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USE OF PROGESTOGENS AND THEIR IMPLICATIONS IN BITCHES - LITERATURE REVIEW

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Abstract: The estrous cycle is divided into four phases: proestrus, estrus, diestrus and anestrus. During proestrus, there are signs such as enlargement of the vulva and serosanguinous secretion, with altered behavior due to the increase in estradiol. In oestrus, the bitch accepts the male and ovulation occurs after the LH peak and a progressive increase in progesterone. Diestrus is marked by the refusal of the male and a high concentration of progesterone, while in anestrus, the reproductive system goes into rest, with the bitch showing no signs of sexual activity. Oestrus can be controlled surgically by ovariohysterectomy, which is recommended because it is effective in controlling disease and reproduction. However, there is resistance from part of the population due to the cost and invasiveness of the procedure, making it unfeasible on a large scale for population control. Alternatives such as chemical castration and immunocastration are a reality, although they still face challenges, especially in females. Progestogens are a pharmacological option that prevent or postpone oestrus by blocking ovarian function, inhibiting ovulation and prolonging the luteal phase. Despite their low cost to owners, there are strict criteria that should be followed for their correct use. They also have the potential to cause adverse effects, such as pyometra and mammary tumors. In conclusion, despite advances in reproductive control methods, there are still challenges in implementing them safely and effectively in female dogs. Although progestogens are widely used for reproductive control in bitches, especially to suppress or postpone estrus, they present health risks, such as pyometra, mammary hyperplasia and an increased incidence of mammary tumors. These adverse effects result from the prolonged and inappropriate use of these hormones, which emphasizes the need for strict veterinary supervision and cautious use. It is suggested that surgical castration is a safer alter-

native for reproductive control, as it eliminates the risks associated with the use of hormones

Keywords: Canine. Estral cycle. Contraception. Castration. Female dogs

INTRODUCTION

The interaction between man and animal has been going on for a long time, growing gradually and vigorously, where various benefits surround this relationship, which is not only one of service, but also one of affection (Serpell, 2004). The various physical and emotional benefits resulting from this bond make pets, especially dogs, part of the family (Dalfarra, 2003; Gardemann *et al.*, 2009).

According to the Brazilian Association of the Pet Products Industry, Brazil ranks third in the world in terms of pet population (ABINPET, 2022). This large number also contrasts with a common problem in most metropolitan areas, the lack of responsible custody of these animals, especially dogs and cats, resulting in abandonment, suffering and often death (Nogueira, 2009).

The growth of the dog and cat population is mainly related to the fact that these species reach puberty early, have a short gestational period and produce many puppies per pregnancy (Bueno; Rédua, 2020; Fernandes *et al.*, 2020; Silva *et al.*, 2020; Lima *et al.*, 2022). Therefore, the promotion of health education for guardians to correctly carry out responsible guardianship, with the adoption of preventive measures against pathologies, the promotion of animal welfare, including population control, whether by castration or contraceptive methods, are extremely important and directly related to the promotion of public health (Langoni *et al.*, 2011).

Controlling unwanted pregnancies is necessary for population control. Estrus can be prevented by surgical sterilization, which is considered the most effective, or by pharmacological contraception (Silva *et al.*, 2019).

Among the possibilities for pharmacological contraception, there are contraceptive drugs from the progestogen group. These are more affordable and can be administered via tablets or injections, and aim to inhibit behaviors related to the female's sexual acceptance (Vigo *et al.*, 2011).

However, inappropriate use of this method can lead to undesirable effects such as cystic endometrial hyperplasia, mammary hyperplasia, pseudocyesis, retained placenta and even fetal death (Laufer *et al.*, 1998).

The indiscriminate use of contraceptives for reproductive control is due to ineffective regulation, since these drugs are sold without veterinary medical restrictions, and mainly to the lack of knowledge among owners about the adverse effects caused by these drugs (Barcardo *et al.*, 2008; Silva *et al.*, 2012).

Thus, this literature review is justified by the need to address the implications of the use of progestogens in bitches, presenting their correct applicability and their benefits, with a view to preventing harm. The aim of this literature review is to address the implications of the use of progestogens in bitches in order to properly utilize their benefits and consequently prevent harm.

