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**Heat disorders in the bitch: treatment protocols  
(Review of literature)**



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## 1 Abstract

Infertility in female dogs is frequently brought about by irregularities in the sexual cycle, with conditions like anestrus, split estrus, and prolonged estrus having a variety of etiological factors. Follicular- or luteal-phase problems may be used to treat bitches with anomalies of the sexual cycle. To properly comprehend these conditions, pet owners should have a solid understanding of their animals' sexual cycles. This study investigates the sexual cycle disorders and their treatments

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## **List of abbreviations**

RBC : Red Blood Cells

OHE : Ovariohysterectomy

OE : Ovarioectomy

LH: Luteinizing hormone

FSH: Follicular Stimulating Hormone

OMH: Anti- Müllerian hormone

Ft4: Free Thyroxine

TSH: Thyroid-Stimulating Hormone

T3: Triiodothyronine

T4: Thyroxine

Hcg: Human Chorio-Gonadotropin

TRH: Thyrotropin-Releasing Hormone

IU: International Unit

Ng: Nano Gramm per Milliliter

µmol: Micromole

PMDS: Persistent Müllerian Duct Syndrome

TGAA: Thyroglobulin Autoantibodies

PMSG: Pregnant Mare Serum Gonadotropin

# 1 Introduction

## **Origin of the domestic dogs**

Domestication of animals, which began more than 15,000 years ago with a long-term interaction between wolves and hunter–gatherers, was one of the most significant transformations in human history.(Frantz *et al.*, 2020) Dogs were the first domesticated species, the only major carnivore to be domesticated, and the only animal known to have entered into a domestic relationship with humans throughout the Pleistocene. Dogs may have had a tremendous influence on the trajectory of early human history and the formation of civilization because of their earlier relationship with humans (Bergström *et al.*, 2020).

## **Canine Estrous Cycle**

The canine estrus cycle comprises 4 stages: **proestrus, estrus, diestrus, and anestrus**. Each stage has its own set of symptoms, including behavioral, physical, or clinical alterations, hormonal, physiologic, and cytologic changes.(Meyers-Wallen, 2007) The stages and changes associated with each stage are described in the following paragraphs. Bitches usually achieve puberty two to three months after they reach adult body size. With a range of four to twenty-four months, the average is six to twelve months.(Feldman and Nelson, 1996a) Smaller breeds mature earlier and, as a result, reach puberty earlier than larger ones. The first estrus might be protracted or split, which is unusual. When young bitches are kept alongside mature cycling bitches, it usually happens sooner.(*Abnormalities in the Sexual Cycle of Bitches* | IntechOpen, no date)

Furthermore, for the detection and identification of the estrus cycle phases, exfoliative vaginal cytology, a non-invasive technique is used. In addition to that, this gynecological examination method is performed in cases of estrus cycle disorders.(Antonov, 2017)

**Proestrus** is the first stage of noticeable changes in the dog. This stage usually lasts 9

days, however it can range anywhere from 3 to 21 days. Male canines will be attracted to the female at this time, but she will not be responsive. This phase is marked by elevated levels of estrogen and the development of follicles which present clinical signs such as a swollen vulva with discharge. Nevertheless, in the case of vaginal cytology, presentation of different types of cells is present such as red blood cells and neutrophils.(Root Kustritz, 2012)

During **estrus**, the female is receptive to the male. Based on behavioral changes, this stage lasts an average of 9 days, but it can last anywhere from 3 to 21 days. During this time, the fertile phase occurs. The vulva grows in size but softens slightly.

The amount of blood in the vulvar discharge has decreased. Estrogen levels are decreasing slowly, while on the contrary progesterone levels are increasing.

Upon vaginal cytology, presentation of cornified epithelial cells will be dominant in this phase.(Root Kustritz, 2012)

***Diestrus*** occurs after estrus, when the female is no longer responsive to the male. The duration of this phase is approximately 2 months pregnant bitches 62- 64 days and non-pregnant bitches 49-79 days. Estrogen levels are low, but progesterone levels peak 3 to 4 weeks after diestrus begins and subsequently drop to baseline by the end of this phase. Whether or not the dog is pregnant, this surge and then reduction in progesterone will occur. Unlike in proestrus, abrupt return to noncornified epithelial cells is noticed and large number of PMNs early in stage

***Anestrus*** is the period between the diestrus and the next estrus cycle. This stage usually lasts approximately 1 to 8 months. FSH receptors are present on the ovary, but follicular growth is repressed until very late in this stage, and serum concentrations of FSH stay steady throughout anestrus at anywhere between 50 and 100% of preovulatory concentrations.(Concannon, 1993)Cycles that are less than 4 months or longer than 10 months are considered abnormal. (Johnston, 1991)

Anestrous cycles can be either primary or secondary. It is considered primary in case the bitch doesn't show any signs of estrus and the cycle hasn't started yet by 24 months.

Generally, cycle abnormalities are usually not investigated until the dog reaches the age of

two years since its delayed in large dogs compared to small dogs that might experience first estrus cycle at the age of 6 months.(Risvanli *et al.*, 2016)

Anestrous, on the other hand, is termed secondary if estrus has not transpired for 10 to 18 months despite the fact that the initial estrus phase occurred previously.(Risvanli *et al.*, 2016) Some factors that lead to primary anestrous can be an early ovariohysterectomy or ovariectomy, usage of glucocorticoids or progesterone, or even due to genetical disorder. If the female dog is adopted or taken from another person, the owner can check for the scar on the ventral wall of the abdomen region to verify if a past surgery was done (Johnston, 1991).

Nevertheless, another three procedures can be done to verify if an OHE or OE was done which are measuring either the AMH (Anti-mullerian hormone) or the LH serum level (Place *et al.*, 2011; Turna Yilmaz *et al.*, 2015). Now in the case of measuring AMH, it is shown that bitches that have undergone surgery have lower levels compared to non-sterilized ones. By measuring the LH serum level, sterilized bitches show a much higher level due to the absence of negative LH feedback. As a third option, serum estrogen level can be measured before and after an hour post intravenous injection of 0.02-0.03 µg/kg buserelin. If estrogen levels are above 15–20 pg/mL, then OHE has been performed (Risvanli *et al.*, 2016).

