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INFLUENCE OF REPRODUCTIVE HORMONES ON *DIABETES MELLITUS* AND INSULIN RESISTANCE IN BITCHES

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Abstract: *Diabetes Mellitus* is a multifactorial condition that affects both neutered and non-spayed bitches. However, the estrous cycle has a significant influence on the development of the disease, affecting blood glucose levels and insulin resistance due to hormonal changes. Therefore, the management of *Diabetes Mellitus* involves therapeutic approaches such as insulin therapy and surgical intervention, but also ovariohysterectomy, which are fundamental for controlling the disease. In addition, pyometra, a bacterial infection that compromises the reproductive system of bitches, may be related to insulin resistance, since progesterone, released during the estrous cycle, can interfere with the action of insulin receptors. This literature review highlights the importance of early diagnosis and regular veterinary monitoring for effective treatment and improved quality of life for affected animals.

Keywords: Surgery. Diabetes. Hormones. Insulin. Pyometra.

INTRODUCTION

The interaction between man and dog has become increasingly affective, with characteristics similar to the relationship between parents and children. This has led to an increase in animal health care, as well as studies into the pathologies that affect domestic animals, specifically dogs, such as diabetes. The cost of caring for a diabetic dog is relatively high, as it is a condition that requires permanent care. Such care has an even greater influence on the strength of the owner-animal relationship (Pöppl, 2013).

Diabetes Mellitus (DM) is considered one of the most common endocrinopathies seen in dogs. It is a chronic metabolic disorder in which insulin production becomes insufficient or absent, completely altering the metabolism of carbohydrates, lipids and proteins. It is characterized by two main clinical signs, polydipsia and polyuria. There are two types of classification for DM, insulin-dependent diabetes and non-insulin-dependent diabetes, type I and type II DM, respectively (Imai, 2009).

Type I DM is more prevalent in non-castrated female dogs and is related to diestrus and pregnancy as influencers for the development of DM. As for type II DM, its incidence is higher in cats (Oliveira, 2019).

DM is a multifactorial disease and can result from infectious diseases, hormonal diseases, neoplasms, a sedentary lifestyle, obesity and stress. Another important factor in the occurrence of DM is the hormonal action that occurs during diestrus and pregnancy, as mentioned above, because progesterone, estradiol and growth hormone (GH) cause hyperglycemia by affecting the response of insulin to its receptor, resulting in insulin resistance (IR) (Oliveira, 2019).

Insulin is the hormone responsible for maintaining glycemic control in the blood. It is synthesized in the ß cells of the Islets of Langerhans, located in the endocrine portion of the pancreas. Insulin promotes glucose uptake in certain cells, such as muscle and fat cells, and is also responsible for stimulating the synthesis of stored carbohydrates (glycogen) and lipids (triacylglycerol), as well as inhibiting the breakdown of fat stored in adipose tissue (Amato, 2020). When there is IR, insulin loses its effective action, i.e. it continues to be excreted into the bloodstream, but there are difficulties for its receptors in receiving glucose from the tissues (Amato, 2020)

Pyometra is a pathology of the reproductive system, usually affecting middle-aged bitches, which occurs in the diestrus phase of the reproductive cycle of bitches and is related to IR. It is characterized by a uterine bacterial infection in which bacteria present in the vaginal microbiota cross the cervix in the post-estrus and estrus phases, triggering the uterine inflammatory process and exacerbating uterine responses (Popple *et al.*, 2024). The relationship between pyometra and DM lies in the fact that the hormonal action of proges-

terone during the diestrus phase can influence the binding of insulin to its receptors, as well as the direct action of bacterial infection, leading to DM (Lima *et al.*, 2023).

The aim of this study is to carry out a bibliographical review, searching the literature for information that indicates the relationship between the estrous cycle of female dogs and some reproductive pathologies and insulin resistance, which can trigger endocrine pathologies such as DM.

LITERATURE REVIEW

PHASES OF THE ESTROUS CYCLE

BITCHES' ESTROUS CYCLE

The bitch is a monoestrous, non-seasonal species, with a proestrus/estrus period lasting two to three weeks, with spontaneous ovulation, followed by a luteal phase lasting around 65 days when pregnant, or a little longer when not pregnant. After this phase, there is an obligatory anestrus. The interval between estruses varies from 6 to 7 months (Silva, 2016).

PROESTRO

Proestrus is the initial phase of the female dog's cycle, lasting approximately 9 days. During this period, behavioral changes occur, in which the female attracts the male, but does not yet accept copulation. Physical changes also occur, such as vulvar edema, vaginal discharge and more evident vaginal folds, triggered by serum estradiol, which increases from 5 to 15 pg/mL initially, to reach peaks of 40 to 120 pg/mL later (Silva, 2016). Vaginal cytology can be performed to estimate serum progesterone values, which gradually increase from the end of proestrus. The epithelial cells found in this phase of the cycle can vary in shape and nucleus, and anechoic follicular cystic structures are also found in the ovaries, which gradually increase in size (Crusco, 2022).

