating and sperm survivability and can vary from 57 to 72 days. In the female cat, induced ovulation occurs and pregnancy lasts approximately 63 - 65 days after mating/LH peak (65.1 days in 90.2% of cases). However, its duration can vary from 61 to 69 days.

Ultrasound in canine and feline obstetrics is used primarily to diagnose physiologic and pathologic conditions of reproductive system, and in pregnancy to evaluate development and viability of embryos and fetuses, diagnose pathologic conditions, determine litter size and predict delivery. In a bitch, the actual pregnancy duration depends on endocrine factors that is: 65 ± 1 days from the LH peak (P4 $\uparrow$), 63 ± 1 days from ovulation, and 57 ± 1 days from the onset of dioestrus, although the apparent length of pregnancy depends on timing of mating and sperm survivability and can vary from 57 to 72 days. In the female cat, induced ovulation occurs and pregnancy lasts approximately 63 - 65 days after mating/LH peak (65.1 days in 90.2% of cases). However, its duration can vary from 61 to 69 days.

Other factors such as female size, litter size, and breed may also affect gestation length in both species. Single or twin pregnancies in dogs are associated with later parturition. With multiple litters, labour usually begins 1-2 days earlier than pregnancies with fewer puppies or kittens. The German Shepherd is a dog breed that has a shorter gestation period of about 2-3 days than the species average, while the West Highland White Terrier has a longer gestation period of about 2 days. In cats, Siamese and Oriental

breeds pregnancies last about 66 days, while Korat pregnancies last about 63 days. There are many evidence pregnancy in giant cat breeds such as the Maine Coon is several days longer than in smaller cat breeds.

Parturition date

Accurate determination of the time of delivery in dogs and cats facilitates the course of natural delivery, providing professional care for newborns. In addition, it is helps to plan a caesarean section in brachycephalic breeds, valuable bitches and kittens, pregnancies with P4 secretion disorders, pregnancies with few fetuses or after uncontrolled mating. Until now, predicting the date of parturition in the bitch has been done by regular follow-up tests of endocrine alterations during the proestrus/oestrus period (measurement of LH and progesterone), cytological inspection of vaginal smears (determination of the onset of dioestrus) or possibly ultrasound monitoring of ovulation. In female cats, on the other hand, it is based on the timing of mating. Based on the results of these tests, the date of delivery can be determined with a fairly high chance. The progress of a bitch's pregnancy can also be assessed by interpreting the size of certain foetal structures or the timing of their visibility upon ultrasound examination. It has been reported that the first gestational ampullae in dogs are already visible approx. 18 days after ovulation and heartbeat may be detected after 23 days. Other structures may be visualised slightly later: foetal stomach: 29 - 33 days, urinary bladder: 31- 35, kidneys: 42 - 43 days, and foetal movement around day 32 - 34. Based on this information, the veterinarian can make a preliminary assessment of the normal development of the pregnancy and identify pathological conditions. None of the currently known methods of predicting the date of delivery in bitches and queens is perfect. They are characterized by well-defined stages of pregnancy, some difficulties in interpretation and invasiveness (blood sampling). A method that offers the possibility of overcoming these difficulties is ultrasound fetometry. It is being more and more developed and willingly used by practitioners (different species, breeds, sizes of specimens).

Ultrasound fetometry

Ultrasound fetometry is based on measurement of selected gestational structures and body parts of fetuses and evaluation of their growth. This method is based on the assumption that the development of particular parts and organs of the fetuses during pregnancy is linear and proportional. It is possible to predict the date of delivery using appropriate charts or mathematical formulas. Ultrasound biometry is used to assess foetal size, calculating the due date and diagnosing pathological conditions. Its greatest advantages are long time of application, non-invasiveness and high efficiency. So far, measurements of various structures have been used in different animal species: BD - body diameter, CRL - crown-rump length, HD - head diameter, EVD - embryonic vesicle diameter, ICC - inner chorionic cavity, BP - biparietal diameter, DPTV - deep portion of diencephalo-telencephalic vesicle (transversal diameter of thalamus and basal nuclei primordia), stomach, eye, foetal femur. In dogs and cats, of all the fetometric parameters, ICC and BP proved to be the most practical.

In dogs, because of the wide variety of sizes and breeds, specific parameters and formulas apply according to body weight; in cats, this topic is just being developed, although in the clear direction of breed differentiation. Calculation formulas and charts have been published for all sizes of dogs and some breeds, and several formulas for cats. In addition, it has been confirmed in practice that in feline pregnancy, a fetal BP of 2.5 cm indicates full maturity.

Pregnant bitches and queens can be examined at any stage of pregnancy (from 14 to 21 days after mating until delivery). The ultrasound is performed through the abdominal wall on a standing or lying animal, after shaving. Several embryos or fetuses need to have their structure measured, ignoring abnormal embryos/foetuses. Then the average of the measurements needs to be taken and applied to a specific formula.

Statistically, both ICC and BP parameters are similarly effective. In practice, ICC is the least specific and the most versatile, sometimes giving up to 100% effectiveness with

an accuracy of ± 1 day. With the same accuracy BP effectiveness decreases from 79% in 5-6th week of pregnancy up to 51% in the last week before delivery. With an accuracy of up to 2 days, it fluctuates around 80% by 8 weeks and drops to 70% a week later. Other authors have confirmed a similar trend in BP measurements - especially between 5 and 9 weeks of gestation. It has been shown that ICC measurements are most effective in the early stages of pregnancy, up to about 28-30 days after ovulation. Some authors suggest, in order to increase the accuracy of prognosis, joint interpretation of several measurements (ICC + BP), at different periods of pregnancy, which from the point of view of veterinary practice seems to be an important information.

Determining in practice

ICC – innerchorionic cavity diameter

The structure examined during first half of pregnancy - from the implantation to about 25 - 27 days before delivery, when it is possible to conveniently visualize the gestational sacs. The value of ICC is significantly related to the stage of development of the pregnancy and its advancement. The result of a single examination is the arithmetic mean of measurements of 2 internal diameters of chorionic cavity positioned at right angles (90°). It is recommended to measure at least 2 pregnancy ampoules from two uterine horns. Measurement results are recorded in millimetres (mm). Several mathematical formulas relating to the weight of the bitch or breed and corresponding formulas for cats are available. The success rate of this method is high and usually varies from about 80% to 90% with an accuracy of 1 day, guaranteeing in clinical practice reliable operations and safe procedures e.g. for caesarean sections. The relatively easiness to perform the necessary imaging and measurements decides about their high efficacy. According to the scientific reports litter size and foetal sex do not affect the accuracy of determining the date of delivery using this parameter, making it even more versatile.

BP - biparietal diameter

It's the most commonly used index during the second half of pregnancy (<25 days before delivery). During this period, the cranial bones are progressively calcified and with each day the parietal bones become more visible via ultrasound. When measuring BP, it is important that the foetal head is properly positioned in the sagittal projection. Next, the distance between parietal bones is measured. The arithmetic mean of the measurements of at least two fetuses from opposite uterine corners is then calculated. The result is presented in millimetres (mm). Again, as with the ICC, formulas for groups of dogs and cats of a certain weight

Table 1: Selected fetometric formulas. (DTD - days to delivery, mm - result in millimetres)

	ICC	BP
Dog (≤ 5 kg)	DBP = $(0,63 \times \text{mm}) - 44,04$	DBP = $(1,62 \times \text{mm}) - 39,70$
Dog (≤ 10 kg)	DBP = $(\text{mm} - 68.68)/1.53$	DBP = $(\text{mm} - 25.11)/0.61$
Dog (11-25 kg)	DBP = $(\text{mm} - 82.13)/1.8$	DBP = $(\text{mm} - 29.18)/0.7$
Dog (26-40 kg)	DBP = $(\text{mm} - 105.1)/2.5$	DBP = $(\text{mm} - 30)/0.8$
Dog (> 40 kg)	DBP = $(\text{mm} - 88.1)/1.9$ lub $(\text{mm} - 79.88)/1.71$	DBP = $(\text{mm} - 29)/0.7$ lub $(\text{mm} - 29.06)/0.67$
Maltese	DBP = $63.2 - (18.58 + 0.71 \times \text{mm})$	DBP = $63.2 - (24.7 + 1.54 \times \text{mm})$
Yorkshire Terrier	DBP = $63.4 - (18.92 + 0.65 \times \text{mm})$	DBP = $63.4 - (23.89 + 1.63 \times \text{mm})$
German shepherd	DBP = $44.76 - (4.34 \times \text{cm})$	DBP = $38.65 - (12.86 \times \text{cm})$
Cat	DBP = $(\text{mm} - 62.03)/1.1$	DBP = $(\text{mm} - 23.39)/0.47$
Maine Coon	DBP = $(\text{mm} \times 0.79) - 57.9$	DBP = $(\text{mm} \times 1.86) - 49.3$

Examples of calculations using ICC and BP formulas:

1) Bitch, 8kg, ultrasound - 04.01.2008, average ICC - 13.10 mm
 $(13.10 - 68.68)/1.53 = - 36.33$ days (36 days to delivery)
 expected date of delivery: 09.02.2008, it took place on 09.02.2008

2) Bitch, 22kg, ultrasound - 29.07.2008, mean BP - 13.82 mm
 $(13.82 - 29.18)/0.7 = - 21.94$ days (22 days to delivery)
 expected date of delivery: 20.08.2008, delivery took place on 21.08.2008

or breed-specific formulas apply. Although the predictive performance of cranial measurements is generally lower than that of gestational ampullae, it is still very helpful in obstetric practice. It has been suggested that breed-specific cranial characteristics and growth rate of individual animals may determine the accuracy of results using this parameter, but the sex of the puppies or kittens are irrelevant to the prognosis.

Conclusion

The effectiveness of fetometrics is not inferior to traditional methods of determining the date of delivery, giving the advantages like simplicity of performance, possibility to use at any point or providing additional information. There is a strong correlation between the stage of pregnancy and the size of ICC and BP. The efficacy of both parameters is also not significantly different between experimental groups of animals. The specific patterns determined by growth curves may have practical applications in determining the timing of parturition in dogs and cats of different weights or specific breeds. Research is needed on further groups of companion animals such as brachycephalic breeds of dogs or further breeds of cats.

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Fig. 1. ICC measurement



Fig. 1. BP measurement



Fig. 2. ICC measurement in pregnant Maine Coon 45 days before delivery



Fig. 3. BP measurement in pregnant Maine Coon 24 days before delivery



Fig. 4. ICC measurement in pregnant Border Collie 39 days before delivery



Fig. 5. BP measure in pregnant Border Collie 5 days before delivery

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Mammary gland tumours in bitches

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Abstract

Canine mammary gland tumours (CMTs) are the most commonly diagnosed tumours in bitches and represent nearly half of all the cancers diagnosed in bitches [1]. According to some studies, the risk of disease reaches 53.3% [1]. Additionally, it is estimated that the incidence of mammary gland cancer in bitches is three times higher than that of breast cancer in women [2]. Considering that more than 50% of them are malignant and, in turn, half of them have already given metastasis at the time of diagnosis, they represent a significant clinical problem [1-5]. They usually occur in older bitches aged 8-10 years and mostly affect intact or spayed bitches at an older age [1-5]. It is estimated that the risk of mammary gland tumours in intact bitches is 7 times higher than in spayed bitches.