The major signs of *anestrus* can be the disappearance of discharge from the vagina and the decrease in vulvar puffiness. This period is used by the body to prepare the uterus for the next prospective pregnancy. (Root Kustritz, 2012)

### **Estrus cycle disorders**

Infertility in bitches is defined as the incapability of the female dog to deliver puppies. In many cases the incapability of the female to deliver is due to poorly timed breeding. In this case, many owners will attempt to breed their dog at an immature age which results in inability to deliver(Grundy, Feldman and Davidson, 2002). Other etiologies that can be due to breed liability range from anestrus to split estrus to prolonged estrus. Sexual cycle anomalies can be categorized as either luteal or follicular phase disorder(Risvanli *et al.*, 2016). However, in order to better comprehend these illnesses, pet owners should have a strong working knowledge of their animals' sexual cycles to have a successful outcome. Nevertheless, most female dogs follow the estrus cycle of their dam if they live in the same

house, so it will be easier for the owner to know the exact timing of the dog (Wilborn and Maxwell, 2012)

However, there are situations in which a young puppy might skip a heat period. This could be considered normal since in most cases the cycle will regulate itself in the end. However, in case the situation does not regulate itself back to normal, it can be categorized according to the seven prominent anomalies in estrus cycle.

These anomalies are Anovulation, Silent heat, Split heat, Prolonged heat, Prolonged inter-estrous interval, Shortened inter estrous interval, and finally Premature ovarian failure.

## 2 Objectives

As infertility is a common problem, the question has arisen to whether there is a therapeutic treatment to solve this anomaly? Which of the ovarian disorders are treatable? What breeds are more predisposed to have a genetic defect that causes infertility? How effective are these treatments? The aim of the research was to confirm or refute these questions. We hypothesize that these estrous disorders have a successful treatment outcome which allows the bitch to conceive and deliver.

## 3 Literature Review

During breeding phase of the bitch preovulatory serum progesterone concentrations are measured to calculate the day of LH peak, not only to precisely timing insemination and anticipate parturition, but also to identify irregular or uncommon estrous cycles caused by ovarian malfunction(Risvanli *et al.*, 2016). The importance of early detection of these illnesses, both therapeutically and economically, cannot be overstated. The following are the topics covered in this review: Anovulation, Silent heat, Split heat, Prolonged heat, Prolonged inter-estrous interval, and finally Shortened inter estrous interval.



## **Anovulation**

Anovulation is characterized as a failure of serum progesterone level to reach 2 ng/ml. Despite the noncornified cell type in vaginal cytology, there is no diestrous-specific progesterone increase, and the bitch falls into anestrus (Risvanli *et al.*, 2016). However, decrease of serum progesterone levels and the absence of ovulation during the days following copulation in a bitch with proestrous and estrous signs are the most common symptoms. (Gobello, Castex and Corrada, 2002; Meyers-Wallen, 2007)

When it comes to the absence and loss of heat, **hypothyroidism** can be a factor (Risvanli *et al.*, 2016). A complete thyroid blood test is commonly used to determine this (Wilborn and Maxwell, 2012). There are unusual causes as well, such as Cushing's syndrome which was shown in one study that 75% of affected bitches lacked cyclicity (*Infertility in the Dog and Cat - PMC*, no date), although in this situation, females should have roughly 8 years or more to live, so they won't be able to reproduce anyway.

## **Diagnoses of Hypothyroidism**

In case of hypothyroidism, 3 popular diagnostic methods are used which are Blood tests (complete blood count, blood test of thyroid function), Radiography, and Ultrasonography. (Mooney, 2011) In case of the blood test of thyroid function, total serum and fT4 are measured in addition to serum TSH. However, measuring serum T3 levels is not recommended as it is not a poor indicator of thyroid function due to its location and the secreted amount compared to T4. (Mooney, 2011)

As for the complete blood count, anemia is most likely seen in case of hypothyroidism due to the fact of decrease of erythropoietin. Nevertheless, fasting hypercholesterolemia is seen in most cases of hypothyroidism in a serum biochemistry screening which both the synthesis and degradation of lipids is affected negatively.

Furthermore, in radiography, a delayed epiphyseal ossification and epiphyseal dysgenesis (i.e., irregularly formed, fractured, or stippled epiphyseal centers) are typical radiographic anomalies in congenital hypothyroidism. (*Recent approaches in diagnosis and management of canine hypothyroidism: A review* | *Semantic Scholar*, no date)

The use of ultrasound to confirm hypothyroidism may also be beneficial. A hyper-echoic capsule outlines the normal thyroid gland, which is homogeneous and well defined. The

parenchyma is hyperechoic to the muscles around it, and its size is proportional to the dog's size (body surface area).(Mooney, 2011)

**Ovarian hypoplasia** is a condition in which the ovaries fail to sexually mature and are incapable to produce enough estrogen. The vulva and mammary glands are still undeveloped. Sex chromosomal disorders (example Trisomy-X) could cause ovarian hypoplasia. A few cases of missing heat may also be linked to an immune-mediated inflammation of the ovaries. Ovarian tumors have also been linked to a lack of heat (Christensen, 2012).

There's also Karyotyping, which is a chromosome analysis that comes in handy for females who haven't had a cycle in the last six months of research. Estrus and ovulation can be produced by FSH and hCG under a regimen specified by your veterinarian, even if the chromosomes are normal and the cycle won't occur for another 2.5 years.(Christensen, 2012)

### **Silent heat**

Silent heat is characterized as maintenance of normal ovarian activities without the appearance of the external and internal signs of heat which are vulvar edema, vaginal discharge and attracting male dogs (Risvanli *et al.*, 2016). However, it is very hard for the owner to determine whether there was vaginal bleeding, since the dog usually licks herself clean.

In young bitches of small breeds, it is more seen for several cycles before proestrus. However, in other cases if silent heat is suspected serum progesterone levels are measured monthly to confirm normal ovarian function which should be normally above 2 ng/ml (Risvanli *et al.*, 2016). Another simple test is to get a male dog and see how the two dogs behave with one another; if the female appears receptive and docile, she is most likely in silent heat (therefore still in heat).(Grundy, Feldman and Davidson, 2002)

### **Split heat**

Split heat is defined as short intervals of proestrus signs ranging from 2 weeks to 2 months correlated with interestrus periods and usually is seen more often in young bitches. In this case, even if a “successful” copulation takes place pregnancy would not be achieved

(Risvanli *et al.*, 2016). However, the next estrus cycle would be a normal one but in case split estrus is seen more frequent this would suggest chronic premature luteolysis or even hypothyroidism (Risvanli *et al.*, 2016). Hypothyroidism is an indirect disease that affects the estrus cycle of bitches by having an increase in prolactin levels, which significantly leads to the impairment of follicular development by inhibiting GnRH. This disease will also lead to the deterioration of the serum total and free thyroxin levels due to the destruction of the thyroid gland. As a consequence, the TRH and TSH hormones will increase. (Mooney, 2011)

### **Prolonged heat**

On average estrus lasts 9 days in an adult dog, but it can last up to 3 weeks in some cases. Prolonged estrus is characterized as estrus lasting more than 21 days without ovulation at the course of that time (Risvanli *et al.*, 2016). In this case, prolonged estrus is associated with consistently high levels of estrogen during the cycle which remains high during the cycle. This condition is common in younger dogs, particularly during the second cycle (Risvanli *et al.*, 2016). Cornification of vaginal epithelial cells, continued urge for copulation, vulvar edema and swelling, and hyperemia in the vaginal mucosa for more than 21 days are the most common clinical indicators of estrus continuation. The level of progesterone in the blood is low, but estrogen is high.(Risvanli *et al.*, 2016)

An anovulatory follicle, follicular cysts, or functional ovarian tumors are the most common sources of persistent estrus (granulose cell tumors) (Risvanli *et al.*, 2016). The follicles that form in bitches given exogenous gonadotropins to induce estrus might sometimes result in delayed estrus. Exogenous estrogen therapy for urinary incontinence or vaginitis, hormone replacement therapy, and pregnancy prevention may all cause persistent estruses (Risvanli *et al.*, 2016).