LITERATURE REVIEW

STAR CYCLE

The marker of the sexual activity phase in female dogs is puberty, which varies between 6 and 18 months of age, depending on the breed and/or individual. After this, the typical hormonal activities of the estrous cycle last approximately 3 months, and synchronize with anestrus (another 2 to 4 months), the last phase that establishes an interval between cycles (Wanke; Gobello, 2006). This estrous cycle is divided into four different phases: proestrus, estrus, diestrus and anestrus, with anestrus being an obligatory phase in all cy-

cles of recovery of the reproductive system for a new cycle, whether from a previous pregnancy or not (Silva, 2019). Thus, bitches have an average of two estrous periods per year, but with only one oestrus in each (monoestrous) (Concannon *et al.*, 1989).

Proestrus is characterized by an enlarged vulva and serosanguinous secretion (Zoppei *et al.*, 2019). Behavior during this period may be altered, with restlessness, polyuria, polydipsia, and the male's rejection of mounting, remaining with his tail towards the perineum (Christiansen, 1988). Diapedesis and the rupture of capillaries in the vaginal region occur, due to the increase in follicular estradiol in the bloodstream, where bloody vaginal discharge can be observed during the proestrus period, which lasts an average of nine days and can vary from 3 to 21 days (Concannon, 2005; Sbiacheski; Cruz, 2016).

These changes during the proestrus period, both behavioral and physiological, are due to the increased concentration of estradiol in the body; the maturing ovarian follicles synthesize this hormone in response to the effect of Follicle Stimulating Hormone (FSH) on them. Progesterone in proestrus remains basal until 24 to 48 hours, however, during the last 12 hours there is a decline in the amounts of oestradiol, and an increase in progesterone values (Ettinger *et al.*, 2004).

ESTRO

Oestrus is characterized by the male accepting the female for mounting, with an average duration of 12 days, ranging from four to twenty-four days (Olson *et al.*, 1986; Guido, 2003). This change in behaviour is a consequence of the decrease in oestradiol, as follicular maturation is in its final phase; at the same time, serum levels of progesterone and Luteinizing Hormone (LH) increase, and 24 to 48 hours after this event, ovulation occurs (Benetti *et al.*, 2004). The pituitary gland and hypothalamus will re-

ceive positive feedback as a result of the decrease in oestradiol and the increase in progesterone, which will result in the pre-ovulatory wave of LH and the release of follicle-stimulating hormone (FSH) (Reynaud *et al.*, 2005).

After the pre-ovulatory peak of LH, ovulation occurs and the follicles mature and begin to synthesize even more progesterone, which increases their serum levels (Allen, 1995; Feldman; Nelson, 2003). Ovulation will end within twelve to twenty-four hours, depending on each animal organism (Feldman; Nelson, 2003).

Bitches under the effects of increased progesterone express behaviors typical of this phase, such as lateralization of the tail to one side, firming of the paws on the ground and elevation of the tail to welcome penetration. Morphological changes can also be noted, such as translucent or straw-yellow discharge, with no blood in the vulva and a less swollen endometrium (Christiansen, 1988).

According to Holst and Phemister (1975), between days zero and five, maximum fertility can be achieved with coverings after the LH surge. On the other hand, Feldman and Nelson (2003) deny the effectiveness of pregnancies from coverings practiced nine to ten days after the LH peak.

In order to precisely define the end of oestrus, vaginal cytology can be carried out with a predominance of keratinized superficial epithelial cells. As for behavioral characterization, there is the rejection of the male by the female (Feldman; Nelson, 2003).

DIESTRO

Diestrus is the phase that occurs after oestrus and lasts between 60 and 90 days, characterized by a refusal to mount (Zoppei, 2019). During this period, bitches that have not been pregnant may show signs such as breast growth, pseudocyesis, nest joining and various other behaviors (Nelson; Couto, 2015).