ESTRO

The oestrus phase lasts between 4 and 24 days, with an average of 9 days. Its main characteristic is the receptivity of the female to the male. During oestrus, cellular luteinization of the dominant ovarian follicles occurs under the action of luteinizing hormone (LH), allowing ovulation (Oliveira, 2019). This phase is therefore marked by ovulation, oocyte maturation and possible fertilization (Crusco, 2022).

Progesterone (P4) synthesis continues to gradually increase its production after the LH peak, reaching between 10 and 25 ng/dl around the tenth day. At the end of estrus, estrogen also peaks (Oliveira, 2019). In bitches, unlike other species, the formation of the corpus luteum precedes ovulation by up to 48 hours. During this process, the follicle ruptures approximately 48 to 72 hours after the increase in LH, completing the development of the corpus luteum and moving into the diestrous phase (Sousa, 2015).

DIESTRO

In diestrus, the female's receptivity to the male ceases and the secretion of P4 by the corpus luteum predominates (Cardoso, 2017). Serum progesterone declines slowly between days 55 and 90 (Silva, 2016).

Diestrus is a crucial phase in the reproductive cycle of bitches, regardless of the presence of pregnancy. During this period, the uterus enters a state of quiescence, which is essential for maintaining pregnancy when it occurs, as well as for the receptivity of the embryo (Barni, 2012).

During this phase, P4 maintains endometrial growth, decreases myometrial activity and keeps the cervix closed. This occurs regardless of whether or not pregnancy is present (Pöppl *et al.*, 2024).

ANESTRO

Anestrus lasts approximately 125 days and is defined by the end of the luteal phase and the beginning of proestrus again. Uterine regression occurs, decreasing the activity of endometrial cells, and this is the period of sexual rest (Santos, 2022).

ESTRAL CYCLE AND MAMMARY GLANDS

During the estrous cycle, P4 plays a fundamental role in the development of the mammary glands, while estrogen is responsible for the development of the mammary ducts. In diestrus, there is an increase in prolactin levels, the hormone responsible for milk production, which is secreted by the pituitary gland (Pöppl *et al.*, 2024).

DIABETES MELLITUS

The cause of DM is multifactorial and involves various clinical signs, such as polydipsia, polyuria and polyphagia. Hyperglycemia exceeds the capacity of the renal tubular cells to reabsorb glucose, causing polyuria, which in turn is compensated for by polydipsia, in order to avoid dehydrating the animal. Excess glucose and incorrect insulin production also deregulates the satiety center in the hypothalamus. With the low amount of insulin produced, satiety does not occur properly and, consequently, polyphagia occurs (Imai, 2009).

PATHOGENESIS

Insulin is produced by the pancreas, and this hormone is responsible for regulating blood glucose levels. In order for glucose to be used by the cell as a source of energy, the process of anaerobic respiration is necessary. Cells have receptors, called tyrosine kinases, which are responsible for capturing glucose from the bloodstream. If insulin is not adequately produced, blood glucose levels rise because glucose utilization decreases, causing hyperglycemia. Insulin's main role is to take sugar from the blood to the body's cells (Santoro, 2009).

Some factors can influence the possible risks and complications of the disease, such as time of diagnosis, type of diabetes, obesity and age of the animal. Generally, if treated correctly, DM has a favorable prognosis; however, this prognosis can change if there are concomitant diseases such as neoplasms (De Almeida, 2017).

PYOMETRA AND ITS POSSIBLE CORRELATION WITH INSULIN RESISTANCE AND DM

Pyometra is a common disease in middle--aged to elderly intact bitches, characterized by an acute or chronic bacterial infection of the uterus after oestrus, with accumulation of inflammatory exudate in the uterine cavity, resulting in various clinical signs, both local and systemic. Pyometra may or may not be associated with cystic endometrial hyperplasia (CEH) and is a serious condition which, if not treated correctly, can lead to the animal's death (Rossi, 2022).

Some bacteria are normally found in the vaginal microbiota of bitches, such as *E. coli,* when there is pyometra. Other bacteria have been isolated in bitches' uteri, such as *Staphylococcus, Streptococcus, Pseudomonas, Proteus*, among others (Pöppl, 2008).

The cause of pyometra has not yet been fully clarified, but it is believed that the main triggers are high levels of progesterone and estrogen after estrus or the exogenous administration of these hormones. Studies suggest that the high levels of progesterone in diestrus increase the amount of estrogen receptors, acting directly on the endometrial tissue, leading to an increase in progesterone receptors (Schweigert *et al.*, 2009).

According to Silva *et al.* (2022), pyometra can cause critical effects of insulin resistance, due to its inhibitory effect on GH during the diestrous phase, as well as its inflammatory and highly septic potential (Silva *et al.*, 2022).