Persistent estrus can also occur as a result of malignancies of the hypophysis or hypothalamus, or as a result of a hepatic condition known as portosystemic shunting, in which an atypical vascular junction is present (Risvanli *et al.*, 2016). Ovarian tumors require immediate surgical intervention in order to be excised. Ovarian cysts must be monitored for a period of time to see if they regress; if they do not, surgery will be required (Risvanli *et al.*, 2016). This monitoring period will be accompanied by the help of

ultrasound to check any persistent abnormal structure such as an ovarian cyst or granulosa cell tumor. However, if the ovarian structures cannot be identified then it is possible to perform a laparotomy followed by biopsy if needed (Risvanli *et al.*, 2016).

Prolonged estrus caused by follicle cysts or follicles may be cured on their own. If estrus lasts more than three weeks, medical intervention is recommended to prevent bone marrow hypoplasia or even pyometra (Risvanli *et al.*, 2016). As for the medical intervention, OHE can be an option if a puppy is not expected.

On the other hand, if the owner wants a puppy it is possible by administering GnRH or hCG injections into follicles to permit ovulation/luteinization. It is recommended to administer 22 IU/kg hCG via intramuscular route for 3 days and GnRH at a dose of 10 IU/kg for 3 days via the same route (Risvanli *et al.*, 2016). However, the owner must know that copulation is not recommended since this procedure is not for the induction of ovulation but for the cessation of prolonged estrus signs.

Another way to minimize the signs of prolonged estrus is the administration of megestrol acetate. (Risvanli *et al.*, 2016)

For two weeks, low doses of megestrol acetate should be taken orally. For the first week, a dose of 0.1 mg/kg is ideal, and for the second week, a dose of 0.05 mg/kg is suitable.

Although megestrol acetate progesterone therapy is beneficial in bitches with prolonged estrus, it has the tendency to cause cystic endometrial hyperplasia (Risvanli *et al.*, 2016).

As a result, the procedure is not recommended for bitches that will copulate in the future.

In bitches with persistent estrus, ovariohysterectomy is usually performed 3 weeks following progesterone medication (Risvanli *et al.*, 2016).

### **Prolonged inter-estrous interval**

When the interestrus period exceeds 12 months it is characterized as a prolonged interestrus interval. While some adult bitches experience estrus 12–13 months after the previous estrus (prolonged interestrus interval), others are not seen to experience estrus for a long time (secondary anestrus) (Risvanli *et al.*, 2016).

It's important to keep in mind that breeds such as Tibetan Mastiff and Basenji have a longer interestrus period in comparison with other breeds (Risvanli *et al.*, 2016).

Hypothyroidism, hyperadrenocortisolism, hyperprolactinemia, progesterone-secreting

ovarian cysts, progestogen, androgenic, or anabolic steroid drugs, systemic disorders, inadequate nutrition, or dwelling in an improper environment can all cause secondary anestrus (Risvanli *et al.*, 2016). It is crucial to evaluate the thyroid function in female dogs with prolonged interestrus period. When the underlying cause is addressed, cycles usually return back to normal.

### **Shortened inter estrous interval (Recurrent estrous)**

The normal inter estrus interval is usually 7 to 8 months in most dog breeds. However, some breeds have a shorter period such as in German Shepherds, Akitas, Rottweiler and Labrador Retriever (Risvanli *et al.*, 2016). During Anestrus, the uterine epithelium will be recovering from the extensive hormone exposure in estrus and diestrus (Grundy, Feldman and Davidson, 2002). If the inter estrus interval is shorter than the normal range, the endometrium may not be able to support the embryo. This anomaly can be caused by ovulatory failure that is due to an overstimulation of the ovaries or a decrease of progesterone levels during estrus phase (Grundy, Feldman and Davidson, 2002).

Nevertheless, by using mibolerone a weak androgen, the shortened inter estrus interval can be extended to reach 6 months. The suggested dose for estrus suppression is usually 30–180 µg per day (Risvanli *et al.*, 2016).

### **Diagnoses of heat disorders**

Vaginal cytology, physical examination, ultrasound

#### **Physical examination**

Before beginning an assessment of the reproductive system, a full physical examination should always be performed. Following the physical examination, the veterinarian should assess the external genitalia, paying special attention to vulvar structure, and then perform a digital examination of the vaginal canal and rectum. Furthermore, the abdomen should be palpated with thoroughness. The stage of the estrous cycle should then be determined by vaginal cytology (Grundy, Feldman and Davidson, 2002). Nevertheless, an abdominal ultrasound should be performed to investigate the structure of the ovaries and uterus if the vaginal cytology is indicative with either diestrus or anestrus.

In order to develop a successful breeding scheme, a bitch should be tested for infertility as soon as possible before the commencement of proestrus. Ovulation time can be used to assess ovarian function if the bitch is in proestrus or estrus. In this circumstance, the bitch's fertility may be determined while also ensuring that she gets bred at the appropriate periods. This combination of breeding and evaluation may be limited if a bitch is presented at other periods. The clinical approach and management, however, are identical.

A core database consisting of a complete blood count, serum biochemistry profile, urinalysis should be assessed for indication of systemic disease after the physical examination is finished (Grundy, Feldman and Davidson, 2002).

At this time, the patient's vaccination and deworming history, as well as his or her heartworm status, should be evaluated. Significant anomalies discovered through a basic database or physical check should rule out the use of an animal for breeding (Grundy, Feldman and Davidson, 2002).

### **Problem: Failure to Cycle**

From history, it is simple to recognize these animals. Identifying primary anestrus, silent estrus, and secondary failure to cycle is useful.

Silent heats, or bitches that are just "not ordinary" in regards of the interestrus interval, are the most frequent causes of failure to cycle. Delay in puberty, prior ovariohysterectomy, disorders in sexual development, ovarian aplasia, ovarian cysts, and immune-mediated oophoritis are less frequent differential diagnoses for primary anestrus (Fontbonne, 2011). Secondary failure to cycle can have less frequent differential diagnoses, such as systemic illness, ovarian neoplasia, endocrinologic dysfunction such as hypothyroidism or other disorders (Grundy, Feldman and Davidson, 2002).