Aetiology

The sex hormones, oestrogen and progesterone, are believed to have a major role in the aetiology of mammary gland tumours. It has been shown that the use of hormonal drugs: oestrogens and progestogens can increase the risk of these tumours. Indeed, the vast majority of mammary gland tumours show expression of progesterone and oestrogen receptors. Therefore, it is still believed that early spaying can effectively prevent their occurrence in older bitches [1-4]. In a frequently quoted study, it was shown that spaying performed before the first heat, after the first heat, or at a later time is correlated with the risk of mammary tumour equal to 0.5 %; 8% and 26% [1]. However, the supporting results are based on a retrospective study and there is no solid evidence to support this theory. In another research, spaying has been shown to reduce the risk of new tumours development in bitches with benign mammary tumours after mastectomy and should therefore be performed simultaneously with this surgery [5], while in the case of malignant tumours such relationship was not reported [6]. However, there have also been several studies related to the lack of effect of spaying on the development of mammary gland tumours in bitches [7,8].

Another important risk factor is breed. The risk varies geographically, but several studies have shown that English cocker spaniels, English springer spaniels, dachshunds, poodles, German shepherds,

Yorkshire terriers, Maltese, Pointers and Irish setters are the most predisposed breeds [1,2]. The increased risk in these breeds may indicate a possible genetic background of the disease. So far, however, studies have not shown the presence of a common genetic mutation, although some studies have identified mutations in the p53 gene, HER2, BRCA1 or BRCA2 mutations.

It is also known that an important risk factor, as in most diseases, is obesity. It should be also kept in mind that adipose tissue is an important source of oestrogen, due to the activity of aromatase, which converts androgens into oestrogens. Thus, excess adipose tissue causes excessive oestrogen to affect the mammary gland, and thus may be a promoter of oncogenesis. One study has shown that the risk of mammary gland tumour is lower in bitches that were thin at 9-12 months of age. Also, the presence of obesity at the age of 1 year or one year before development of the disease is considered an increased risk [2].

Histopathological types of mammary gland tumours

Tumours of the mammary glands of bitches are characterized by varied morphology. We distinguish between simple tumours (ductal epithelial or myoepithelial cells), complex tumours (ductal epithelial and myoepithelial cells), mixed tumours (ductal epithelial and/or myoepithelial cells and bone/chondral metaplastic tissue) as well as mesenchymal tumours. The lesions seen in

Table 1. Histopathologic types of mammary gland tumours in bitches based on Goldschmidt et al. 2011.

Malignant		Benign	
1. Simple carcinoma*	Adenomatous carcinoma	1. Adenoma*	Simple adenoma
	Adenocarcinoma *		Intraductal papillary adenoma
	Solid carcinoma		Complex adenoma
	Anaplastic carcinoma		Basaloid adenoma
2. In situ carcinoma		2. Fibroadenoma*	
3. Complex carcinoma*		3. Benign mixed tumour*	
4. Special types of carcinoma	Spindle cell carcinoma	4. Duct papilloma	
	Squamous cell carcinoma	5. Myoepithelioma	
	Mucinous carcinoma	Mammary hyperplasia and dysplasia	
	Lipid-rich carcinoma		
	Inflammatory carcinoma		
5. Carcinoma arising in a mixed tumour		Ductal hyperplasia	
6. Carcinosarcoma		Lobular hyperplasia	
7. Sarcoma	Fibrosarcoma	Cysts	
	Osteosarcoma	Duct ectasia	
	Other sarcomas	Fibroadenoma-like lesion	
8. Comedocarcinoma		Gynecomastia	

*The most common types of tumour

patients are often multiple and it is not uncommon to have different types of tumours within the same or other glands. This makes it even more difficult to finally diagnose, select the appropriate treatment and determine the probable prognosis.

Histological types of mammary gland tumours were developed by The World Health Organization (WHO). The International Histological Classification of Mammary Tumours was established for the first time in 1974 and then modified in 1999. However, nowadays it is recommended to apply the classification developed by Goldshmidt et al. in 2011 (Table 1). Among malignant lesions, adenocarcinomas, papillary carcinomas, solid carcinomas, and complex carcinomas are the most common types [1,9]. Benign tumours of the mammary gland are mainly fibroadenomas, ductal papillomas, benign mixed tumours, and adenomas [1,9].

Generally, larger lesions are considered malignant, but there are also malignant lesions smaller than 1 cm in diameter, which emphasizes the importance of early diagnosis and removal of even the smallest lesions. Clinical features of mammary gland malignancy also include rapid tumour growth, large tumour mass, presence of ulceration or necrosis on the surface of the lesion, lack of mobility in relation to surrounding tissues, and enlargement of regional lymph nodes.

Clinical staging is also performed (TNM system) and it includes evaluation of the size of the primary tumour (T-tumour); regional lymph nodes (N-noduli) and the presence of metastasis (M- metastasis) (Table 2) [10,11].

Table 2. Clinical staging of canine mammary tumours according to TNM system based on WHO guidelines. (Owen,1980)

T- primary tumour			
T ₁	Maximum diameter < 3 cm		
T ₂	Maximum diameter 3-5 cm		
T ₃	Maximum diameter >5 cm		
N- regional lymph nodes			
N ₀	No metastasis		
N ₁	Metastasis present		
Metastasis present			
M ₀	No distant metastasis detected		
M ₁	Distant metastasis detected		
Stages			
1	T ₁	N ₀	M ₀
2	T ₂	N ₀	M ₀
3	T ₃	N ₀	M ₀
4	T ₁ T ₂ or T ₃	N ₁	M ₀
5	T ₁ T ₂ or T ₃	N ₀ or N ₁	M ₁

Among malignant tumours, 3 grades of malignancy are distinguished: Grade I - low degree of histological malignancy (well-differentiated tumour cells); Grade II - moderate degree of histological malignancy (moderately differentiated tumour cells); Grade III - high degree of histological malignancy (poorly differentiated tumour cells). The degree of malignancy depends not only on the type of tumour, but also on the presence of significant pleomorphism of nuclei and cells, mitotic index, presence of necrotic areas, peritumour infiltration and presence of metastasis to regional lymph nodes [9].

Diagnosis

Bitches at the time of diagnosis of mammary gland tumour usually show no alarming clinical signs other than enlargement of the mammary gland. Glandular lesions are found incidentally by owners or during a visit to the veterinary clinic. Depending on the time elapsed from the onset of tumour growth to diagnosis and the nature of the lesion, the clinical presentation of mammary gland tumours in bitches may vary (images 1-3). It is clinically important to estimate the rate of tumour growth, the exact size at the time of diagnosis, the consistency of the tumour and its structure, its mobility in relation to the base, the presence of ulcerations and lesions on the skin surface as well as the enlargement of the surrounding lymph nodes.

So far, there have been no screening tests available to detect the disease before clinical symptoms appear (presence of a tumour), although more and more researches are being conducted on the possibility of implementing various tumour biomarkers (chemical substances detectable in blood and other tissues, e.g. tumour tissue, whose levels change during ongoing neoplastic processes) in the early laboratory diagnosis of CMT. Such biomarkers include: Ki-67, VEGF, E-cadherin, COX-2 or still less known CEA, MUC-1 and numerous miRNAs.

When mammary gland lesions are clinically identified, performance of fine needle aspiration biopsy from the lesion is recommended to identify the histopathologic type of the lesion. Studies have shown that the result of such cytologic examination is highly compatible with the result of histopathologic examination after removal of the lesion and ranges from 81-93% depending on the type of lesion [12]. Obtaining the result of the cytologic examination is very useful for proper planning of the surgical procedure (determining the type of procedure to be performed and establishing the surgical incision margin).

Before proceeding with the treatment, in addition to a detailed history and clinical examination, it is necessary to perform

basic blood tests, chest X-ray (3 projections: left-lateral, right-lateral and dorsal-abdominal) - to exclude macroscopically visible lung metastases, as well as, in the case of older bitches, echocardiography is recommended. Occasionally, a chest CT scan may also be necessary if microscopic lung metastases are suspected.

Treatment

The treatment of choice for mammary gland tumours in bitches is their surgical removal - mastectomy. Different surgical techniques may be used depending on the extent and nature of the lesion and the lymphatic vasculature. The lymphatic system contributes to metastasis and therefore lymphatic connections between mammary glands must be taken into consideration during mastectomy. The axillary lymph nodes supply glands 1, 2, and partially 3; the lymph flows directly from gland 1 to the axillary lymph node and has no lymphatic connections to the other glands (Image 4). The situation is similar for the inguinal lymph nodes, which supply 3, 4 and 5th pair.

In the case of very small nodules, up to 0.5 cm in diameter, nodulectomy may be performed - removal of the tumour, but only if it's well demarcated and there are no other changes in other mammary glands. If cytology confirms that the lesion is benign, removal of the affected mammary gland alone - a mamnectomy - may be considered. On the other hand, partial mastectomy is a procedure that involves the removal of several glands that share a common network of lymphatic vessels within a single milk line. Thus, it is recommended to remove glands 1 and 2 (and sometimes 3) or 3, 4, and 5 at the same time. Currently, however, if any lesion is found within a milk line, unilateral mastectomy is recommended - that is, removal of all the mammary glands of one milk strip due to the possibility of lymphatic metastasis to the remaining glands. If tumours are found in both milk lines, total mastectomy (removal of both milk lines at the same time) may be an option, but it carries an increased risk of wound dehiscence, prolonged healing and recovery (Image 5).

For malignant lesions with metastases to lymph nodes and in advanced stages of the disease, chemotherapy is recommended as an auxiliary treatment (or sometimes, if surgery is not possible it is the only treatment). There are many chemotherapy protocols and chemotherapy should always be tailored individually. The most common cytostatic agents chosen to treat CMT are doxorubicin, cyclophosphamide, fluorouracil or carboplatin. A combination therapy with more than one chemotherapeutic drug is recommended because it is more effective.



Fig. 1. Neoplasm of mammary gland with diameter of 1 cm in female rough-haired dachshund. (photo: Ilona Kaszak)



Fig. 2. Mammary gland tumour with skin hyperpigmentation in a female Labrador retriever. (photo: Ilona Kaszak)



Fig. 3. Neoplasm of the mammary gland at an advanced stage. A large area of wet gangrene is visible. (photo: Ilona Kaszak)

Radiation also gives very good results. Radiotherapy involves ionizing radiation. Usually a cycle of 3-6 exposures is performed. However, this is a very expensive therapy and not all pet owners decide for it.

Due to the presence of oestrogen and progesterone receptors in the mammary gland tissue in dogs and cats, a hormone therapy may also be considered. It is especially recommended for benign tumours. The most common is tamoxifen 0.4 mg/kg/day p.o. for 4 to 8 weeks (an anti-oestrogen drug). On the other hand, using aglepristone 20 mg/kg s.c. as a single dose or 1, 2, and 7 and 8 days before surgery, a reduction in tumour size and progesterone receptor numbers was observed.

Also, nonsteroidal anti-inflammatory drugs, mainly cyclooxygenase 2 inhibitors are used to control inflammation and pain, but they also show potential anti-tumour effects.