This is the phase in which the serum concentration of progesterone is similar in non-pregnant bitches and pregnant bitches, due to the inability of uterine prostaglandins to cause the destruction of the corpus luteum (CL) (Silva, 2019). The main functions of progesterone are to increase endometrial glandular activity, preserve the uterine environment favorable to pregnancy, occlude the cervix, reduce the inflammatory response and uterine contractility (Oliveira, 2018).

In non-pregnant bitches, CL luteolysis occurs slowly due to low progesterone levels. In pregnant bitches, this process is linked to the events that trigger childbirth, beginning with the release of cortisol from maternal and fetal prostaglandins, although the specific mechanisms are not clear (Silva, 2019).

Anestrus lasts approximately 125 days and is a phase of reproductive quiescence in which there are no clinical signs, the female does not allow cover or the males to mount her; the vulva is already at its normal size, with no mucous secretion (Zoppei, 2019). At this stage, the uterus is recovering from the physiological changes, the uterus is involuting and re-establishing itself for a new cycle (Concannon, 2008).

Hormonal events related to the pituitary-ovarian axis and the uterus remain alive, however, the ovaries show a low response to gonadotropins (FSH and LH), due to the effect of prolactin in the circulation (Jeffcoate, 1993). Anestrus is considered a phase of sexual rest, and its end is signaled by an increase in FSH and estradiol levels, with the end of prolactin action and the recruitment of a new follicular wave, thus starting a new cycle (Kooistra *et al.*, 1999).

CONTROL

Among the alternatives for oestrus control is ovariectomy, the procedure most indicated for elective castration in veterinary clinics and hospitals (Silva, 2016). The indication is directly related, not only to repro-

ductive control, but also because it allows the control of diseases that may affect the female's reproductive system, with the control of pathologies such as mammary tumors, metritis, pyometra and stagnation of some systemic diseases, such as diabetes, which is directly affected by the gonadal hormones, estrogen and progesterone (Fossum, 2005).

On the other hand, there is part of the population that assumes that surgical sterilization is an adverse procedure to nature and animal welfare (INSTITUTO PASTEUR, 2000; Soto *et al.*, 2006). What's more, the costs, the demand for trained professionals, post-surgical care, time consumed and possible risks, both anaesthetic and surgical, end up making the procedure unfeasible when the aim is population control through mass sterilization (Johnston; Rhodes, 2015; Soto *et al.*, 2005; Kutzler; Wood, 2006)

An alternative is chemical castration. These are considered to have considerable advantages: low cost and the possibility of mass sterilization, which can encourage public health organizations and governments to participate, even if they don't have large resources (Garde *et al.*, 2016). The chemical option is carried out through intrathecal injection, directly into the gonad, of the specific formulation (e.g. Zinc Gluconate), which causes local inflammation, reactions and lipid peroxidation. As a result, there is irreversible degeneration of the gametes, causing infertility (Oliveira *et al.*, 2013)

However, although research into these methods is well-defined for male animals, there is a great lack of similar studies when it comes to females. Cavalieri and Hayes (2017) observed the intra-abdominal positioning of the ovaries as an immense challenge, which limits the administration of chemo sterilants. Therefore, once laparotomic access is achieved, ovariohysterectomy can be performed. Therefore, chemical castration is a controversial procedure in females.

New research has been carried out looking for non-surgical methods that are simple to apply. One of the alternatives found is immunocastration based on hormonal antibodies and other pharmacological techniques, promising for use in both sexes (Palmer *et al.*, 2018). Immunocastration induces the production of antibodies against gonadotropin-releasing hormone (GnRH), generating an immunological barrier between the hypothalamus and the adenohypophysis, preventing the normal activity of GnRH-I, a key hormone in the reproductive system (Herbert, 2005). Immunocastration creates some specific antibodies that will act by recognizing and binding to GnRH-I, making a barrier and preventing it from binding to the pituitary receptors, consequently inhibiting the secretion of FSH and LH, resulting in infertility (Root Kustritz, 2018). However, vaccines are still in the experimental field, with promising responses, but still with challenges such as reactions at the application sites (Baden *et al.*, 2009; Vargas-Pino *et al.*, 2013).