When a cycle fails, infertile bitches are often in anestrus or diestrus. A bitch's anestrus and diestrus cannot be distinguished by vaginal cytology alone.

Both phases of the estrus cycle can be seen on vaginal cytology, with the majority of parabasal cells and a few intermediate vaginal epithelial cells, irrespective whether they include white blood cells or bacteria (Grundy, Feldman and Davidson, 2002). Diestrus and anestrus can be distinguished from one another by analyzing the vaginal cytology in conjunction with serum progesterone levels (Grundy, Feldman and Davidson, 2002).

Nevertheless, the proper vaginal cytology and a serum progesterone concentration of between 1 and 2 ng/ml are both indicators of a bitch being in anestrus (Grundy, Feldman and Davidson, 2002).

While bitches in diestrus will have vaginal cytology identical to those in anestrus, their serum progesterone levels will be higher generally than 2 ng/ml (generally more than 5 ng/ml). An ovarian cycle should have taken place within the previous 45 to 90 days if diestrus is found (Grundy, Feldman and Davidson, 2002). The bitch may have a luteinized ovarian cyst if diestrus is protracted, In that circumstance, it is advised to perform an abdominal ultrasound now to picture the ovaries and uterus in order to exclude out pregnancy (Grundy, Feldman and Davidson, 2002). Estrus induction can be taken into consideration if anestrus is proven, protracted (>2 years), and all differentials have been ruled out. However, induction with Prolactin inhibitors, such cabergoline, can successfully induce ovulation at a high cost which is about \$1,000 per bitch (Grundy, Feldman and Davidson, 2002).

If superficial cells are present in the vaginal cytology, the bitch is either in proestrus or estrus. Both breeding and ovulation timing must be accomplished. She may have an ovarian follicular cyst if proestrus or estrus lasts a long time (more than 30 days). To find ovarian cysts, the ovaries should undergo an ultrasound examination (Grundy, Feldman and Davidson, 2002).

### **Silent or Missed Heat**

The vast majority of owners base their determination of the beginning of proestrus, thus, the beginning of an estrous cycle, on the presence of a bloody vaginal discharge. Receptivity of the female is typically utilized to predict advancement to the fertile phase if a male is available. Estrus identification is typically simple, despite variances in reproductive behavior (Grundy, Feldman and Davidson, 2002). Owners should be advised to keep the bitch with an intact cycling female and to submit weekly vaginal cytology smears for a prospective evaluation for estrus or monthly serum progesterone samples for an evaluation of ovarian activity.

Continued anestrus is confirmed by the absence of superficial cells on vaginal cytology and the persistence of baseline serum progesterone levels usually 1 to 2 ng/ml during a 12-month period. Additional testing for failure to cycle should be carried out as necessary,

including a thyroid panel, an abdominal ultrasound, a chemistry panel, and a complete blood count. Estrus induction may be considered if all other potential explanations of a secondary failure to cycle have been ruled out (Grundy, Feldman and Davidson, 2002).

### **Delayed Puberty**

Beginning of pubertal estrus can happen in a bitch either as early as 6 months or as late as 18 to 24 months (Johnston, 1991). Since it is reported in various cases that pubertal bitches lack sexual receptivity, it is constantly advised delaying a bitch's diagnostic evaluation till she is at least 24 months old . All bitches exhibiting an estrus cycle failure should be evaluated for a silent or lack of estrous cycle to confirm sustained aestrus. However, estrus induction should only be used on healthy bitches with extended interestrus intervals (Grundy, Feldman and Davidson, 2002).

Before considering estrus induction, a patient's physical examination, database, dietary status, and environmental stressors should all be ruled out as potential causes(Grundy, Feldman and Davidson, 2002).

It is possible to try to induce estrus in three different ways: (1) by directly stimulating the ovarian follicle (equine chorionic gonadotropin, estrogen, follicle-stimulating hormone [FSH], and LH), (2) by vivifying the release of pituitary gonadotrophins (gonadotropin-releasing hormone [GnRH] and its analogues), or (3) by shortening the anestrus with prolactin inhibitors or dopamine agonists (cabergoline, bromocriptine), however viable estrus induction does not always result in a prolific ovarian cycle (Concannon, 1989; Johnston, 1991)

Possible side effects of estrus reduction are typically drug-related and include nausea, diarrhea, and thrombocytopenia (Concannon, 1989) . Cabergoline (5 x g/kg/day) is the most clinically effective treatment because it induces fertile estrus and ovulation more predictably than gonadotropins or estrogen and has less gastrointestinal side effects than bromocriptine (Grundy, Feldman and Davidson, 2002).

Nonetheless, as it was already stated, the medication is costly and needs to be formulated for dosing in dogs. Inadequate gonadotropin stimulation, ovarian aplasia, or anomalies of sexual differentiation can all lead to failure to respond to estrus induction. In order to check for irregularity of sexual development, a lack of reaction is a sign that karyotyping, imaging, and maybe surgical examination of the female reproductive tract are necessary.



Only abdominal radiography, magnetic resonance imaging, and ultrasound are available for noninvasive imaging of the ovaries (Grundy, Feldman and Davidson, 2002).

It's vital to understand that abdominal ultrasonography cannot reveal the reproductive tract's histology or function.

On ultrasound, in some cases it been had seen what appeared to be "regular ovaries" that later turned out to be ovotestes. It is necessary to do a laparoscopy or exploratory celiotomy to collect ovarian tissue samples for biopsy (Grundy, Feldman and Davidson, 2002).

### **Previous Ovariohysterectomy**

As a result of early neutering programs, some puppies are neutered as young as 7 weeks old. As a consequence, a bitch's owner might not be aware that the bitch has been spayed. There are no physical traits that are useful for diagnosis for an ovariohysterectomized animal, making it extremely difficult to discern between an anestrus bitch and an ovariohysterectomized bitch (Grundy, Feldman and Davidson, 2002). The absence of the ovary's negative feedback is a consequence/result from ovariohysterectomy surgery, which also causes substantial rises in the levels of LH and FSH in the blood. However, there are just a few commercially available quantitative gonadotrophin assays. Only direct assessment of the reproductive system can serve as a substitute for ovariohysterectomy documentation (Grundy, Feldman and Davidson, 2002).

### **Abnormalities of Sexual Development**

The three basic forms of disorders of sexual differentiation are: (1) abnormalities of chromosomal sex; (2) abnormalities of gonadal sex; and (3) abnormalities of phenotypic sex. The chromosomes that determine sex are among the distinctive chromosomes found in an average dog. The X chromosome comes from the dam, and the X or Y chromosome from the sire (Christensen, 2012).