Prognosis

The disease has a rapid course very sporadically. It progresses slowly and the average survival time is estimated to be 2 years, although for very malignant tumours this time may be only 2 months. The best prognosis is, of course, for noncancerous growths and benign tumours. The prognosis depends not only on the histopathologic type of the tumour and the degree of malignancy, but also on the age of the patient, size of the tumour, stage of the disease at which the it was diagnosed, the applied treatment and the presence of coexisting diseases. Most metastases occur within a year after mastectomy. The most common metastatic organs are the lungs, liver, kidneys and bones. A patient with metastatic disease is usually in poor condition, with apparent weight loss, muscle atrophy, weakness, and often polydipsia and polyuria (Image 6). However, the most common cause of death is cardiopulmonary failure due to the presence of tumour metastases in the lungs.

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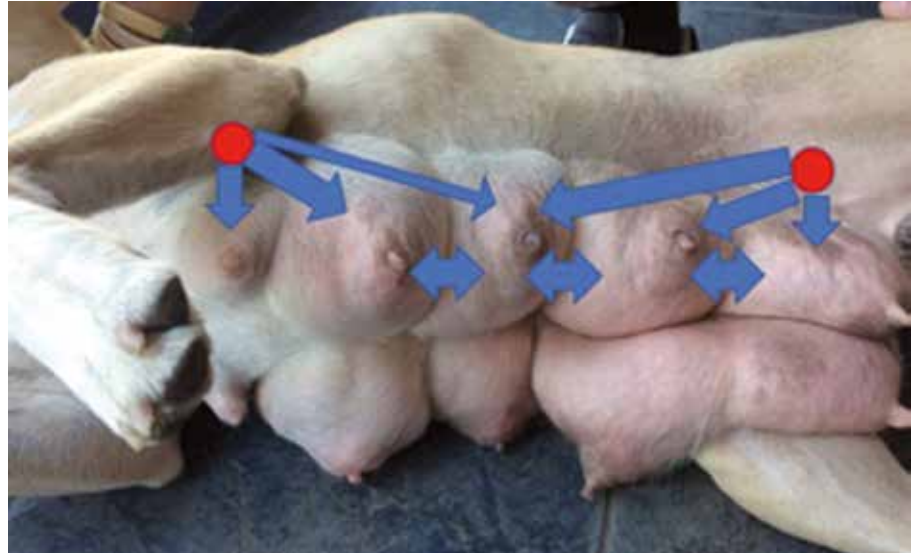


Fig. 6. Cachexia and muscle atrophy in a bitch with advanced disease. (photo: Ilona Kaszak)



Fig. 5. Wound healing process after mastectomy. Visible purulent exudate and dehiscence of the wound edges. (photo: Ilona Kaszak)



Fig. 6. Visualization of the lymphatic drainage routes in normal canine mammary glands. (photo: Ilona Kaszak)

Polycystic ovarian syndrome in domestic cavies

Małgorzata Ponikowska DVM, specialist in non-domesticated animals



One of the most common pathologies of the reproductive system in domestic cavies (guinea pig, *Cavia porcellus*) is polycystic ovary syndrome (syndrome ovariorum polycysticum, PCOS). Despite its common occurrence, it is often overlooked, diagnosed accidentally or too late. Guinea pigs usually do not show the symptoms until the late stages of the disease, and the symptoms are nonspecific and vary depending on the type of cysts. Treatment and prevention consists primarily of ovariectomy, preferably at a young age when such surgery may be performed as an elective procedure and is safe. A very important aspect here is to raise awareness among owners, discourage pseudo-breeding practice, and dispel myths about health-promoting effects of pregnancy and the high mortality rate of cavies during anaesthesia.

Three types of ovarian cysts are encountered in domestic cavies. The first and most common, ovarian omentum cysts. Less common are follicular cysts, occurring according to different researchers in 0-22.4% of cases, and occasionally periovarian cysts are diagnosed. Few researches showed the occurrence of neoplastic lesions on the ovary (6).

The problem most often affects both ovaries; one-sided malformations are less common. Polycystic ovary syndrome affects older animals, mainly after the age of three years. Studies conducted on several groups of domestic cavies showed the presence of pathology in 66% of cavies aged between three months and five years (1), 75% of cavies between three months and five years (2), 76% of cavies between eighteen and sixty months (3), 93% of cavies older than two years. The prevalence of polycystic ovaries varies according to the study group selected and the designated cut-off values for follicle size which is considered pathological. In all available studies, pathologies were found in more than 50% of the females studied. Normal ovarian follicles do not exceed 0.7mm in diameter (7). Cysts can range in diameter from 0.5cm to 7cm, occurring as individual lesions or multicystic aggregates (Fig.1) (3). Larger cysts are more common in older animals, and there was no relationship between the size of the cysts and reproductive history (7). There was also no relationship between duration of the heat or prevalence and cyst occurrence (6). The causes have not yet been clearly established; possible factors include omentum epithelial cell proliferation, ion pump dysfunction, omentum

epithelial permeability, and provisions of phytoestrogens in food (6).

Polycystic ovarian syndrome in domestic cavies is often asymptomatic and may not be detected until the routine clinical examination. The occurrence of symptoms varies depending on the type of a cyst in an individual. The most common symptom of hormonally active cysts noticed by owners is symmetrical alopecia, occurring without pruritus or skin lesions, starting on the sides of the abdomen and spreading to the thighs, entire abdomen, and sometimes even on the back (Figure 2). Additionally, skin lesions may appear around the nipples, where the skin often shows excessive keratinization (3,6). A change in behaviour, agitation, or aggression may be noted, especially when steroid-secreting follicular cysts are present (6). Rarely, vulvar swelling or clitoral hyperplasia may be observed. Ovarian lesions

may be accompanied by other pathologies of the reproductive system, and the frequency of their occurrence is shown in the table (Table 1). Due to the prevalence of ovarian cysts, a possible lack of correlation should be taken into account (6).

As the cyst grows, the body of a guinea pig becomes more pear-shaped and an increase in body weight maybe observed. In case of larger cysts there is a mass effect which is more typical of the ovarian omentum cysts. Compression on abdominal organs, mainly the intestines, can cause nonspecific symptoms such as a change in stool consistency or absence of stool, lack of appetite, and apathy. The abdominal cavity is tense, significantly filled but usually not painful.

Diagnosis is based on clinical and imaging examination. Larger ovarian cysts may be palpated during abdominal examination, either on one or both sides of the body, posteriorly to the kidneys. They are round, firm, non-painful structures, often with an irregular surface that are susceptible to pressure. To measure their size, an abdominal ultrasound is performed in which the cysts are clearly visible, filled with anechoic fluid, and often multilocular (5). Radiological examination does not allow for clear visualization of the cysts which are difficult to differentiate from nodular lesions, so it is not an examination mode of choice (6). Testing of blood hormone levels shows a limited usefulness.

Table 1. Prevalence of other reproductive system pathologies in guinea pigs (3)

Pathology	Prevalence in guinea pigs diagnosed with ovarian cysts	Prevalence in guinea pigs without ovarian pathologies
Uterine myomas	6/54	0/17
Cystic endometrial hyperplasia	7/54	0/17
Endometritis	3/54	1/17
Mucometra	2/54	0/17
Placental tissue pathology	3/54	0/17



Fig.1. The uterus and ovaries with cystic changes after ovariectomy



Fig.2. Skin lesions associated with polycystic ovarian syndrome in domestic cavies



Fig.3. Blood collection in a guinea pig

The treatment of choice is ovariectomy. The procedure should be performed in line with general aseptic principles and under general anaesthesia. A complete clinical examination and a basic biochemical blood test are necessary to qualify the patient for the procedure (Fig. 3). Reference values for biochemical tests are shown in the table (Tables 2). The patient preparation consists of discontinuation of bloating products for one day before the procedure, domestic cavies should have access to hay and water until the very moment of premedication, their gastrointestinal tract will never be emptied. The anaesthetic protocol should be selected according to the general condition of the patient, the technical capabilities of the clinic and technical skills and theoretical knowledge of the anaesthesiologist. Ovariectomy in domestic cavies can be performed through white line incision or a lateral incision. Postoperative care includes

Table 2. Reference values of biochemical test results for guinea pigs (8)

Parameter	Values
ALT	10-25 U/l
Bilirubin	0,3-0,9 mg/dl
Triglycerides	0-145 mg/dl
Cholesterol	20-43 mg/dl
BUN - blood urea nitrogen	9-32 mg/dl
Creatinine	0,6-2,2 mg/dl
Ca	7,8-10,5 mg/dl
P	5,3 mg/dl
Total protein	4,6-6,2 g/dl
Albumin	2,1-3,9 g/dl
Globulin	1,7-2,6 g/dl
Glucose	60-125 mg/dl

maintenance of adequate body temperature, observation of appetite, and administration of analgesics. Dosages of the analgesics which are safe for this species are shown in the table (Table 3). Antibiotic therapy is rarely indicated after sterile surgery; if necessary, an antibiotic which is safe for this species should be chosen. The return of peristalsis after the surgery is critical for rapid recovery. If peristalsis has been impaired, prokinetic drugs such as metoclopramide 0.5-1 mg/kg or cisapride 0.1-1 mg/kg may be administered and forced feeding is helpful (8). Domestic cavies are rarely interested in their sutures, and postoperative clothing or collars are not required. It is important to provide a clean, dry pad because of the close contact of the abdomen with the floor. The possible complications after ovariectomy include hematomas, suture material reactions, nonspecific symptoms related to intestinal decompression, decreased blood pressure, abnormal response to anaesthesia, and peritonitis (6).

Alternative therapies may include the use of hormonal drugs. It is possible to use human chorionic gonadotropin at 1000UI in intramuscular administration every 7-10 days (4). GnRH can be administered twice at the dosage of 25µg/kg, 2 weeks apart. Both of

Table 3. Dosages of selected analgesics for guinea pig (8)

Active substance	Dosage
Tolfenamic acid	2 mg/kg
Meloxicam	0,1-0,3 mg/kg
Metamizole	20-50 mg/kg
Tramadol	5 mg/kg
Buprenorphine	0,05 mg/kg
Butorphanol	1-2 mg/kg

these therapies will have no effect on cysts originating from the ovarian omentum. As this is the most common type of cyst in domestic cavies, hormonal therapy should be considered impractical.

If performance of the surgery is impossible and the ovarian cysts are severely enlarged, ultrasound-guided puncture and aspiration of the fluid may be performed (4). This method gives relief to patients with symptoms related to the size of the cysts and their compression on surrounding tissues. Relapse of the problem and re-enlargement of the cysts, even after a few days is probable (6). Possible complications of this procedure include ovarian bleeding and peritonitis.

In order to prevent the prevalence of polycystic ovary syndrome in guinea pigs, young animals should be subjected to ovariectomy. This procedure is still not popular in Poland, but the owners of guinea pigs should be informed about its benefits for health and life of their pets.

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Progesterone - not just a canine hormone. Indications and options for testing progesterone levels in cats

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Evaluation of progesterone levels is almost an integral part of canine reproduction management. But only few veterinarians and animal breeders find this hormone equally useful in feline reproduction. In the following article, I will attempt to provide some insight into several options for assessing progesterone levels beyond its standard use.

