Applicability of Pap smear and progesterone dosage in artificial insemination in

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ABSTRACT

Pets have been bred to live with human beings for different reasons, the main one is affective relationships, but also for the purpose of therapy, company for people with special needs, sports, participation in tournaments and exhibitions and reproduction. The pet sector in Brazil is divided into 3 pillars, industry, commerce and animal husbandry services. As a result, animal reproduction and genetic improvement have been gaining ground, due to an increase in the demand for specialists and consequently a significant increase in research in the area. Artificial insemination is the most used biotechnology in this species, especially when natural mating is unfeasible due to anatomical and behavioral issues, in addition to the improvement in genetic gain. In addition, it minimizes the stress caused by handling the animals during conventional mounting and prevents diseases transmitted during copulation. The success of this procedure requires a deep understanding of the reproductive physiology of the species in question. The evaluation of sows and sires involves detailed examinations, including gynecological examination, Pap smear, serum progesterone dosage, and andrological examination. Vaginal cytology aims to identify the phase of the estrous cycle and possible pathologies through cells of its epithelium, while serum progesterone measurement aims to have a greater accuracy of the moment of insemination and the andrological examination aims to evaluate the fertility of the sire. In the general clinical evaluation, several parameters are considered, such as reproductive and pathological history, vaccination and parasitic protocols. Pap smear is considered a more practical, lower-cost and easier to apply method, reflecting changes in estrogen concentration and not progesterone. The hormonal dosage of progesterone is performed at the end of proestrus or the beginning of estrus, which is when it precedes the LH peak. Upon reaching peak LH, progesterone levels increase. This means that there will be ovulation, which is the ideal time to perform intravaginal artificial insemination.

Keywords: Vaginal cytology, Reproductive physiology, Semen, Bitches.

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INTRODUCTION

In recent years, domestic animals have become important members of the nuclear family. The dog, due to its easy and pleasant adaptation, is the most present companion in the lives of many people. The wide variety of patterns, sizes, aptitude, and even the different characteristics of each breed brings a range of options capable of meeting the life needs of different owners. The more humanized treatment aims to ensure a better quality of life for pets, with better health, nutrition and well-being conditions. Tutors are increasingly interested in obtaining animals of high genetic value, according to their lifestyle, or even in the intention of serving a differentiated market. (Santana *et al*., 2017; Silva and Brito, 2023).

According to the Pet 2023 yearbook of the Pet Brasil institute, Brazil is the third largest market in the world, with a share of 4.6% (about U$5.4 billion). Between 2019 and 2022, Pet retail revenue increased by 70.5%, jumping from 35.3 billion to 60.2 billion. The pet sector in Brazil is divided into 3 pillars: industry, commerce and services & animal husbandry. General services already correspond to R$5.3 billion and veterinary services earned R$5.6 billion. On the other hand, the sale of pets directly from legal breeders is the second most important segment of the market, moving R$6.3 billion. The number of companies in the Brazilian pet sector exceeded 292 thousand establishments and 107.4 thousand breeding sites. It is estimated that the stock of jobs in the pet sector, considering both the formal and informal sectors, was approximately 3.1 million in Brazil in 2022. There was an increase of 30 thousand jobs in 2022 compared to 2021. The specialized services sector (activities registered with the Federal Council of Veterinary Medicine) generated 122,000 jobs (Medeiros and Larissa, 2023). According to ABINPET, it is estimated that there are a total of 167.6 million pets in Brazil, of which 67.8 million are dogs and 33.6 million are cats. With this in mind, the pet segment has everything to always be in operation and evolution, since it has a loyal audience that is constantly growing. There are more than 285 thousand companies focused on pets in Brazil, being the third largest pet market in the world. Pets are animals bred to live with humans for affective reasons, generating a beneficial relationship. Their main destinations are: therapy, companionship, leisure, assistance to people with special needs, sports, ornamentation, participation in tournaments and exhibitions, conservation, preservation, breeding, genetic improvement and special work. The trajectory of the Brazilian pet sector follows the path towards urbanization and the growing integration of pets in the lives of families. They became companions in everyday life. This causes families of all types and sizes to increase pet health care. The Brazilian pet sector has gained international prominence, and is constantly among the main markets in the world (GOV.BR, 2023).

According to Silva and Brito (2023), the breeding of purebred dogs has been increasing significantly, consequently the demand for specialized veterinary services in this new market has also increased. The area destined for animal reproduction is one of the most prominent, due to the search

for improvement in the quality of the herd, since by increasing biotechniques it is possible to boost the growth potential of breeding (Silva; Brito, 2023). The first studies related to artificial insemination and conservation of canine semen date back to the end of the eighteenth century, developed by Lázaro Spallanzani according to England (1993). Christensen and Meyers (2023) further report that the dog was the first mammal to reproduce from an artificial insemination in 1776. Therefore, biotechnologies applied to reproduction, such as intravaginal artificial insemination (IVAI) with fresh semen can be used for this purpose. IVAI is an important technique developed for the genetic improvement of animals, being the most common technique to be used, because it is easy to perform and offers good results. For this, the veterinarian working in the area of animal reproduction must have a broad knowledge about the reproductive morphophysiopathology of the species, cynophilia, have a notion of market preferences, that is, know which breeds are the most sought after and acquired by people, in addition to having the confidence to practice general and reproductive medicine (Gonçalves *et al.*, 2021; Silva and Brito, 2023).