A dog's typical karyotype is 78, XX for a female and 78, XY for a male. The gonads become the ovaries in a female (XX) animal, and the absence of testosterone causes the mullerian ducts to grow into the female reproductive system (Christensen, 2012). The testicles mature and secrete testosterone and a chemical that inhibits mullerian growth

when the Y chromosome is present. As a result, the mullerian ducts degenerate and the wolffian ducts transform into the male reproductive tract(Christensen, 2012).

The gonadal sex type of an animal is determined by the sort of gonads it develops (Christensen, 2012). If an animal grows ovaries, for instance, its gonadal sex type is female. The term "phenotypic sex" describes an animal's outward appearance for example, a phenotypic female appears externally to be female (Christensen, 2012). The last step of sexual development is phenotypic sexual differentiation. Throughout these stages, abnormalities can happen at any time.

Chromosome sex abnormalities, such as XXY, XO, or XXX, have been observed in dogs (Wikström and Dunkel, 2011). These dogs typically have undeveloped genitalia but otherwise appear to be normal males or females. Only by karyotyping may chromosomal sex abnormalities be identified (Christensen, 2012).

Animals with abnormal gonadal sex have chromosomal and gonadal sex imbalances. For instance, XX true hermaphrodites, XX male syndrome, or XX sex reversal. Breeds include cocker spaniels, beagles, Chinese pugs, Weimaraners etc. are said to have a family history of gonadal sex abnormalities (Meyers-Wallen and Patterson, 1988; Christensen, 2012). The gonads are histologically examined, and karyotyping is used to make the diagnosis. When karyotype and gonadal sex concur but are discordant with phenotypic sex, phenotypic sex is abnormal. For instance, a 78, XX animal has male external genitalia and ovaries.

Pseudohermaphrodites is the name given to these animals (Christensen, 2012).

### **Phenotypic Abnormalities**

When the chromosomal and gonadal sex are in agreement but the phenotypic disagrees or is unclear, the condition is referred to as pseudohermaphrodites (Christensen, 2012). The histology of the gonads is usually required to distinguish between these situations, which are frequently misinterpreted as sex reversals. When female fetuses are exposed to endogenous or exogenous synthetic progesterone or androgens during development, they can become female pseudohermaphrodites (Curtis and Grant, 1964; Knighton, 2004).

However, there are two different types of male pseudohermaphroditism that have been described and have distinct etiologies: (1) persistent Müllerian duct syndrome (PMDS) and (2) failure of androgen-dependent masculinization (Christensen, 2012).

For a precise diagnosis, it is essential to look at the external genitalia, perform karyotyping and histology on the gonads to identify the type or types of gonadal tissue present as well as the phenotypic and chromosomal sex.

For animals with aberrant sexual development, there is no cure. It is advisable to neuter these animals and have the gonads histologically examined. The genetic effects of these illnesses should be discussed with the breeder.

### **Ovarian Aplasia**

This uncommon congenital condition results from improper prenatal germ cell migration, which impedes the ovaries development (Johnston, 1989, 1991; Feldman and Nelson, 1996a).

### **Luteal Cysts**

Ovarian folliculogenesis is inhibited by progesterone-secreting luteal cysts, which are functional ovarian cysts (Feldman and Nelson, 1996a). A prolonged rise in blood progesterone concentration more than 2 ng/ml for more than 10 weeks, along with abdominal imaging showing cystic ovarian structures, can be used to diagnose progesterone-secreting ovarian cysts (Feldman and Nelson, 1996a). In a breeding dog, the preferred course of treatment is a unilateral ovariectomy because it is challenging to remove the cyst itself. In this specific case histology is required (Feldman and Nelson, 1996a).

### **Iatrogenic/Drug-Induced Infertility**

Through adverse feedback on the pituitary, any pharmaceutical, nutraceutical, or herbal supplement that has a glucocorticoid, estrogenic, progestational, or androgenic impact may induce a reduction in blood gonadotropin concentrations. Glucocorticoids and androgens are among the most typical drugs used. Whatever medicine taken regularly should be examined for a potential role in infertility (Feldman and Nelson, 1996a; Wilborn and Maxwell, 2012).

### **Thyroid Dysfunction**

Our understanding of how thyroid disease affects a bitch's reproductive cycle is limited and contentious. This is most likely due to the difficulties in making a conclusive diagnosis of

hypothyroidism, which makes it impossible to demonstrate a causal link between a real hypothyroid state, thyroxine supplementation, and a restoration to normal reproductive function (Grundy, Feldman and Davidson, 2002).

The impairment of the thyroid gland is the common primary cause of hypothyroidism in dogs. One of most typical causes include atrophy brought on by lymphocytic thyroiditis (Feldman and Nelson, 1996a). The levels of serum total and free thyroxine drop as a result of thyroid gland failure. In response, the hypothalamus produces more thyrotropin-releasing hormone (TRH), which prompts the pituitary to release more thyroid-stimulating hormone (TSH). In dogs with primary hypothyroidism, this physiological mechanism causes the classic serum biochemical results of decreased free and total thyroxine concentrations and elevated serum TSH concentrations (Grundy, Feldman and Davidson, 2002).

Only if clinical and biochemical symptoms of hypothyroidism are present in an infertile animal should thyroid testing be carried out (Grundy, Feldman and Davidson, 2002). Gaining weight, mental fatigue, exhaustion, a dry, fragile hair coat or alopecia, cycle failure, or other anomalies of the reproductive cycle are only a few of the clinical signs of thyroid dysfunction that can be non-specific. Thrombocytosis, hypercholesterolemia, and a non-regenerative anemia are examples of clinicopathologic irregularities (Feldman and Nelson, 1996b). Because a variety of unrelated variables can reduce the quantities of circulating thyroid hormone, interpreting thyroid gland function tests can be challenging. Any disease may have a significant impact on the serum thyroid hormone levels and the thyroid's ability to respond to TSH and TRH. The altered thyroid condition that develops related to illness and may be mistaken for a true hypothyroid state is referred to as euthyroid sick syndrome (Feldman and Nelson, 1996b). In order to prevent unnecessary thyroid testing and the wrong diagnosis of hypothyroidism, thyroid testing in breeding bitches must be based on clinical indicators and biochemical abnormalities other than infertility (Grundy, Feldman and Davidson, 2002).

Histologic analysis of the thyroid glands is necessary for a conclusive diagnosis of hypothyroidism (Grundy, Feldman and Davidson, 2002). Thyroid function testing in a clinical environment should evaluate the levels of total and free serum thyroxine, serum TSH, and serum thyroglobulin autoantibodies (TGAA). Total thyroxine and free serum thyroxine will drop in a true hypothyroid state, TSH will rise, and TGAA may be positive

if immune-mediated lymphoplasmacytic destruction of the thyroids, a hereditary illness, is taking place. All hypothyroid animals receiving thyroxine supplements should have their clinical response to treatment documented (Grundy, Feldman and Davidson, 2002).