Pharmacological contraception has also been established. This is a low-cost, simple-to-acquire method that is assigned an important task as a contraceptive option that acts to prevent or prolong oestrus (Silva *et al.*, 2019).

One example of this contraception is progestogens, which act by blocking ovarian function, inhibiting follicular growth and ovulation and consequently increasing the luteal phase. Before using them, it is advisable to carry out complementary tests associated with the animal's clinical condition, as there are several reports of unwanted effects, which can be avoided if used safely (Luz, 2023), as will be described below

Another recent option is treatment with GnRH agonist drugs, such as desrolerin. These act in two stages: firstly, the pituitary gonadal axis is stimulated, consequently increasing the secretion of FSH and LH, resulting in an increase in steroid sex hormones (*flare-up*).

However, with chronic use, desensitization of the pituitary gland occurs, interrupting the secretion of gonatrophins. As a result, there is no production of sex steroids, inhibiting reproductive function (Luz, 2023).

Previously, daily intravenous or subcutaneous injections were used, as the drugs had a short half-life. Nowadays, more viable commercial options have been developed, with the use of implants with a constant release of deslorelin with a duration of action of 3 to 12 months (Suprelorin®, Virbac) (Luz, 2023). However, *flare-ups* in the days following implantation in females can result in signs of estrus, as well as the possible occurrence of ovarian cysts, lactation, pyometra and changes in behavior (Fontaine, 2012).

THE USE OF PROGESTOGENS IN BITCHES

Progestogens, popularly known as “contraceptives”, are easily found in farm stores and pet stores and are sold without the need for a prescription from a veterinarian (Honório *et al.*, 2017). They are available in the form of injectables or tablets, which in the body of bitches, will delay the male acceptance phase, through negative feedback on the hypothalamus/pituitary-gonadal axis (Sbiacheski; Da Cruz, 2016).

The most commonly used progestogens for reproductive control in female dogs are megestrol, medroxyprogesterone and proligestone (Honório *et al.*, 2017). These are synthetic compounds analogous to progesterone, but with similar effects and mechanism of action to the natural hormone (Binsfeld *et al.*, 2014), acting against GnRH-I, which is manufactured and secreted in the hypothalamus, resulting in its adhesion and action on the adreno-pituitary receptors being prevented, resulting in the non-stimulation of the release of the reproductive hormones FSH and LH, and therefore estrogen, which is important for the start of the proestrus phase and progression to estrus (Root Kustritz, 2012).

ACETATE

When it comes to oral progestogen treatment, megestrol acetate is the drug of choice as a contraceptive method, as it is slightly metabolized and more harmless. Its administration varies according to the phase of the estrous cycle. It can be administered during anestrus in order to avoid proestrus, and therapy must be applied at the end of anestrus, once a day at a dose of 0.55 mg/kg for 33 days; or, during proestrus, to suppress estrus where treatment should be administered in the first three days of proestrus, once a day at a dose of 2.2 mg/kg for 8 days in bitches (Santos, 2003).

However, if treatment is administered after the first three days of proestrus, megestrol acetate can mimic the pre-ovulatory drop in the estrogen/progesterone interrelationship, which will consequently stimulate the release of LH, ovulation and fertile estrus. It is therefore advisable to monitor the estrous cycle by means of colpocytological examinations, so that there are no gaps in the timing of the estrous cycle. The female should be separated from the males within three to eight days until proestrus is suppressed, as during this period she may still be receptive to males. Reproductive cyclicity will normally reappear between four and six months, however, it can reach up to nine months after administration (Apparício; Vicente, 2015). This drug can be found commercially as Preve-gest®, Megestar® and Gynodal® (Santos, 2003).

MEDROXYPROGESTERONE ACETATE

Because medroxyprogesterone acetate is a long-acting progestogen with slow hepatic metabolism and high progestational activity, it has high side effects. As a result, its sale is banned in some countries such as Australia and New Zealand. However, it is still one of the most widely used drugs in Brazil. When using this drug, it is recommended not to exceed the recommended dose and not to exceed

the frequency of applications, as its side effects are directly linked to prolonged use, overdose and administration of the treatment at a time in the estrous cycle other than anestrus (Apparício; Vicente, 2015). Among the other progestogens, it has the greatest androgenic and immunosuppressive effects (Luz, 2023).