The selection of breeding dogs is essential to achieve success in artificial insemination. For this, it will be necessary to evaluate both the male and the female, through an anamnesis, physical examination with inspection and palpation of the reproductive organs, and complementary tests in reproductive management: Pap smear and progesterone dosage and andrological examination (CBRA, 2103; Santos *et al.,* 2016, Souza *et al.*, 2023).

To obtain results with artificial insemination, it is essential to know the estrous cycle of the species. The bitch is a species whose estrus varies on average every six months, being classified as monoestrous. The estrous cycle of the canine female has four phases: proestrus, estrus, diestrus and anestrus (Silva, 2016; Secco and Moya, 2021). Proestrus has an average duration of 9 days, which can vary for more or less days, and is characterized by the interest of the male in the female, but the female refuses to mount. The clinical signs present at this stage are vaginal hyperemia, serosanguinous secretion and vulvar edema. The end of proestrus is marked by the beginning of the acceptance of the female by the male's courtship (Silva, 2016; Silva and Lima, 2018). The beginning of the fertile period occurs during estrus, the female begins to accept covering, in the heat position*,* and does not present vaginal discharge. Estrus lasts about 5 to 15 days, with an average of 9 days. In this phase, estrogen levels decrease before the Luteinizing Hormone (LH) spike. When the LH peak is reached, immature oocytes ovulate will occur, which will mature over 48 to 60 hours after this peak, thus increasing the synergistic production of progesterone, reaching about 10 to 25ng/mL. The diestrus or luteal phase is characterized by a decrease in progesterone. In a pregnant dog, there will be the formation of the corpus luteum, which will keep progesterone levels high to maintain pregnancy, and this corpus luteum will undergo abrupt luteolysis in the prepartum period, by prostaglandin f2 alpha. However, in a non-pregnant bitch, this same corpus luteum will not undergo

abrupt luteolysis, but a slow regression lasting 72 days, thus slowly decreasing progesterone. As a result, the bitch is the only species that has a luteal phase longer than gestation. Lastly, anestrus is the phase of total sexual inactivity (Johnston and Kustritz, 2001; Rodrigues *et al*., 2019; Silva and Lima, 2018). Because it presents this peculiar and still poorly understood reproductive physiology associated with the anatomical characteristics of the female reproductive tract, the development of assisted reproduction techniques in the canine species is still a challenge (Suzuki *et al.,* 2022).

The andrological examination has also been of great value in canine reproduction, as it evaluates the fertility of the dog in the natural breeding program or artificial insemination, as well as in the conservation of genetic material, in the selection of sires and diagnosis of infertility, in addition to investigating the effect of prostate disease on semen quality. Factors such as the small amount of sperm fraction volume have limited semen cryopreservation, as well as the lack of standardization of dilutors, highlighting the need for further studies and caution in seminal evaluation. (Saints *et al*., 2016; Mason, 2018; Tesi *et al*., 2018; Rodrigues *et al*., 2019).

Andrological examination is of paramount importance to detect clinical conditions and estimate the reproductive efficiency of breeders. This exam evaluates all anatomical structures, such as scrotum, testicles, epididymis, prostate, foreskin and penis, evaluates symmetry, presence or not, position, location, consistency of the testicles and epidymis. Testicular biometry is measured by length, width, and scrotal circumference (CBRA, 2013; Luz and Silva, 2019). Semen collection is performed with the digital manipulation method and two evaluations are performed on the spermogram: macroscopic and microscopic. The macroscopic evaluation consists of evaluating the volume, color, density, odor and pH of the semen., In the microscopic examination, sperm motility, vigor, concentration and morphology are evaluated (CBRA, 2013; Silva and Brito, 2023; Souza *et al*., 2023).

Seminal quality directly affects fertility, so in a breeding dog it must be evaluated in order to ensure reproductive efficiency. The correlation of seminal characteristics with the characteristics of the sire, such as age or weight, and fertility are factors that need to be more relevant in the routine of reproductive evaluation. The spermogram is extremely important and ideally it should be performed regularly (Mason, 2018; Tesi *et al*., 2018).