### **Uterine Infection**

Several aerobic bacteria can be found in the uterus and vaginal vault of healthy female reproductive tracts including mycoplasma species. Several investigations have demonstrated that healthy bitches can harbor mixed vaginal cultures; the most prevalent isolates include *Pasteurella multocida*, hemolytic streptococci, *Escherichia coli*, and *Mycoplasma* spp (van Duijkeren, 1992; Watts, Wright and Whithear, 1996). The only bacterial species that is proven to be a specific cause of infertility in the bitch is *brucella canis* (van Duijkeren, 1992). Recent development of transcervical uterine cannulation has enabled intra-uterine cultures and biopsies, to be collected noninvasively (Watts, Wright and Whithear, 1996; Wilson, 2001).

During normal canine estrus, bacteria ascend the reproductive tract and are present within the uterus. The existence of bacteria in the uterus at various times of the estrus cycle has been examined in two studies that have been published in literature. In both investigations, an increase in intrauterine bacteria was seen during proestrus and estrus and thereafter spontaneously disappeared (Watts, Wright and Whithear, 1996; Wilson, 2001).

Consideration should be taken when assessing vaginal and intrauterine cultures because many bacterial populations are part of the normal bacterial flora and do not always signify disease or provide a justification for infertility (Bjurström, 1993). The proliferation of bacteria from the vagina or uterus is typically considerable and requires the use of antimicrobial medications in conjunction with clinical symptoms of excessive or atypical vaginal secretions, vaginal mucosal inflammation, peripheral leukocytosis, and systemic disease (Grundy, Feldman and Davidson, 2002). If at all possible, cytology or a biopsy of the uterus should be screened for signs of infection or inflammation. It is illogical to presume that all positive vaginal or uterine bacterial cultures are related to infertility or to treat all positive vaginal cultures with antibiotics (Feldman and Nelson, 1996a).

## **Vaginal cytology**

After completing the physical examination, it is suggested to perform vaginal cytology which is the most used diagnostic test as part of the gynecological checkup. It is based on the identification of cellular types that helps in determining the cycle's phase (Antonov, 2017).

A sterilized speculum and a saline-moistened cotton swab could be used to sample vaginal cells . It's crucial to keep the swab away from the vestibule since its cells don't react as quickly to a spike in blood estrogen levels as the vaginal mucous membrane does (Wehrend, von Plato and Goericke-Pesch, 2013) . Nevertheless, vaginal cells could also be collected by only using a cotton swab.

The cotton-tipped end of the swab is inserted into the vulva's dorsal commissure, gently pressed in the caudo dorsal direction until it passes over the ischial arch, and then rotated completely in each direction before being withdrawn (Aydin *et al.*, 2011). From one end of a glass microscope slide to the other, a cotton tip is delicately rolled (Feldman and Nelson, 1996b).

Vaginal cells can be obtained by injecting sterile saline into the vaginal cavity and aspirating it using a plastic catheter. On a glass microscope slide, the liquid is applied, distributed into a thin coating, and air-dried (Antonov, 2017).

Although the approach is non-invasive, a change in cell shape and a reduced absolute cell count have been noted in some cases (Olson *et al.*, 1984).

Trichrome or new methylene blue could be used to stain the smears. Smears stained with new methylene blue could be examined right away, but as for RBC will not be stained, and the smears cant be saved for subsequent study (Johnston, Root Kustritz and Olson, 2001). Another stain that is can be easily implemented in daily clinical work is a Diff-Quick which is a Wright-Giemsa stain. Smears are fixated with methanol and after stained with Diff-Quick stain. The advantage of these types of stain is that its long storage period. It can be used for later on examination unlike the New methylene blue (Johnston, Root Kustritz and Olson, 2001).

As for the vaginal cytology evaluation, a light microscope with magnifications ranging from 100 to 400 is used to examine the vaginal smears (Antonov, 2017) . There are various types of vaginal cells. The cells are divided into basal, parabasal, intermediate, superficial, and squamous cells (Wehrend, von Plato and Goericke-Pesch, 2013).

These cell types are correlated to the cycle's phase which serves vaginal cytology as a significant diagnostic tool in evaluating gynecological disorders.

The smallest cells that are found in the vaginal wall (10–20 µm) are known as basal cells. Since they reside on the basement membrane, they are virtually fully occupied by the nucleus and are rarely seen in smears (Olson *et al.*, 1984). However, parabasal cells are relatively small too (15–25 µm), characterized with an oval shape, and have a large nucleus. It's also possible that parabasal cells include granulocytes of neutrophils in the cytoplasm (Olson *et al.*, 1984). These types of cells are likely seen on smears obtained during diestrus and anestrus.

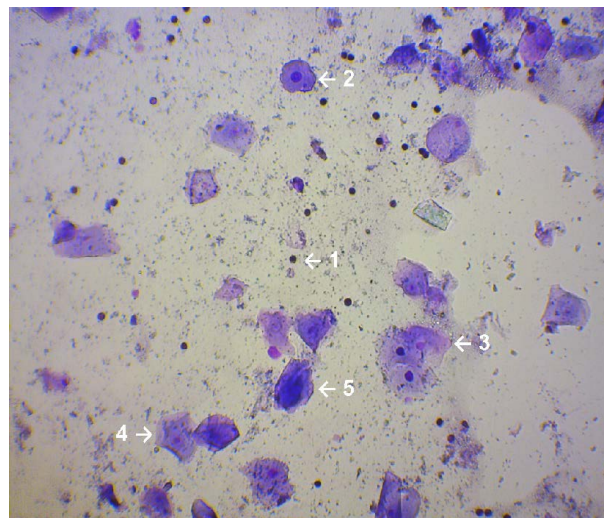


Figure 1 Fig. 1. Vaginal smear of a bitch in proestrus: 1 – red blood cell; 2 – small intermediate cell; 3 – large (Antonov, 2017)

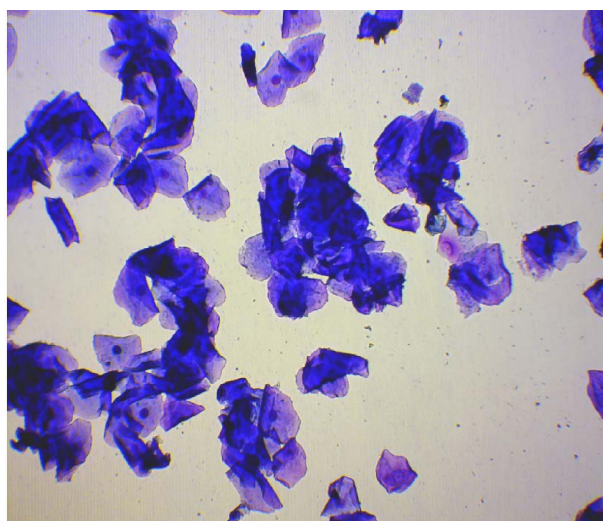


Figure 2 Vaginal smear of a bitch in estrus: 1 – superficial cell; 2 – squamous cell (Antonov, 2017)