Introduction

Progesterone level testing is the most common test performed in breeding dogs and very rarely in cats. It should be kept in mind, however, that these two animal species undergo different reproductive cycles and the results should be interpreted differently. More importantly, the indications for progesterone level measurement in dogs and cats are significantly different.

Progesterone testing in dogs - a summary of the most important information

Testing serum, or less commonly, plasma progesterone levels are the most commonly performed tests in canine reproduction. Their primary goal is to establish the optimal time of mating. Determination of the moment of LH peak and ovulation allows to specify the optimal fertility period and insemination time. When choosing the breeding day we need to consider the method of mating, e.g. natural mating, insemination with fresh semen collected in the presence of the bitch, insemination with cooled or frozen semen.

When organizing and planning mating, it is particularly important to be able to perform a progesterone level measurement in the clinic and obtain the result within minutes. This allows to take actions more quickly, especially when the owner doesn't know when the heat has started. This happens especially in a situation where the owner has several dogs staying outside. This sometimes results in elevated hormone values during one of the first tests. In such situations it is very helpful to get a result in a short period of time, and not the next day or after several hours.

It is also worth remembering how valuable it is to determine the optimal time of mating i.e. ovulation. It also gives the possibility of specifying the precise delivery date. It is considered that the time of birth from ovulation occurs on day 63, with a tolerance of ± 1 day. For comparison, the gestation period, counted from the day of mating is 63 days $\pm 5-7$ days (i.e. the gestation period may be 57-70 days). If the moment of ovulation is not determined and no ultrasound fetometry is performed, there is a risk of incorrect estimation of the pregnancy length and expecting the delivery earlier than it should be. This may look like a prolonged pregnancy or a preterm births and mistaken for miscarriages.

When managing problematic dog pregnancies, it is still very helpful to measure progesterone. Further evaluations of progesterone levels are performed to rule out corpus luteum hypoplasia and to start pregnancy support therapy if necessary.

The last period when it is worthwhile to check progesterone levels again is during the perinatal period, because a drop in progesterone levels below 1-2ng/ml is commonly observed 24-48 hours before delivery. Its detection may be useful in deciding whether to perform a caesarean section and in assessing the actual duration of pregnancy when the owner has doubts in terms of a due date (the phenomenon of apparent prolonged gestation).

It is interesting to measure progesterone levels after performance of a scheduled caesarean section when the date of the surgery was established based on the mating time, without any symptoms of labour and or lactation. Showing persistent progesterone levels at this stage may suggest a premature procedure and guide us on the treatment aimed at abolishing the influence of progesterone on the bitch's organism. This should

stimulate the lactation and lead to opening the cervix and proper cleaning of the uterus after birth.

Introducing progesterone measurements considering specific cycle of cats

In case of cats we should begin with the expression "cat is not a small dog". This expression is particularly true when comparing the cycles of these two animal species. Therefore, certain determinations of sex hormone levels should be selected and interpreted differently.

Felines show signs of reproductive activity in seasons – they are seasonally polyoestrous. It means that during mating season (usually January to October) the symptoms of heat may be repeatedly observed. Their reproductive activity is correlated with the daylight duration. We should bear in mind that the vast majority of farm cats are kept in confinement in the home and are exposed to artificial light on a rather constant basis than free-ranging cats. Thus indoor cats come into heat even during the winter season.

Possible cycle course:

1. End of winter/early spring $\rightarrow$ heat $\rightarrow$ interoestrus $\rightarrow$ heat $\rightarrow$ interoestrus $\rightarrow$ heat $\rightarrow$ interoestrus $\rightarrow$ heat $\rightarrow$ $\rightarrow$ anoestrus during short daylight period (winter)
2. Heat $\rightarrow$ successful mating $\rightarrow$ pregnancy $\rightarrow$ lactation $\rightarrow$ next heat
3. Heat $\rightarrow$ ineffective mating (but effective ovulation) $\rightarrow$ false (phantom) pregnancy $\rightarrow$ next heat
4. Heat $\rightarrow$ ineffective mating (no ovulation) $\rightarrow$ interoestrus $\rightarrow$ next heat

The first situation described occurs when the queen, along with increasing daylight, regularly comes into oestrus which usually lasts 4-7 days and occurs at intervals of 1-2 weeks. At the end of the autumn, as the daylight duration shortens, free-ranging cats start the period of sexual rest i.e. winter anoestrus.

The second situation described occurs when a successful mating takes place during one



Fig. ICC measurement



Fig. 2. Example of photometric measurement of fetal head structures

of the oestrus periods. The female becomes pregnant for about 65 days (60-72 days) (8). This is followed by parturition and lactation, which suppresses the ovaries only for a short period of time. It is not uncommon for oestrus to occur as early as the 2nd-3rd week of lactation. Moreover, some of these cycles may result in another pregnancy if mating is not controlled.

So in conclusion the queen may fall pregnant again while still nurturing the previous litter.

The third situation mentioned takes place during the physiological heat when mating is unsuccessful i.e. when a male is infertile or simply when fertilized gametes fail to develop. Oestrus symptoms then disappear. However, this period lasts about 30-45 days and is shorter than the real pregnancy. It

should be emphasized that false pregnancy in bitches, and more specifically the associated drop in progesterone levels, lasts longer than real pregnancy.

The fourth described situation occurs when a female cat is in heat and mated, usually expressing only weak behavioural signs. In the absence of adequate stimulation of the vaginal vestibule there is no hormone release and thus no ovulation. After a normal period of interoestrus, the queen will come into the next oestrus.

One might ask why progesterone should be tested in queens if for years it was possible to proceed without it. The answer is simple: "to know more ...", to establish if there was a successful mating, if male and female can be separated, if there is hope for successful pregnancy, to be able to guide breeders more

successfully. On the other hand one may say that there is no problem since the oestrus in the season occurs so often. However, it's a false assumption because catteries are being run more and more efficiently, and therefore owners care about good effects of mating - sometimes in a very limited time frame during which a male and a female stay together.

Unlike during the oestrus in bitches, it is not when we perform hormone measurements in cats. In bitches there is a pre-ovulatory rise in progesterone levels independent of mating or the presence of a male.

Ovulation in queens usually occur 1-1.5 days after mating (1). This is called an induced ovulation. However, in recent years it has been reported that some females ovulate spontaneously without mating (1).

An indirect confirmation of successful mating is an increase in blood progesterone levels. Effective mating leads to ovulation, i.e. the oocytes have been released and there is a chance of fertilization and pregnancy.

It is worth noting that an increase in progesterone level in this test (performed 5-7 days after mating) is not a confirmation of pregnancy, but only an indication that pregnancy is likely, because mating was long enough to trigger a hormonal response and ovulation in the queen. Now there are two possible outcomes:

- Pregnancy
- Phantom pregnancy

We can monitor the levels of this hormone every few days; in the case of a real pregnancy the values will gradually rise, especially after starting the production of placental progesterone. In the case of false pregnancy, on the other hand, these values usually drop significantly after 2-3 weeks.

It is important to analyse the results in relation to the history, the clinical condition of the queen and the results of ultrasound examination. If pregnancy is confirmed by ultrasound but progesterone levels are falling, we may suspect abnormal progesterone production, indicating pregnancy pathology. However, detection of this decrease in progesterone allows us to take measures which are essential to support pregnancy development.

The fact that ovulation has been confirmed is sometimes useful in situations when a queen has been staying with a male cat far from her home for a long time. For example when the owners of a tomcat wonder if cats can be separated already believing that the successful mating occurred since estrus symptoms have ended. It is also possible then that oestrus has come to an end without mating and in a week or the queen will come into heat again. Therefore this information is very useful in terms of the

organization of mating, especially when a female and male live far from each other.

The progesterone testing after the time spent is also a source of valuable information in the case of repeated unsuccessful mating.

Reproductive problems may be suspected in case when the queen has already been with the male many times and even though the mating has been seen there is no pregnancy. Then measurement of progesterone level allows to find out if ovulation has taken place. If not, there are usually two solutions

- changing a male cat to induce ovulation ("more temperamental")
- pharmacological induction of ovulation :
 - as far as mating occurs with the deposition of sperm in the vagina
 - artificial insemination

What is the purpose of progesterone testing in cats :

- Confirmation of ovulation = effective mating (1)
- Exclusion of unwanted mating = no increase in progesterone = no ovulation (1)
- Confirmation of adequate level for pregnancy maintenance
- Detection of corpus luteum insufficiency
- Detection of habitual spontaneous ovulation (7)
- Detection of lutein cysts or hormonally active tumors (7)

Importantly, there is one very significant difference in the perinatal period between dogs and cats. Namely, as mentioned in the first part of the article related to bitches, progesterone levels drop to the baseline level 1-2 days before delivery. This effect is not observed in cats, making it unreasonable to test progesterone level to assess a queen's "readiness" for parturition (4, 5).

This difference is associated with the fact that the only source of progesterone during pregnancy in the bitch is ovarian corpus luteum. In the second half of pregnancy in cats, , placentae of the developing kittens are becoming, beside the ovaries, a very important source of progesterone (6). Thus, the final drop in progesterone does not occur until after parturition and expulsion of all placentas.

Examples from daily clinical practice

1. a queen with repeated unsuccessful mating which rapidly goes into heat within 1-2 weeks.

The progesterone level was 1.08 nmol/l = 0.34 ng/ml 7 days after mating. At this stage we can already conclude that the mating did not lead to ovulation.

2. A female cat with repeated failed ovulations, intervals between ovulations longer than 3-4 weeks

The progesterone level was 10.3 ng/ml on 7th day after mating which indicates ovulation and the formation of corpus luteum.

3. Progesterone testing in a queen after changing a tomcat. Previous mating had been ineffective. However, this time the progesterone test on the 7th day after mating gave a result of 9.72ng/ml (Vcheck) using the conversion factor $\times 0.7 = 6.804$ ng/ml. This confirms ovulation. Moreover a repeated test 6 days showed a further increase in progesterone of 20.87 ng/ml (Vcheck), which after conversion $\times 0.7$ gives 14.609 ng/ml. On the 25th day after the mating we confirmed a normal multiple pregnancy with ultrasound.

An interesting situation is fibroadenomatosis which is a hormonal, non-inflammatory and non-cancerous progesterone-dependent proliferation of the mammary gland in females. It is usually associated with hormonal contraception in cats, but it may also be a consequence of endogenous progesterone increase, for example following spontaneous ovulation (3). This can be confirmed by elevated serum progesterone levels.

Summary

For many years, measurement of blood progesterone levels have been the main diagnostic tool for canine reproduction, and in recent years, the availability of progesterone tests has increased due to the further development of the equipment used in veterinary clinics. Of course, the wide availability of different measurement tools and methods is associated with the fact that even from the same blood sample significantly different values may be obtained depending on the applied method. However, knowing how to interpret results obtained with different devices enables quicker diagnosis of pregnancy disorders or problems with mating in the case of bitches. Progesterone testing

is an interesting method that will certainly become more and more adjusted for the application in cats as catteries are becoming increasingly popular. But most of all, the breeders are willing to cooperate in order to improve reproductive capabilities.