The recommended dose is 1.5 to 3.0 mg/kg/IM, applied to the inner thigh, every 3 to 6 months, and should be administered during the anestrus phase. Estrus returns 3 to 9 months after administration. It is worth noting that the younger the female, the lower the dose administered and the shorter the period of use (maximum 3 cycles in a row), the lower the risk of adverse effects in bitches (Luz, 2023). Its commercial presentations include Singestar®, Promone®, Anticion® and Provera® (Santos, 2003).

Research by Ptaszynska (2010) shows that progesterone, a second-generation progestogen, has a weak progesteric action and greater antigonadotrophic specificity as its most striking feature, thus causing fewer uterine and mammary gland disorders than other progestogens.

Administration on the inside of the thigh is recommended to avoid adverse reactions at the application site such as discomfort and alopecia. The treatment should be carried out during anestrus, when the aim is to avoid proestrus, or during proestrus, when the aim is to suppress estrus (Apparício; Vicente, 2015). The protocol for administering doses of immunocastration in bitches, with the first application carried out at birth (day 0), followed by additional doses at 3 and 7 months of age, aims to be effective in delaying oestrus. The recommended dose is 10 mg/kg intramuscularly (IM) for large bitches and 33 mg/kg IM for small bitches. After these initial doses, applications should be made every 5 months. This strategy not only aims to be more than 95% effective in controlling the reproductive cycle of bitches, but is also based on the need to avoid overpopulation and the

consequences associated with the estrous cycle, such as the risk of disease and behavioral problems. In these cases, it is more than 95% effective (Luz, 2023).

The female will return to cycling after administration of the progestogen, depending on the number of applications made. If a single application was given, the female will return to cycling between three and six months. If a second application was administered, the return will take place between six and twelve months (Apparício; Vicente, 2015). Proligesterone is commercially available as Covinam® (Santos, 2003).

According to Araújo (2016), various problems are caused by these hormones, all directly related to their incorrect application and/or the incorrect timing of the bitch's estrous cycle. Therefore, it is essential to carry out complementary tests with cell analysis, ultrasound, among others, to define the phase of the estrous cycle.

COMPLICATIONS ARISING FROM THE USE OF PROGESTOGENS

As a result of this anti-gonadotrophic attribute, the first hormone used to suppress the reproductive cycle in bitches was progesterone, which was first reported in 1952 (Apparício; Vicente, 2015). However, the exogenous administration of progestogens can contribute to the occurrence of pyometra, mammary hyperplasia (Anjoletto, 2013), urinary incontinence, infertility, obesity, adrenal gland suppression, *diabetes mellitus*, anemia and uterine bleeding (Aguiar; Moreira; Porto, 2016). If applied to pregnant bitches, it can cause fetal retention, dystocia and abortion, which can eventually risk the female's life (Monteiro *et al.*, 2009; Papich, 2012).

Among these possible complications, the most common reported is pyometra, which is directly related to the use of contraceptives due to the fact that progestogens reinforce the hormones that are already active in the animal's

body (Silva *et al.*, 2012). This will amplify the secretory action of the endometrial glands, leading to greater production and accumulation of fluids, as well as contraction of the cervix and impaired contractile activity of the myometrium, which are relevant to the ineffective drainage of intrauterine fluid, which favors the condition (Hardy; Osborne, 1974). Another action of these hormones is to induce the growth of endometrial glands, which, when in excess, can lead to cystic endometrial hyperplasia (CEH) (Hardy; Osborne, 1974).

The exogenous progesterone administered stimulates growth hormone through lobe-alveolar enlargement in the mammary glands, eventually leading to the production of malignant or benign tumor masses (Mol *et al.*, 1997). In pregnant females, the acting progestogen will prevent uterine and abdominal contractions, which will consequently result in fetal death and retention, as estrogen, prostaglandins and oxytocin will not act during labor (Lopes, 2002). Montanha, Corrêa and Parra (2012) reported that the administration of contraceptive drugs is contraindicated in the following phases of the estrous cycle: late proestrus, estrus and metestrus, as it can fail to prevent mating and result in pregnancies with the potential for complications in signaling and triggering childbirth.