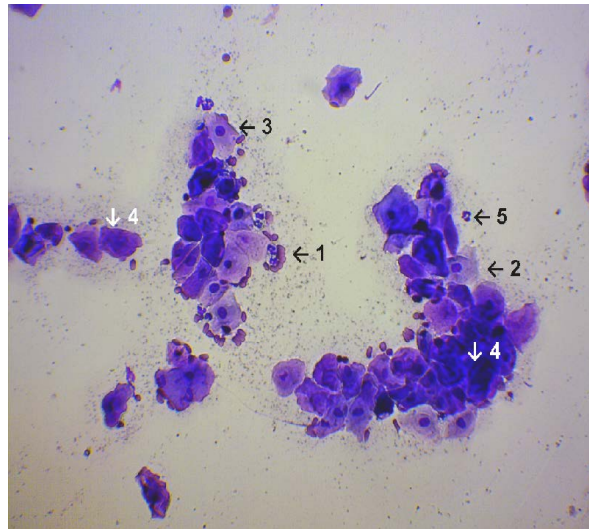


Figure 3 Vaginal smear of a bitch on the first day of diestrus: 1 – red blood cell; 2 – small (Antonov, 2017)

Since intermediate cells vary greatly in diameter, they are classified as either small (20  $\mu\text{m}$ ) or large (30  $\mu\text{m}$ ) (Olson *et al.*, 1984; Wehrend, von Plato and Goericke-Pesch, 2013). The nuclei of both types are well-shaped. The small intermediate cells are oval shaped with a large notable nucleus, meanwhile the large intermediate cells are polygonal shape characterized with a small/ irregular cytoplasmic/nuclear ratio (Johnston, Root Kustritz and Olson, 2001). Due to the similarity of both sizes, the large intermediate cells are sometimes mixed up with superficial cells. Nevertheless, superficial cells are considerably large cells having a diameter of 30 to 75  $\mu\text{m}$ . Their margins are uneven, and the nucleus is either missing or pyknotic(dim/dark). They are mostly seen during proestrus due to the peak of estrogen levels (Johnston, Root Kustritz and Olson, 2001).

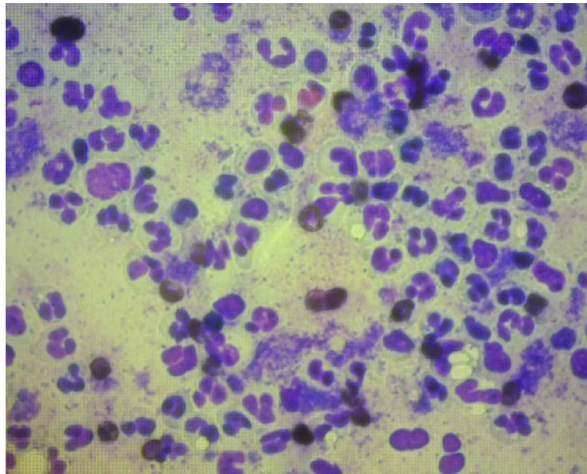
Finally, the last type of cells is the squamous. Squamous cells are basically huge cornified superficial cells that have disintegrated into dead cells lacking a nucleus after degeneration. During estrus, they normally are stained with a dark blue-purple color (Johnston, Root Kustritz and Olson, 2001).

An abdominal ultrasound should be completed to evaluate the morphology of the ovaries and uterus. If proestrus/estrus is prolonged (>30 days), she may have an ovarian follicular cyst. The ovaries should be evaluated with ultrasound to detect the presence of ovarian Cysts (*Granulosa Cell Tumor in a Bitch - Case Report | IVIS*, 2012).



## **Vaginitis**

The vaginal smear in dogs with vaginitis usually consists of polymorphonuclear leukocytes, with or without bacteria (Johnson, 1991).



*Figure 4 Vaginal smear from a bitch with vaginitis (Antonov, 2017)*

## **Ovarian remnant syndrome**

Ovarian remnant syndrome is defined as the presence of functional ovarian tissue left in the abdomen in a previously ovariohysterectomised bitch. The clinical presentation is characterised by signs of proestrus and estrus, including vulvar swelling and haemorrhagic discharge and behavioural changes, such as flagging (Antonov, 2017). Due to the estrogen produced by the remnant, vaginal exfoliative cytologic examination may reveal cornified epithelial cells, similar to normal proestrus and estrus stages (*Granulosa cell tumor of the ovary in dog: Case report from Tehran | Semantic Scholar, no date*).

## **Ovarian cysts and tumours**

Bitches with ovarian cysts or granulosa cell tumour have hormonal imbalances such as excessive estrogen production, exhibiting prolonged estrus, vulvar swelling, dermatological changes and cystic endometrial hyperplasia with persistent serosanguineous discharge (Olson *et al.*, 1984). Vaginal cytology is characterised with more than 80–90%

superficial and squamous cells, similarly to the normal estrus stage, so abdominal ultrasonography is required (Antonov, 2017).

### **Mismating**

Vaginal cytology is the best evaluation method for diagnosing a mismated bitch.

Spermatozoa could be found in vaginal smears for 24–36 hours after mating.

Lack of sperm does not eliminate mating (Antonov, 2017). It could be impossible to find spermatozoa because there are none present if a mating did not take place or there was in fact a copulation but no sperm have been recovered. In most cases where the female dog is suspected to be infertile, it is mainly due to mismanagement of breeding (Antonov, 2017).

The optimum time for normal breeding or AI is the estrus stage, when the rate of superficial and squamous cells in vaginal smears is over 80% (Johnston, Root Kustritz and Olson, 2001).

### **Vaginal tumours**

The transmissible venereal tumour is the most frequently diagnosed neoplasm in the bitch using vaginal exfoliative cytology (Antonov, 2017). Vaginal smears contain copious amounts of erythrocytes, neutrophils, lymphocytes, parabasal, intermediate cells and round to-ovoid shaped cells containing intracytoplasmic vacuoles (Antonov, 2015).

## **4 Methods and Results**

### **Treatment protocols for heat disorders**

To begin with, the pet's owner should have a thorough understanding of the signs of estrus, its cycle, the dog's age and if any previous surgeries have been performed such as OH/O.

Nevertheless, confirmation of any previous drug administration should be known.