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Product description in Vet Pharmacy

Stud dog – basic algorithms of assessing breeding value

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The term 'breeding soundness examination' is used in Poland in relation to livestock, but not so commonly used in the context of companion animals, such as dogs or cats. In English literature and practice, this term 'breeding soundness examination' (BSE) is used to describe the suitability for breeding of both male and female animals of all species. In dogs this examination is usually carried out in case of fertility problems. Infertility or reduced fertility in dogs are becoming increasingly common these days, and sometimes the reason remains unknown (Domosławska & Zduńczyk 2020; Feldman & Nelson 2004; Fontbonne 2011; Memon 2007).

In case of dogs the evaluation should be based on a very detailed history, clinical examination, semen analysis, tests for infectious diseases like *Brucella canis* and others, blood tests (CBC, biochemical parameters, hormonal profile).

History

The first step in making a proper diagnosis is the clinical history, which should be as detailed as possible. Health maintenance and nutrition can have a significant impact on its reproductive potential. If infertility is the result of a male's clinical history, it is important to determine whether the mated bitch has been properly tested to determine the appropriate time of mating and whether she has previously had litters with other males. This is very important to decide whether the problem is attributed to the male or female.

Another point of the questionnaire is how many times the bitch was mated, the number of litters and their size, the intervals

between matings, whether the male has suffered from infectious diseases (if it has been vaccinated against them - if so, which ones and when), whether it has become infected with diseases leading to an elevated body temperature such as babesiosis (fever can affect semen quality temporarily or permanently) (Domosławska & Zduńczyk 2020). The administered medications should also be reported in the clinical history. Some of them can decrease or suppress libido and spermatogenesis (Amory 2007). Family history is also important: whether there is a history of diseases that may affect fertility such as endocrinopathies (hypothyroidism or hyperadrenocorticism) in the breed line,

parents or siblings (Feldman & Nelson 2004; Johnson et al. 2001).

Clinical examination

The next step of the andrological examination is the general clinical examination which should not be omitted. Dogs with joint or spinal disease or in poor clinical condition may never be able to bear offspring naturally. In such cases, insemination is usually performed, but the owner should be aware of the problem of spreading hereditary conditions to the offspring.

The external reproductive organs - penis, prepuce, scrotum and testicles should be examined very carefully to exclude such anomalies as persistent penile frenulum, penile or prepuce inflammation, abrasions, ulcers. Abrasions or petechiae on the penis may be caused by natural mating, which can lead to contamination of the ejaculate with blood. Palpation of the testicles allows for the assessment of their consistency, turgor or pathological formations within the tissue. Small and soft testicles are most often associated with poor semen quality. Their enlargement is often associated with inflammation of the testes or epididymis. Testicles in older males usually have a decreased turgor and are less flexible. In all cases, palpation of the testes and epididymis should not be painful, the testes should be easily movable in the scrotal sac and two of them should be palpable. In case of cryptorchids which should be eliminated from breeding,



Fig. 1. Ultrasound image of the prostate in a dog



Fig. 2. Ultrasound image of prostatitis



Fig. 3. Polycystic prostate

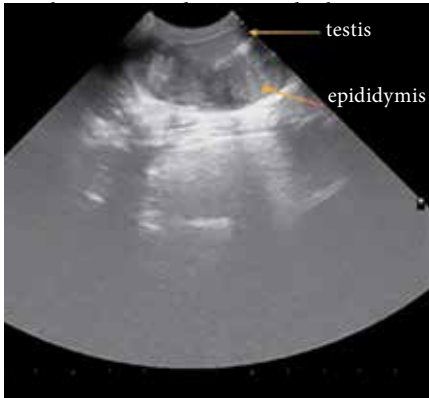


Fig. 4. Epididymitis

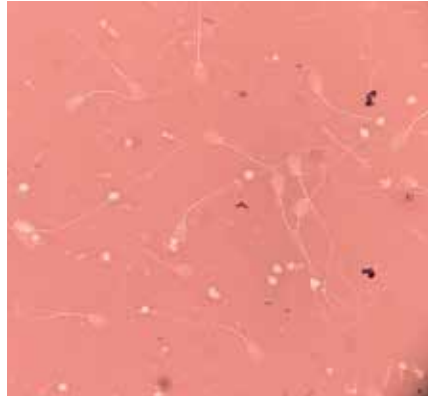


Fig. 5. Eosin-nigrosin sperm cell staining

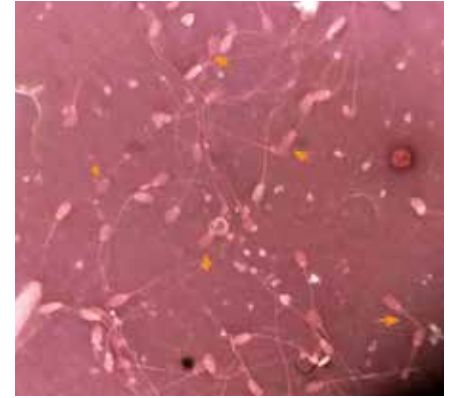


Fig. 6. Dead sperm cells (pink) and cells with a coiled tail

nation which is performed per rectum and with ultrasound (Levy et al. 2014). The per rectum examination evaluates the position of the prostate in relation to the rectum, its surface area and any soreness. Ultrasound examination through the abdominal wall allows for a more accurate assessment. We evaluate the echogenicity of the parenchyma, its integrity, size, course of the urethra, presence or absence of cysts, abscesses or inflammation. In dogs, prostate problems affect fertility and they are one of the main causes of infertility or reduced fertility. Benign prostatic hyperplasia (BPH) is the most common and mainly affects dogs over the age of 5. The prostate capsule of dogs is thin which results in outward growth of the prostate lobes, then palpation per rectum of the prostate is painful, the urethra is pressed which causes difficult urination and dripping blood. In many cases, this is the earliest presenting symptom and much more common than defecation of feces in a form of a flat tape. Introducing pharmacologic treatment in sire dogs is not troublesome. Inhibitors of 5- reductase are used to counteract the conversion of testosterone to dihydrotestosterone. This allows to avoid surgical or chemical castration in many cases. Treatment of BPH is important because prostatic hypertrophy is predisposing to the development of inflammation, or prostatitis. The examinations of the prostate mentioned above are among the non-invasive tests. An invasive test is a prostate biopsy, which allows for the collection of a sample and its evaluation. It is performed especially in cases of suspected neoplastic conditions. Cytological examination of the fluid and cells collected from the prostate with a catheter inserted through the urethra is also very useful in this case.

Other conditions of the prostate gland, alone or in combination with or as a consequence of BPH, in most cases always have an effect on the semen quality and fertility of the dog. In the case of prostatitis, hematuria is the most common symptom and the third

fraction of the ejaculate may contain large numbers of leukocytes. The main tests to be performed are culture of the third ejaculate fraction and urine culture. In prostatitis, mainly *E. coli*, *Staphylococcus* spp, *Streptococcus* spp., *Proteus* spp, *Klebsiella* spp, *Pseudomonas* spp, *Mycoplasma* spp, *Ureaplasma* spp are isolated. Fungal infections of the prostate gland are very rare. Untreated prostatitis may lead to prostatic ulceration, sepsis, and peritonitis in extreme cases (Levy et al. 2014; Memon 2007).

Semen examination

The clinical examination is followed by a detailed examination of the semen. In most cases, the ejaculate is collected in most cases by manual masturbation. It is recommendable to collect the semen in the presence of a bitch in heat or out of oestrus. It is also a good idea to have frozen swabs with the scent of a bitch in heat. Be sure to perform the semen collection procedure on a suitable surface so that the dog does not slip. The ejaculate consists of three fractions: I - initial from the prostate gland (0.5-2 ml), II- sperm cells (0.5-2 ml) and III - prostate gland with the largest volume - up to 40 ml. The fractions from the prostate gland are transparent and easily distinguished from the fraction containing sperm cells which is milky white. Problems in distinguishing the fractions may occur when the sperm concentration is low. Then fraction II may be only slightly cloudy, very similar in appearance to fraction III. The duration of semen collection - ejaculate (of all fractions) varies and may take up to 10-15 minutes until losing the erection.

The basic examination of semen includes evaluation of its volume, concentration, mobility including progressive movement, percentage of viable sperm cells and their morphology.

Volume varies, depending on the size of a dog and individual characteristics. Volume of the second fraction varies from 0.5-5 ml, but there are record-breaker donors which

give 10-20 ml. Concentration can be determined with a haemocytometer or Computer Assisted Sperm Analysis (CASA), which is used to assess various sperm mobility parameters (Rijselaere et al. 2007). Evaluation of sperm concentration requires appropriate diluents. When performing calculations based on chambers (Bürker, Thom), the sperm must be immobilized. In case of CASA device, diluents that do not affect sperm movement are used, because apart from concentration, their movement is also assessed. Computer-assisted analysis of sperm motion includes: percentage of motile spermatozoa (MOT), percentage of spermatozoa with progressive movement (PMOT), velocity average pathway (VAP), velocity straight line (VSL), velocity curvilinear (VCL), showing the averaged total path the spermatozoa travels in a given unit of time, thus giving a message about its viability, amplitude lateral head (ALH), beat cross frequency (BCF), straightness (STR), linearity (LIN), and subcategories of sperm movement speed - rapid, medium, slow and static.

Evaluation with CASA system is very useful and helpful, however it is quite expensive and in veterinary medicine it is used mainly in scientific and academic centres and less often in private clinics.

Apart from motility of spermatozoa, assess their viability and morphology are also evaluated. For this purpose, among others, appropriate stains are used. The simplest staining is based on eosin-nigrosin which allows to assess the percentage of viable spermatozoa that do not absorb the dye, i.e. do not stain. Another method of assessing sperm viability is HOST - hypoosmotic swelling test. This is a semi-quantitative test based on the principle of semi-permeability of the cell membrane in an intact living sperm. In this test, only alive sperm cells with an intact cell membrane will "become swollen" in a hypotonic environment. This test is easy to perform and evaluate and provides additional information about sperm tail membrane integrity.

Morphology can also be assessed using eosin-nigrosin or other stains such as hemacolor (Diffquick) which is used in private veterinary practice for staining of other samples such as skin scrapings and blood. Determination of sperm morphology is extremely important for diagnosis - whether the problem is located in the testes during sperm production or in the epididymis and sperm ducts.

A summary of physiological parameters of dog semen is given in Table 1.

Tab.1. A summary of physiological parameters of dog semen.

Parameter	Reference values [5,9]
Volume (ml)	0.5-2.0
Concentration (x10⁶/ml)	292.6 ± 208.3
MOT (%)	88.3 ± 18.4
PMOT (%)	60-70
VAP (µm/s)	124.3 ± 19.7
VSL (µm/s)	113.0 ± 20.2
VCL (µm/s)	160.7 ± 19.7
ALH (µm)	5.0 ± 0.7
BCF (Hz)	26.2 ± 4.4
STR (%)	88.9 ± 3.4
LIN (%)	70.1 ± 7.5
RAPID (%)	65.2 ± 21.7
MEDIUM (%)	3.4 ± 2.4
SLOW (%)	19.7 ± 12.8
STATIC (%)	11.8 ± 14.4
Normal morphology (%)	75-90
Viable (%)	90-95

A more detailed evaluation of sperm parameters, and thus breeding soundness evaluation of the male, is performed with specific reagents and equipment. In some cases, even though the preliminary examination revealed a good semen quality its further evaluation may suggest or confirm fertility problems. It may be associated, for example, with acrosome integrity, mitochondrial activity, apoptosis, DNA degeneration or oxidative stress (Domosławska & Zduńczyk 2020).