Although there are several reports of side effects, these events can be avoided or minimized, as long as they are administered thoughtfully, following certain precautions (Apparício; Vicente, 2015):

- Do not exceed the indicated dose.
- Do not use progestogens for a long period of time. If necessary, it is recommended to stop taking them every four to six months.
- In animals with existing diseases such as neoplasms, breast lesions, uterine bleeding, cystic endometrial hyperplasia or *diabetes mellitus*, the use of progestogens is strictly contraindicated.

- Do not use in pregnant women, as not only will the gestational period be prolonged, resulting in fetal death due to detachment and ageing of the placenta, but it may also cause masculinization of female fetuses in early pregnancy.
- In bitches aiming for future procreation, opt for treatment with another progestogen, avoiding the use of medroxyprogesterone, as reports of cystic endometrial hyperplasia and pyometra can reach more than 60%.
- Avoid treatment during the diestrous period, with anestrus being the period of choice for treatment.

In general, the best scenario for treatment with progestogens is in adult females, free of endocrine and reproductive pathologies, and who are in a period of anestrus (Apparício; Vicente, 2015).

Therefore, the recommendations should only be used in adult females in the correct estrous phase, preferably in anestrus, and it is necessary to have a veterinarian with the appropriate knowledge to evaluate the estrous cycle in the bitch before administering the progestogen. In view of this, it is clear that the misuse of progestogens, without proper monitoring by a professional, can lead to adverse and mostly irreversible effects (Bueno; Rédua, 2020).

PERSPECTIVES ON THE USE OF PROGESTOGENS

Progestogens have been widely recognized in global reproductive guidelines, which highlight the importance of progestogens in the management of reproductive control, confirming their role. Given their proven relevance, it is foreseeable that these drugs will continue to be made available on the market. Continuing to market them not only guarantees patients access to effective treatments, but also supports the evidence-based practice recommended by international guidelines (Squires *et al.*, 2024).

However, its ease of access, indiscriminate sale and low cost means that it is widely used by owners in Brazil without proper monitoring (Moura *et al.*, 2016). Most people administer these drugs without any control of the estrous phase, without knowing if the female has already mated or even during pregnancy. This results in an intensification of adverse effects (Prado *et al.*, 2020).

Stricter measures to prevent adverse effects and abuse are being taken to ensure that these drugs are used safely. There is currently an approved bill (PL n° 4.853/2020) which proposes a ban on the sale of these contraceptive drugs to female dogs and cats, without the prescription of a veterinarian, for the entire national territory (BRASIL, 2020). If approved, this could be a start towards the correct use of progestogens, minimizing their side effects. Stricter supervision will help to ensure that the rules are respected and that possible infractions are identified. In this way, the goal of using progestogens safely and effectively will be achieved, guaranteeing the well-being of bitches and cats.

FINAL CONSIDERATIONS

Progestogens are widely used in bitches for reproductive control, mainly to suppress or postpone estrus (heat). Although effective, their prolonged use can lead to a number of health problems, including pyometra, mammary hyperplasia and an increased risk of mammary tumors. These side effects occur due to the prolonged action of the hormones on reproductive and mammary tissue, which can lead to severe complications, especially when administered inappropriately.

To avoid such problems, it is essential that progestogens are used with caution and under strict veterinary supervision. Treatment should be indicated for bitches who really need it, preferably on a temporary basis and with doses adjusted to each animal's condition. In addition, alternatives such as surgical castration are recommended for population and reproductive control, which eliminate the risks associated with hormones.

Progestogens, despite their risks, remain on the market due to their effectiveness and the demand in cases where other options are not viable. Even so, the conscious and responsible use of these drugs is emphasized in veterinary guidelines, which cite the importance of considering the health risks for bitches, recommending that they be a short-term solution and accompanied by regular monitoring of the animal's health. Correct use, when necessary, can minimize risks, but it is essential that both professionals and owners are aware of the potential adverse effects.

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