Before any intervention, the level of serum progesterone in anestrus bitches should be evaluated periodically for 6 to 8 months (Risvanli *et al.*, 2016).

In an average female population, the progesterone level after estrus exceeds 2 ng/ml, however if the levels are below 2ng/ml for about 7 months it definitely signifies prolonged anestrus (Risvanli *et al.*, 2016). Weekly vaginal smears should also be used to check for any changes in vaginal epithelial cells. Following a general inspection, anestrous bitches should have routine blood and urine tests (full blood count, biochemical analysis), as well as thyroid function testing (Risvanli *et al.*, 2016). When a luteal cyst or tumor of the ovaries is suspected, a progesterone test and an ultra-sonographic examination of the ovaries should be performed. If genital organ developmental defects are suspected, a karyotype analysis should be conducted (such as hermaphroditism) (Risvanli *et al.*, 2016). The etiology of primary or secondary anestrus is the right treatment. If a bitch has had an ovariectomy or ovariohysterectomy, ovarian aplasia, or autoimmune oophoritis, there is no treatment available. Autoimmune oophoritis necessitates ovariohysterectomy. In bitches diagnosed with hypothyroidism-related anestrus, hormone replacement therapy may be used (Simpson, Devi and Whitem, 2013).

In case of anestrus bitches that the underlying cause cannot be verified, it is possible to use estrus stimulation test. Accordingly, exogenous gonadotropins (LH, FSH, hCG, PMSG), GnRH agonists (lutrelin, buserelin, fertirelin, deslorelin), dopamine agonists (bromocriptine and cabergoline), synthetic estrogens (diethylstilbestrol), and dopamine agonists (cabergoline and bromocriptine) may be utilized. (Kutzler, 2007; Wiebe and Howard, 2009).

Dopamine agonists are important in improving fertility in most bitches; however, they may need to be used for a long time (Gobello, Castex and Corrada, 2002; Kutzler, 2007). For this, bromocriptine or cabergoline are utilized which act primarily on the D2 receptors of the pituitary gland. Bromocriptine is less liked because it induces vomiting and must be used for long periods of time to stimulate estrus (Gobello, Castex and Corrada, 2002). Cabergoline, while more expensive than bromocriptine, may produce estrus more effectively and safely, with fewer adverse effects. Cabergoline is normally given orally at a dose of 5 g/kg every day until 3–8 days after proestrus has started (Gobello, Castex and Corrada, 2002; Kutzler, 2007).

Estruses can be induced by using GnRH and its analogs. To induce productive estrus, however, long-acting analogs such as lutrelin, deslorelin, or leuprolide must be used for at

least 8 days. Because GnRH should be given as a pulsatile continuous infusion at a dose of 0.2–0.4 g/kg every 90 minutes via the intravenous or subcutaneous route for 3–9 days, it is impossible to induce estrus with short-acting natural GnRH or GnRH agonists. This necessitates 3–9 days in the hospital and the use of a pulsatile infusion pump (Kutzler, 2007). Long-acting GnRH analogs such as lutrelin, deslorelin, and leuprolide have been implanted subcutaneously or submucosally with great effectiveness. Implants containing deslorelin (Suprelorin®, 4.7 mg deslorelin) are most commonly utilized for this purpose (Kutzler, 2007; Walter *et al.*, 2011).

Estruses can also be induced using hypophyseal (FSH and LH) and chorionic (PMSG and hCG) gonadotropins. Chorionic gonadotropins have been proven to be more effective in bitches than hypophyseal gonadotropins. Despite the fact that many regimens have been tried, satisfactory results have been recorded with 20 IU/kg/d PMSG administered subcutaneously for 5 days, followed by 500 IU intramuscular hCG on day 5. PG600, a pig-specific formulation containing PMSG and hCG (80 IU PMSG and 40 IU hCG/mL), is very successful at inducing estrus (Root Kustritz, 2006; Popescu *et al.*, 2012).

## 5 Conclusion

The primary focus of this paper was to scrutinize the sexual cycle disorders and diagnoses in female dogs. As well as highlighting the core treatments that can be used such as synthetic estrogens, dopamine antagonists, GnRH agonists, and exogenous gonadotropins.

The use of dopamine agonists, which predominantly act on the D2 receptors of the pituitary gland, such as bromocriptine or cabergoline, is useful in enhancing fertile estrus in most bitches (Gobello, 2006). In other words, dopamine agonists main role is to suppress lactation. Nevertheless, GnRH agonists such as Deslorelin main purpose is to continuously expose the pituitary gland to GnRH, causing desensitization and a sharp drop in the levels of LH and FSH in the blood. However, it is preferred over other endogenous GnRH due to its higher receptor affinity and less side effects. As for the exogenous

gonadotropins, such as FSH, LH, hCG, and PMSG have been proven to be more effective in bitches than hypophyseal gonadotropins (De Rensis *et al.*, 2006).

Nonetheless, as mentioned previously it is always recommended to start with a full body examination and especially the reproductive system in case of disorder in the sexual cycle. After this step, it can be followed by an ultrasound and vaginal cytology to dissect and evaluate the cause.

To summarize, infertility in female dogs is often brought about by irregularities in the cycle which results in the incapability to deliver puppies. However, in most cases this incapability is due to the poorly timed breeding at an immature age and the other sporadic cases are due to anomalies discussed previously.

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**Title of document (to be uploaded):** Heat disorders in the bitch: treatment protocols  
(review of literature)

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**Publication data of document:** 2023

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### Thesis progress report for veterinary students

Name of student: Karim Bahlawan

Neptun code of the student: AOGBSI

Name and title of the supervisor: Prof. László Zöldág, PhD, DSc

Department: Department of Animal Breeding and Genetics

Thesis title: Heat disorders in the bitch: treatment protocols (review of literature)

.....  
 .....

#### Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2021	05	24	Choosing the right topic	
2.	2021	05	26	Topic, Thesis announcement form	
3.	2022	06	04	Methods of literature research	
4.	2022	07	04	Formal requirements	
5.	2022	08	31	Materials and methods	

Grade achieved at the end of the first semester: .....very good.....(5)

#### Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022	09	20	First draft	
2.	2022	12	04	Implemented second version	
3.	2023	01	08	Last version	
4.	2023	03	20	Last corrections	
5.	2023	03	23	The requirements of the presentation	



Grade achieved at the end of the second semester: *very good (V)*

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

signature of the supervisor

Signature of the student: *Kaplan*

Signature of the secretary of the department: .....

Date of handing the thesis in.....27 March 2023 .....

I hereby confirm that I am familiar with the content of the thesis entitled

Heat disorders in the bitch:  
treatment protocols (review of literature)  
written by Hasim Bahlawan

(student name) which I deem suitable for submission and defence.

Date: Budapest, 27 day 03 month 2023 year

Supervisor name and signature



Department