A second fraction cytology test should also be performed routinely. This can be done with Diffquick staining method. A normal image will show sperm cells, white and red blood cells, epithelial cells and bacteria. The same test applies to the prostate

fraction, which will contain the cells listed above without sperm cells (it may contain single sperm cells). An increase in white blood cells or bacteria number may indicate inflammation, while an increase in red blood cells may indicate prostate disease or bleeding within the penis or foreskin. Note that in dogs there may not be a correlation of an increase in inflammatory cells with an increase in bacteria in the semen sample culture (Root Kustritz et al. 2005).

Additional testing

Any dog suspected of having fertility problems should be tested for *Brucella canis*. Other sexually transmitted diseases and pathogens may not affect dog's fertility, but may impact the fertility of bitches in case of contamination during natural mating.

The hormonal status of males is not routinely evaluated. In dogs with sperm abnormalities, the pituitary hormones and testosterone levels should be measured. In such cases, luteinizing and folliculotropic hormone may be at physiological levels or elevated due to the lack of a testicular feedback mechanism (Johnson et al. 2001). It is extremely important to collect and evaluate semen several times at an average interval of 3 months to rule out other conditions or toxin exposures. The results of a single semen analysis should not be suggestive. In cases of suspected fertility problems, semen should be collected again after approximately one week under the most favourable conditions to ensure minimal stress to the dog. Further procedures should be chosen based on the diagnosis (Johnson et al. 2001).

In conclusion, male dogs should be routinely tested for fertility even if they ensure frequent and numerous litters. This would allow to detect pathological subclinical conditions and appropriate further management.

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Product description in Vet Pharmacy

The impact of vitamin E and selenium supplementation on semen quality in dogs with reduced fertility

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Reduced fertility in male dogs remains a topic that is connected with a number of doubts and inconsistencies, predominantly due to the limited number of studies and publications pertaining to the subject. The deterioration of semen quality may occur at any age. Moreover, the cause of said problem often remains unexplained. Semen production depends on both internal and external factors. Apoptosis and oxidative stress are normally occurring processes that regulate the development of reproductive cells. Sperm is particularly sensitive to oxidative stress. Nutritional deficiencies may have a negative impact on semen quality, especially when it comes to the reduced intake of antioxidants, such as vitamin E, selenium, or polyunsaturated fatty acids.

Antioxidants and their importance

Selenium can be identified in the body in the form of selenoproteins, which contain selenocysteine in their structure. Selenoprotein P is responsible for transporting selenium to the testicles. Glutathione peroxidase is an enzyme and the main selenoprotein found in the testicles. Its role is to decompose hydrogen peroxide. Studies in humans, boars, bulls, and turkeys have all clearly shown a significant correlation between selenium deficiency and fertility disorders.

Vitamin E plays a significant role when it comes to protecting sperm against lipid peroxidation. Supplementation of said vitamin in humans, dogs, and boars has proved to be beneficial with regard to semen quality. Said fact indicates the synergistic effect of the aforementioned compounds and should serve as a sensible reason for simultaneous supplementation.

Research description and course

An examination has been conducted in order to determine the effect of selenium and vitamin E supplementation on semen quality, glutathione peroxidase activity, and total antioxidant capacity (TAC). The piece of research in question has involved 20 dogs of various breeds, aged 4 to 8, which were found to have reduced fertility. Failed insemination during the last two or three mating attempts or having a small litter (depending on the breed) has been considered an indication of possible decreased fertility. All dogs have been in good general condition and have not shown

any anatomical abnormalities with regard to the reproductive system.

The dogs have been divided into two groups: the study group (n=10), which has received daily oral supplementation of selenium (6 µg/kg), vitamin E (5 mg/kg), and evening primrose extract (50 mg) for 60 days (Semevet, Vet Expert) and control group that has not received any supplementation. The study has been conducted for 90 days, meaning 60 days of supplementation and 30 days for monitoring semen parameters after the end of supplementation.

Semen for the study has been collected manually on the following days: 0, 30, 60, and 90. The cellular fraction of semen has been collected into a previously heated test tube. At the same time, blood has been collected for testing purposes and plasma has been immediately centrifuged and frozen.

Semen concentration and motility parameters have been assessed by means of using a Hamilton Thorne Sperm Analyzer, IVOS version 12.3 (HTR-IVOS 12.3). The following indicators have been determined: concentration (CONC), percentage of motile spermatozoa (MOT), percentage of progressive motility spermatozoa (PMOT), velocity average pathway (VAP), velocity straight line (VSL), velocity curvilinear (VCL), amplitude lateral head (ALH), beat cross frequency (BCF), linearity (LIN), and straightness (STR). In each ejaculate, the subpopulation of sperm characterized by rapid movement (RAPID), moderate movement (MEDIUM), slow movement (SLOW), and no movement (STATIC) has been determined. The percentage of live and dead sperm cells has been determined by performing swab tests with swabs stained with

eosin-nigrosin. Morphology-related results have been assessed by performing swab tests with swabs stained in compliance with the principles of the Diff-Quick method. The results of the examinations are summarized in Table 1.

While the volume of the sperm-rich fraction has remained without significant changes in all animals, the study has shown that the majority of examined parameters have improved in the study group (PMOT, VSL, VCL, ALH, BCF, RAPID, MEDIUM, SLOW, STATIC).

A significant increase in the concentration of selenium and vitamin E in plasma has also been demonstrated. In the group being provided with supplements, an increase in the amount of live sperm with normal morphology has been additionally observed. In the control group, there have been no differences with regard to sperm motility parameters. What is more, there has even been a decrease in the number of normal sperm, which has suggested the progressive nature of the issue.

Conclusions

Vitamin E and selenium supplementation has had a significant positive effect on the activity of glutathione peroxidase, showing a significant increase. The total antioxidant capacity has also significantly improved in the study group (Table 2).

The study has shown that supplementation with vitamin E and selenium for 60 days has had a positive effect on semen quality in dogs. The presented data has shown desired quality parameters being maintained even after the conclusion of the supplementation period. Improving semen quality is directly connected with increased sperm resistance to oxidative stress.

Antioxidant efficiency summary

Oxidative stress plays a remarkable role in the etiology of reduced fertility in dogs. Providing the right amount of selenium and vitamin E – being widely known antioxidants – in their diet may be the answer to the discussed problem in many cases. Selenium is an essential ingredient when it comes to the production

Table 1. Semen quality parameters (mean $\pm$ SD) in male dogs supplemented with Se and vitamin E ($n = 10$) and in the control group ($n = 10$) on days 0, 30, 60 and 90.

Parameter	Unit	Day 0		Day 30		Day 60		Day 90	
		Study group	Control group	Study group	Control group	Study group	Control group	Study group	Control group
Semen volume (seminal fraction)	ml	1,65 $\pm$ 0,62	2,00 $\pm$ 1,02	2,12 $\pm$ 0,83	2,49 $\pm$ 0,80	2,49 $\pm$ 0,57	2,52 $\pm$ 0,63	2,79 $\pm$ 0,57	2,46 $\pm$ 0,79
Concentration	$\times 10^6$ /ml	120,77 $\pm$ 32,01 ^a	123,73 $\pm$ 57,34 ^a	131,51 $\pm$ 44,07 ^c	118,86 $\pm$ 62,24 ^c	260,60 $\pm$ 42,86 ^{bde}	104,24 $\pm$ 57,91 ^{bf}	292,07 $\pm$ 35,68 ^{bde}	89,08 $\pm$ 49,88 ^{bdf}
Total sperm count	$\times 10^6$	210,54 $\pm$ 114,36 ^{ae}	255,50 $\pm$ 199,01 ^{af}	295,32 $\pm$ 171,18 ^c	300,24 $\pm$ 193,49	657,21 $\pm$ 218,24 ^{bde}	278,56 $\pm$ 179,52 ^f	816,86 $\pm$ 208,41 ^{bde}	227,96 $\pm$ 152,71 ^{bf}
MOT	%	58,10 $\pm$ 30,51 ^a	59,10 $\pm$ 32,71	73,30 $\pm$ 9,92 ^b	53,40 $\pm$ 31,89 ^f	82 $\pm$ 12,65 ^b	47,40 $\pm$ 30,67 ^f	88,70 $\pm$ 4,85 ^{be}	44,30 $\pm$ 29,39 ^f
PMOT	%	29,40 $\pm$ 18,72 ^a	30,10 $\pm$ 25,07	54,10 $\pm$ 13,17 ^{be}	25,30 $\pm$ 17,34 ^f	61,40 $\pm$ 13,58 ^b	20,60 $\pm$ 16,52 ^f	72,90 $\pm$ 12,34 ^{be}	22,80 $\pm$ 18,64 ^f
VAP	μ m/s	105,75 $\pm$ 20,54 ^a	108,79 $\pm$ 41,45	120,31 $\pm$ 13,63	111,41 $\pm$ 26,07	131,10 $\pm$ 23,63	102,55 $\pm$ 29,69	145,34 $\pm$ 15,51 ^{be}	97,34 $\pm$ 26,97 ^f
VSL	μ m/s	92,03 $\pm$ 17,64 ^a	95,13 $\pm$ 37,79	109,38 $\pm$ 11,91	93,76 $\pm$ 20,07	118,20 $\pm$ 21,17 ^{be}	89,47 $\pm$ 25,69 ^f	133,60 $\pm$ 15,64 ^{be}	85,48 $\pm$ 25,58 ^f
VCL	μ m/s	176,67 $\pm$ 48,97 ^a	169,05 $\pm$ 34,93	184,69 $\pm$ 42,77	185,73 $\pm$ 43,21 ^a	186,05 $\pm$ 38,46 ^e	161,39 $\pm$ 36,44 ^f	194,73 $\pm$ 24,56 ^{be}	152,98 $\pm$ 37,79 ^{bf}
ALH	μ m	7,07 $\pm$ 2,58 ^a	5,75 $\pm$ 2,42 ^a	6,86 $\pm$ 2,90	5,90 $\pm$ 3,39	6,42 $\pm$ 2,35	5,96 $\pm$ 2,92	5,91 $\pm$ 1,57 ^{be}	7,22 $\pm$ 2,31 ^{bf}
BCF	Hz	21,99 $\pm$ 12,24 ^a	16,94 $\pm$ 14,38 ^a	20,97 $\pm$ 11,48 ^c	16,51 $\pm$ 16,60	26,07 $\pm$ 10,65 ^{bd}	22,13 $\pm$ 15,01	23,50 $\pm$ 13,33	26,84 $\pm$ 9,96 ^b
STR	%	79,09 $\pm$ 7,24 ^a	85,30 $\pm$ 7,05	84,63 $\pm$ 2,02	84,70 $\pm$ 9,42	86,72 $\pm$ 5,39	86,80 $\pm$ 4,51	91,18 $\pm$ 4,13 ^b	85,70 $\pm$ 3,16
LIN	%	55,30 $\pm$ 11,75	55,60 $\pm$ 16,07	62,40 $\pm$ 12,75	53,80 $\pm$ 15,61	65,40 $\pm$ 13,33	56,50 $\pm$ 7,57	70,00 $\pm$ 8,94 ^e	56,20 $\pm$ 6,19 ^f
RAPID	%	35,5 $\pm$ 24,86 ^a	34,60 $\pm$ 28,70 ^a	57,20 $\pm$ 14,37 ^{bc}	34,20 $\pm$ 21,36 ^f	67,80 $\pm$ 18,03 ^{be}	24,80 $\pm$ 20,77 ^{bf}	79,40 $\pm$ 11,47 ^{bde}	25,10 $\pm$ 21,13 ^f
MEDIUM	%	22,60 $\pm$ 16,09 ^a	24,50 $\pm$ 14,82	15,90 $\pm$ 7,10 ^c	19,00 $\pm$ 10,99	12,20 $\pm$ 5,78 ^{de}	21,50 $\pm$ 17,65 ^f	9,50 $\pm$ 8,36 ^{bde}	18,90 $\pm$ 12,08 ^f
SLOW	%	6,20 $\pm$ 6,05 ^e	20,20 $\pm$ 20,74 ^{af}	10,60 $\pm$ 10,23	17,20 $\pm$ 18,46	10,10 $\pm$ 9,64 ^e	13,50 $\pm$ 15,24 ^b	5,40 $\pm$ 3,86 ^e	18,80 $\pm$ 15,84 ^f
STATIC	%	35,90 $\pm$ 31,46 ^a	34,20 $\pm$ 26,09	16,00 $\pm$ 8,95 ^{bce}	29,70 $\pm$ 28,29 ^{af}	9,90 $\pm$ 7,96 ^{bde}	40,00 $\pm$ 26,91 ^{bf}	5,90 $\pm$ 5,93 ^{bde}	37,20 $\pm$ 30,18 ^f
Live sperm: (eosin-nigrosine)	%	82,40 $\pm$ 10,97 ^a	72,50 $\pm$ 14,35	88,00 $\pm$ 6,56 ^e	64,70 $\pm$ 26,71 ^f	92,00 $\pm$ 4,54 ^{be}	65,65 $\pm$ 30,16 ^f	93,40 $\pm$ 3,37 ^{be}	64,50 $\pm$ 26,76 ^f
Normal sperm	%	67,10 $\pm$ 21,49 ^a	58,10 $\pm$ 15,50	76,20 $\pm$ 12,62 ^{be}	58,80 $\pm$ 12,92 ^f	80,00 $\pm$ 8,36 ^{be}	56,70 $\pm$ 13,84 ^f	83,70 $\pm$ 6,61 ^{be}	56,80 $\pm$ 13,59 ^f