Pyometra and DM have been correlated in studies for over 50 years, but these have not advanced in this area (Fall, 2010). What is known is that the cystic endometrial hyperplasia-pyometra complex constitutes a chronic inflammatory state that can lead to an inflammatory disorder capable of causing insulin resistance, impairing the activity of tyrosine kinase (specific receptor for insulin) in the membrane of muscle tissue, reducing the affinity between insulin and its receptor in this tissue (Feldman, 2004).

As shown in Table 1, we can see in which breeds the risk of pyometra and DM is higher. Some breeds are more predisposed to developing both pyometra and DM. Therefore, the veterinarian should pay more attention to breeds predisposed to these diseases. In the case of an increased risk of pyometra and DM, we can mention the Poodle toy breed with a higher predisposition to both (Pöppl *et al.*, 2024).

Increased Risk of Diabetes Mellitus	Decreased Risk of Diabetes Mellitus
Australian Terrier	Beagle
Border Colie	Boxer
Border Terrier	Boston Terrier
Finish Spitz	Bulldog
Fox Terrier	Cocker Spaniel
Irish Setter	Collie
Siberian Husky	Dalmatian
Cavalier Springer Spaniel	Golden Retriever
English Setter	Dog Shepard
Cairn Terrier	Doberman Pinscher
Bichon Frise	Shi Tzu
Poodle toy	Basset Hound
Yorkshire Terrier	Greyhound
Increased risk of pyometra	**Decreased risk of pyometra**
Boxer	Coton deTutelar
Bull Terrier	Dachshund
Golden Retriever	Finnish Spitz
Cavalier King Charles Spaniel	Laika
Rotweiller	Maltese
Poodle toy	Tibetan Terrier

Table 1. Dog breeds at increased risk and reduced risk of developing diabetes mellitus and pyometra. Source: Adapted from Pöppl *et al.*, 2024.

CONTROLLING THE INTERFERENCE OF THE ESTROUS CYCLE ON DIABETES

Once established, DM is rarely reversible, but the condition can regress depending on the early identification of the factors that led to it, such as diestrus, pregnancy, obesity and metabolic diseases. Once the cause has been identified, treatment should be established on an individual basis, thus increasing the likelihood of the disease going into remission (Batista, 2021).

It is crucial to note that achieving clinical and glycemic control of diabetes in entire bitches, when influenced by P4 and GH due to diestrus, pregnancy or a possible pyometra, can be extremely challenging and unsuccessful (Pöppl, 2024). Furthermore, waiting for diabetes to be fully controlled before opting for the surgical sterilization procedure can reduce the chances of a possible remission of diabetes in this context (Pöppl, 2013).

According to a study carried out by Fall with a group of 13 bitches, 58% of these animals achieved remission of gestational diabetes after termination of pregnancy (Fall, 2008). According to Pöppl, total remission of the condition occurs with the control of insulin resistance factors, as a result of ovariohysterectomy (OSH), which removes the source of progesterone. This leads to a decrease in the plasma concentration of growth hormone and, consequently, insulin resistance (Pöppl, 2024).

As reported by Lima et al. (2022), following the diagnosis of DM secondary to a case of stump pyometra in a ten-year-old female dog of no defined breed, she was given NPH (*Neutral Protamine Hagedom*) insulin, followed by surgery to remove the remaining ovaries and uterine stump. The insulin therapy associated with the surgery kept the patient's glycemic levels stable, but she became insulin-dependent and totally blind due to a diabetic cataract.

To stabilize the animal treated with insulin, the patient's needs and the level of insulin production must be monitored. The initial dose of insulin can be 0.4 IU/kg, adjusted according to the animal's needs, using blood glucose levels as a parameter, and daily control should be maintained between 80 and 250 mg/d. Surgical treatment thus becomes a more urgent treatment when glucose levels are not being controlled with insulin therapy, as it causes P4 levels to fall. However, even with surgical treatment, some animals need insulin until they achieve complete control of their diabetes, which can take several weeks (Pöppl, 2024).

Treating DM is a challenge, as it can be related to various causes and influenced by other factors. This makes it essential for the veterinary surgeon to take the appropriate course of action in each case, achieving control of the disease and avoiding more serious complications (Assis, 2022).

Routine examinations, as well as regular veterinary follow-up, are essential for a rapid diagnosis of the disease. In the case of DM influenced by diestrus, the veterinarian will be instrumental in advising on the importance of sterilizing these bitches.

FINAL CONSIDERATIONS

This study concludes that DM and the estrous cycle are directly related, due to the hormonal influence of progesterone during diestrus in antagonizing the effects of insulin. Other hormones may also be related to the onset of DM in non-castrated bitches, such as estrogen and GH. The appearance of pyometra may also be related to high levels of progesterone and estrogen, but more studies on this subject are lacking. Therefore, surgical sterilization may be indicated to reduce cases of insulin resistance in female dogs. Veterinarians should be responsible properly diagnosing DM and verifying the influence of the estrous cycle on IR and glycemic control, and may consider surgical sterilization according to the particularities of the breed and/or individuals.

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