Means of a, b and c, d - pairs of statistical differences in the row separately for the supplemented and control groups; means of e, f - pairs of statistical differences in a row between the supplemented and control groups on each day of the study.

Table 2. Values of Se, vitamin E in blood serum and GSH-Px and TAC activities in sperm (mean $\pm$ SD) in male dogs supplemented with Se and vitamin E ($n = 10$) and in the control group ($n = 10$) on days 0, 30, 60 and 90.

Parameter	Unit	Day 0		Day 30		Day 60		Day 90	
		Study group	Control group	Study group	Control group	Study group	Control group	Study group	Control group
Se	μ g/l	214 $\pm$ 71,63 ^a	161,40 $\pm$ 42,43	332,70 $\pm$ 65,18 ^{be}	176,40 $\pm$ 43,50 ^f	356,70 $\pm$ 33,81 ^{be}	198,20 $\pm$ 43,22 ^f	339,20 $\pm$ 31,73 ^{be}	185,30 $\pm$ 39,71 ^{bf}
Vitamin E	mg/ml	21,31 $\pm$ 12,80 ^a	28,62 $\pm$ 9,02	33,61 $\pm$ 11,15	28,29 $\pm$ 9,04	40,09 $\pm$ 7,06 ^{be}	26,53 $\pm$ 8,02 ^f	37,90 $\pm$ 5,62 ^{be}	26,20 $\pm$ 5,23 ^f
GSH-Px	nkat/mg białka	0,38 $\pm$ 0,11 ^a	0,39 $\pm$ 0,13 ^a	0,42 $\pm$ 0,10 ^a	0,48 $\pm$ 0,17	0,67 $\pm$ 0,37 ^{be}	0,56 $\pm$ 0,32 ^{bf}	0,62 $\pm$ 0,12 ^{be}	0,41 $\pm$ 0,03 ^f
TAC	μ mol/g białka	12,81 $\pm$ 7,25 ^a	14,04 $\pm$ 5,75 ^a	12,23 $\pm$ 4,97 ^a	12,89 $\pm$ 5,19 ^b	14,53 $\pm$ 7,80 ^{bce}	11,49 $\pm$ 4,82 ^{bf}	19,31 $\pm$ 4,03 ^{bde}	13,05 $\pm$ 2,56 ^f

Means from a, b - pairs of statistical differences in the row for the supplemented group; means from e, f - pairs of statistical differences in the row between the supplemented and control groups on each day of the study.

Based on: A. Domosińska, S. Zdunczyk, M. Franczyk, M. Kankofer, T. Janowski „Selenium and vitamin E supplementation enhances the antioxidant status of spermatozoa and improves semen quality in male dogs with lowered fertility” Wiley Andrologia, March 2018

and maturation of sperm. As a component of glutathione peroxidase present in sperm, it significantly affects its activity and thus increases the resistance to oxidative stress caused by harmful peroxides. Additionally, selenium is one of the structural elements of sperm. The aim of vitamin E is to reduce free radicals, directly translating into the reduced destruction of sperm cell membranes. Therefore, it can be

stated that the supplementation of the aforementioned vitamin results in improved sperm motility and morphology. Evening primrose oil contained in the examined preparation is a rich source of polyunsaturated fatty acids - building components of sperm cell membranes. At the same time, it has an antioxidant effect, which has a beneficial effect on the quality of semen.

To sum up, the oral supplementation of selenium and vitamin E for 60 days has significantly improved sperm concentration, motility, and morphological parameters in clinically healthy dogs diagnosed with reduced fertility.

Reference materials are available at the editorial office

Phantom pregnancy in female dogs – is it a negligible issue?

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Until quite recently, phantom pregnancy among unneutered female dogs has been a common occurrence. Even though it affects a particular animal, it can also be very stressful for its owners. The increasingly common castration of female dogs has translated into phantom pregnancies being observed much less frequently in clinical practice. Nevertheless, there are still situations that require medical attention. Most cases of phantom pregnancy tend to cease on their own, without the need to opt for complicated treatment protocols. It is worth elaborating on the most important clinical aspects of phantom pregnancy in female dogs, causes and mechanisms of said phenomenon, as well as solutions available when it comes to modern veterinary medicine.

Introduction

Phantom pregnancy (Latin: lactatio sine graviditae, lactatio falsa, pseudograviditas) should be understood as the occurrence of physical and mental symptoms of pregnancy, and sometimes - even signs of pregnancy in non-pregnant female dogs. The discussed phenomenon occurs mainly in female dogs, but is in some scenarios reported in female cats as well. There have been no specific racial predilections with regard to phantom pregnancy. It is observed in female dogs not allowed to mate, as well as in mated or inseminated female dogs that have not become pregnant.

There are numerous hypotheses and myths pertaining to phantom pregnancy. One theory assumes that this is a purely physiological phenomenon, a manifestation of atavism, the roots of which date back to the times when dogs were undomesticated, wild animals. It is believed that today's domestic dog (*canis familiaris*) comes from wolf (*canis lupus*), and the phenomenon of phantom pregnancy was first observed in wild wolves. Wolves, being herd animals, have a perfectly developed hierarchical system, according to which only the leader of a given group (the alpha female) can have offspring. The remaining companions have to make every effort possible to provide the leader and his offspring with comfortable living conditions. That is why, after giving birth, the remaining females in the herd begin lactation to provide the cubs with enough food. It is made possible thanks to the existence of the mechanism of synchronizing sexual cycles in all female animals in the herd. Phantom pregnancy has been around in wild animals for thousands or

even millions of years, but in today's domestic dog, it seems to be just an evolutionary remnant. Supporters of the aforementioned theory claim that it is a typical condition for the discussed species that is related to the hormonal system, the treatment of which is not necessary. Usually, the phenomenon ceases to exist on its own.

In practice, one may also encounter a completely opposite view, which classifies phantom pregnancy as a pathological phenomenon being the sign of hormonal disorders, which - similarly to any other disease - should be treated, especially in the case of the long-term persistence of symptoms (4-8 weeks).

Regardless of the views, phantom pregnancy is often the reason for visiting a veterinarian, especially when it comes to intense lactation or changes in the female dog's behavior observed shortly after the period of heat. It also happens that the initial symptoms go unnoticed by caregivers and only female dogs that have developed mastitis are brought to a given clinic.

Formation mechanism

Causes of phantom pregnancy and associated lactation in female dogs are not fully understood. It is known to occur in the corpus luteum phase, usually 4-8 weeks after the heat period. Nevertheless, it has also been found to occur between the 13th and 14th week and between the 17th and 22nd week after the end of the estrus period.

Negative correlation between progesterone (P4) and prolactin (PRL) plays a fundamental role in the pathogenesis of lactomania. This explains its occurrence in female

dogs after ovariectomy in the luteal phase of the cycle. The female dog ovarian cycle is characterized by long-lasting secretory activity of corpora luteum, which in non-pregnant dogs (similarly to pregnant ones) lasts for a very long time, up to 7-12 weeks. It results in high progesterone concentration. Nevertheless, the concentration of said hormone in the blood in pregnant and non-pregnant female dogs is similar. A sharp drop in progesterone concentration in female dogs is related to the lysis of the corpus luteum. Said phenomenon is not identified in the case of phantom pregnancy. Therefore, the persistently high level of progesterone stimulates the hypertrophy of the secretory part and interstitial tissue of mammary glands. At the same time, during the supposed perinatal period, there is a decrease in the level of progesterone in the blood, with a very significant increase in prolactin (PRL) released from the glandular part of the pituitary gland in female dogs with a phantom pregnancy. The increase in prolactin concentration in dogs with clinical symptoms of phantom pregnancy is much higher than in dogs without symptoms. Prolactin secretion is strictly controlled by the hypothalamus, which produces two antagonistic substances:

- PRF – prolactin releasing factor – prolactoliberin
- PIF – prolactin inhibiting factor – prolactostatin

The PIF factor is controlled by dopamine. The inhibition of PRL release is mediated by the neurotransmitter dopamine, which causes a change in the surface tension of specific PRL-producing cells. The dopaminergic effect of PIF on the permeability of the cell membrane to PRL is disabled under the influence of serotonin. The serotonergic neurotransmitter stimulates the release of PRL in connection with the milk ejection reflex, triggered by puppies sucking mammary glands, licking the skin of the glands by lactomaniac female dogs, or by animal owners' attempts to remove some milk from such glands.

To sum up, a decrease in progesterone concentration causes an increase in prolactin concentration, which leads to cases of phantom pregnancy in non-pregnant

female dogs. Prolactin stimulates mammary glands to secrete milk. It plays a similar role in non-pregnant female dogs, leading to the occurrence of clinical symptoms of phantom pregnancy.

Clinical symptoms

Clinical symptoms are easily noticeable and closely resemble the symptoms of physiological pregnancy and childbirth (Table 1). The mammary gland enlarges. What is more, in its ducts, there is the presence of milky or milk-like secretion, sometimes flowing out spontaneously (Fig. 1-3). Female dogs have an increased appetite and often gain weight. Motor restlessness sometimes appears (in the form of excessive excitability) and the dog may prepare the nest and take care of objects that it thinks are puppies (Fig. 4). A frequently observed accompanying symptom is licking the skin around the glands, especially those located in the inguinal area. In some cases, the mammary gland may become inflamed. Significant enlargement, redness, swelling and pain

are then observed. Mechanical damage can be observed on the skin caused by excessive licking, having the form of wounds, scabs, hypertrophy, or hyperpigmentation. The nipples may be tender when palpated and may leak milk or a milk-like substance when squeezed. Quite often, reluctance to leave home, aggression, body tremors, and swelling of the vulva are observed. Scanty mucous, stringy discharge from the genital tract may be noticed as well. Such symptoms may be accompanied by a simulated labor with movements imitating labor pains. The duration of phantom pregnancy symptoms varies. They may last for several days or even months, but in extreme cases – even for years. The cause of said condition is not fully understood. It probably results from the lack of hormonal interactions between the pituitary gland/hypothalamus and the ovaries. It is hypothesized that simply licking and biting the glands may also lead to chronic lactomania. The milk secretion remaining in the glands then thickens, deposits, and mineral salts are deposited

within the glands, causing pressure, the formation of abscesses, fistulas, and – as a result – cancer in some extreme cases. If severe clinical symptoms of phantom pregnancy persist for more than 2-3 weeks, it is worth checking the concentration of thyroid hormones. It is not uncommon for female dogs with hypothyroidism to have increased prolactin secretion, causing galactorrhea.

Therapeutic treatment

Modern veterinary medicine and widespread access to various types of supplements make it possible to use supporting preparations before the need to opt for standard medications. Two aspects should be taken into account when limiting the symptoms of false pregnancy. The first one is the psychological factor that is often overlooked, but may play a key role in the course of the therapy. One of most vital issues is the cooperation of the caregiver with the doctor, who should explain the etiology of the identified phenomenon to the owner in the simplest possible manner. He or she should also be assured that his or her actions do not cause the pet any harm. The basis of treatment is the elimination of all factors that may affect the maintenance of the pseudopregnancy, i.e. eliminating the nest, throwing away toys, providing more exercise, ensuring a low-protein diet and limiting water intake, or even opting for a 24-hour fast followed by a gradual return to the full daily food intake (3-5 days). Said undertakings ensure that the energy obtained meets the dog's needs and is not used for milk production. If the dog lactates frequently, special clothing items or jackets can be used. An important factor is also limiting petting and, if possible, showing less affection than usually. In most cases, simple actions are enough to spontaneously reverse the symptoms of phan-

Tab.1. Characteristic symptoms of phantom pregnancy

Mammary gland	• significant enlargement and even swelling, especially in the posterior bundles • painful when palpating • leakage of milk or milk-like secretion when squeezed • gland may be warm and hard
Behavioral changes	• excessive arousal or reluctance to move • increased appetite and weight gain • preparation of a nest • aggression • licking the area around the glands, expressing milk
Other	• swelling of the vulva and vaginal discharge • enlargement of the abdomen • simulated childbirth with apparent labor pains

Table 2. Substances used in the treatment of phantom pregnancy and used to inhibit lactation.

Substance	Dose (mg/kg)	Administration method	Contraindications to use	Possible side effects	Application time (in days)
Proligestone	25-35 mg/kg	s.c.	Mammary gland tumors Pregnancy Diabetes	Hyperplasia of the uterine mucosa Mammary gland tumors Pyometra Ovarian cysts	
Megestrol acetate	20-30 mg/kg	p.o.	as above	Hyperplasia of the uterine mucosa Mammary gland tumors	
Estrogens	0,01-0,1 mg/kg	i.m.	Pregnancy Mammary gland tumors	Hyperplasia of the uterine mucosa Myelosuppression Pyometra	
Mobilerone	16-20 µg/kg	p.o. i.m.	Mammary gland tumors Pregnancy Diabetes	Recurrence of symptoms Hypertrophy of the external genitalia Vaginal discharge	5
Testosterone	1 mg/kg	i.m.		Recurrence of symptoms Hypertrophy of the external genitalia Water retention	1
Bromocriptine	10-100 µg/kg	p.o.	Pregnancy	Vomiting Depression Lack of appetite	7-10
Cabergoline	5 µg/kg	p.o.	Pregnancy	Vomiting	7
Metergoline	0,1-0,2 mg/kg	p.o.	Pregnancy	Vomiting Diarrhea Aggression Stimulation Howling	4

tom pregnancy (after 2-3 weeks). Nevertheless, sometimes it may be necessary to implement pharmacological therapy. In addition to agents used generally in the form of pills or injections, local treatment is also important. If the glands are swollen and red, astringent compresses, cooling, and anti-inflammatory ointments can be applied locally. Understanding the mechanism of phantom pregnancy is vital when it comes to the correct use of available pharmacological substances.

Pharmacological substances that one may opt for:

- **progestogens** (medroxyprogesterone, proligestone, megestrol acetate). The use of even small doses of progestogens may lead to epithelial hyperplasia and cystic degeneration of the uterine mucosa, and - as a result - to the development of EPC syndrome (endometritis pyometra complex). Said substances are not recommended in the case of diabetic dogs.
- **estrogens**. The use of estrogens is not very effective. Moreover, their long-term administration may lead to bone marrow suppression, the formation of mammary gland cancerous tumors, or the hyperplasia of the uterine mucosa and EPC.
- **inhibitors**. Prolactin inhibitors have been used for over 30 years to prevent or inhibit perinatal lactation, treat excessive, non-physiological milk production, as well as to support the treatment of acromegaly and Parkinson's disease. They inhibit the secretion of PRL by the pituitary gland. A secondary effect is a sharp decrease in P4 concentration in pregnant and non-pregnant female dogs. Said fact results in a sharp decrease in milk production and secretion, as well as in positive changes in the dog's behavior. If treatment is stopped within the initial 4-5 days, progesterone levels may return to pre-treatment levels, resulting in milk production. Currently, three ergoline derivatives are used to inhibit PRL secretion - bromocriptine, metergoline, and cabergoline. Said substances differ when it comes to the recommended dosage, effectiveness and possible side effects.
- **Bromocriptine**. It is the longest-used inhibitor. It is available in the form of pills administered orally. Its side effect is vomiting, so it is recommended to administer it in gradually increasing doses or simultaneously with antiemetics.
- **Metergoline**. When used, vomiting occurs less frequently, but behavioral disorders such as agitation, aggression, and howling are observed.
- **Cabergoline**. It is administered orally in the form of a solution that can be mixed with food. Occasionally, it causes vomiting. It should be administered for 4-5 days or for a longer period, especially if satisfactory results are not achieved.



Fig. 1. and 2. Enlargement of the mammary gland in a female dog



Fig.3. Mammary gland secretion during phantom pregnancy



Fig.4. Change in the behavior of a female dog in the course of phantom pregnancy - creating a nest and collecting toys

In the case of increased excitability or aggression, additional mild sedatives should be opted for. Despite appropriate actions taken and treatment opted for, the symptoms of phantom pregnancy may return during the next estrous cycle. The only effective method that provides a remarkable certainty with re-

gard to the absence of recurrences is ovariohysterectomy.

Reference materials are available at the editorial office



SemeVet₆₀



Designed specifically for male breeding dogs.

The product supplies substances necessary for the proper formation and maturation of sperm cells, reduces abnormalities caused by oxidative stress. This supplement improves the quality of sperm by 30% so it's perfect for dogs suffering from impaired reproductive function.

Composition: Evening primrose extract 100 mg, vitamin E 100 mg, selenium yeast 60 mg

Instruction for proper use: 1 tablet/10 kg b.w. Product should be administered for at least 30 days, optimally for 60 days.

Packaging: 60 tablets.

ProlactiNO ProlactiNO Large Breed



Designed for female dogs with the typical symptoms of phantom pregnancy.

This product is designed to be used independently or alongside hormonal therapy. It prevents swelling, shows astringent properties and naturally inhibits prolactin secretion. Perfect for use in female dogs after heat, before or after the symptoms have set in. ProlactiNO/ProlactiNO LB counteracts the symptoms of phantom pregnancy in a safe, gentle, natural way, free from adverse side effects.

Composition: vitex (*Vitex agnus-castus* L.) 200 mg, parsley (*Petroselinum sativum*) 50 mg, dandelion (*Taraxacum officinale*) 25 mg, conker (*Aesculus hippocastanum* L.) 20 mg (ProlactiNO); vitex (*Vitex agnus-castus* L.) 700 mg, parsley (*Petroselinum sativum*) 150 mg, dandelion (*Taraxacum officinale*) 80 mg, conker (*Aesculus hippocastanum* L.) 80 mg (ProlactiNO LB).

Instruction for proper use: 1 tablet/5 kg b.w. (ProlactiNO); 1 tablet/15 kg b.w. (ProlactiNO LB)

Packaging: 30 tablets (ProlactiNO); 40 tablets (ProlactiNO LB)

VetoMune



Designed for dogs and cats to help support the immunity.

The preparation made for dogs and cats, aiming at the support of the functioning of non-specific immunity mechanisms. A combined preparation containing, among others: beta-glucan isolated from yeast and β -hydroxy- β -calcium methylbutyrate (Ca-HMB). The preparation may be administered to older and adolescent animals, as well as the ones during reproduction and convalescence phase.

Composition: Beta-glucan 80 mg, HMB-Ca 25 mg, Valeriana officinalis L. 10 mg

Packaging: 60 twist-off capsules



VetAminex



Vitamins and minerals designed for adult dogs and cats.

Vitamin and mineral preparation for dogs and cats. The preparation can be used in animals fed with home-prepared food.

Composition: Calcium 100 mg, vitamin E 6 mg, zinc 5 mg, iron 1.5 mg, vitamin B1 1.5 mg, vitamin B12 2 mcg, vitamin B2 1.5 mg, magnesium 40 mg, vitamin B6 1 mg, copper 0.15 mg, vitamin A 200 IU, acid folic 0.1 mg, biotin 0.03 mg, vitamin D3 25 IU, vitamin B3 7 mg, manganese 0.4 mg.

Instruction for proper use: Cats, dogs and puppies up to 10 kg body weight – 1 capsule every other day. Dogs and puppies over 10 kg body weight – 1 capsule per 10 kg body weight daily.

Packaging: 60 twist-off capsules





NEW

Rapid test for diagnosis of early pregnancy Vet Expert Rapid Test Canine Relaxin

The Vet Expert Canine Relaxin Test is a chromatographic immunoassay for the qualitative detection of canine relaxin (RLN) in canine's serum or plasma. The serum relaxin level of pregnant dogs at 15 to 30 days of pregnancy is significantly higher than in not pregnant. Relaxin level is peaking at 35 day of pregnancy so it can be used as a helpful marker in detection of early pregnancy in dogs.

VET EXPERT RAPID TEST CANINE RELAXIN

- + Quick result - reading of the test result after 5 minutes
- + Easy interpretation of the obtained test result
- + Simple procedure of performing of the test
- + Quality confirmed by internal studies