Complementary tests, such as Pap smear and serum progesterone dosage, are used to monitor the phases of the estrous cycle and estimate the stage of ovulation, in order to identify the best time for artificial insemination. Vaginal cytology is done with a *swab* and is able to detect the predominant cells in each phase of the cycle. In the proestrus phase, parabasal and intermediate cells, with the presence of neutrophils and bacteria, are evidenced. In the estrus phase, the presence of superficial cells, nucleated or anucleated, bacteria and with the absence of neutrophils predominates. In diestrus, parabasal cells, intermediate cells, and metaestrus cells with a reduced amount of bacteria and a large

amount of neutrophils (Silva, 2016; Silva and Lima, 2018; Santos *et al*., 2022). And, finally, in anestrus, there is little or no presence of parabasal and intermediate cells, with or without bacteria and neutrophils (Silva, 2016; Silva and Lima, 2018, Santos *et al*., 2022).

Vaginal cytology is considered the most practical, low-cost, and easiest method to apply, but it is somewhat limited, because changes in the vaginal epithelium reflect changes in estrogen concentration and not in progesterone. This means that the vaginal swab does not show exactly when the dog will be fit to be inseminated, and it is necessary to work with an interval of a few days to estimate the fertile period. Maximum cell cornification can occur from 3 to 6 days after estradiol peak and can extend for 6 days, which in practical terms means that maximum cornification can occur from 4 days before the ideal time to inseminate to 3 days after (Linde-Forsberg and Forsberg, 1989).

Serum progesterone is measured by collecting 5mL of blood about 48 hours after the end of proestrus, which is when it precedes the LH peak. Upon reaching peak LH, progesterone levels increase, reaching 10 to 25ng/ml. This means that there will be ovulation, which is the ideal time to perform intravaginal insemination (Nogueira *et al*., 2019). The bitch has an interesting reproductive biology when compared to other species because the oocytes are eliminated as primary oocytes and after 1 to 3 days they reach the second meiotic division and then they will be considered fertilizable. These oocytes can remain fertilizable for five or more days after ovulation (England *et al*., 2021).

Assisted reproduction techniques in dogs can contribute to genetic diversity and the efficiency of the reproductive program, but it is still limited when compared to those in other mammals and even humans (Suzuki *et al.,* 2022). Artificial insemination (AI) is a very important biotechnique developed for the genetic improvement of animals, being used when natural mounting is impossible, due to anatomical and/or behavioral problems; It can also minimize the stress caused by the manipulation of them for natural mounting. In addition, AI also assists in the prevention of diseases transmitted during copulation and the preservation of genetic material. Four types of artificial insemination in have been described: vaginal insemination, transcervical insemination (Norwegian catheter), endoscope-assisted transcervical insemination, surgical insemination. Intravaginal artificial insemination (IVAI) is commonly used for insemination with fresh or chilled semen and is not recommended for frozen semen (Suzuki *et al.,* 2022).

IVAI with fresh semen is the most commonly performed method, as it is easy to perform and brings good results. This procedure requires a probe that is able to reach the bottom of the dog's vagina and thus deposit the semen (Souza *et al*., 2023). The ideal is the use of malleable probes such as the Osiris probe (IMV-technologies, France) or MAVIC (Minitube, Germany), as such equipment adapts more easily to the curvatures present in the dog's reproductive tract. The more rigid tubes, similar to those used in other species, may present discomfort in their introduction, especially in dogs

of the "bull" type (such as French Bulldog and American Bully), because in most females of these breeds there is a marked clitoral hyperplasia during estrus, which causes this structure to significantly increase its size, making it difficult for any equipment to pass through the vagina. Intravaginal artificial insemination (IVAI) is the most common technique to be used, as it is easy to perform and offers good results. IVAI with fresh semen consists of collection by the method of digital manipulation, semen analysis and deposition, in the cranial region of the vagina at an angle of 45° ventrally, using a pipette. The female's pelvic region is elevated for five to ten minutes, accompanied by massage in the clitoral region, in order to prevent semen reflux and ensure maximum success of the procedure (Souza *et al*., 2023; Silva and Brito, 2023).

For the procedure, it is first necessary to observe and monitor the behavior and signs of proestrus, and then do a vaginal cytology to detect which cells are present and characteristic of the phase. A bitch in the final stage of proestrus begins to accept the male's courtship, thus indicating the beginning of her estrus phase. At this point, a new vaginal cytology should be done to confirm the presence of superficial cells and a serum progesterone measurement, in order to detect the LH spike. At the same time, it is necessary to collect and perform and evaluate the spermogram, to place it in a syringe, which will be attached to the probe (Monteiro *et al.,* 2020).

Subsequently, the vulva will be cleaned with cotton wool and 0.9% sodium chloride solution. To depose the semen, the IMV-technologies insemination pipette is used with about 15 cm (Monteiro *et al*., 2020). In all cases, the pipette is lubricated and introduced into the vagina, guiding itself through the dorsal commissure of the vulva at an angle of 45° from horizontal towards the lumbar spine and cranial towards the vagina. By offering resistance, it will be the moment for the deposition of semen (Mason, 2018; Souza *et al*., 2023).

The timing of artificial insemination is an important factor for the success of the technique. Since the bitch ovulates about 48 hours after the luteinizing hormone (LH) peak and this ovulated oocyte is still immature and takes about another 48 hours to mature in the fallopian tube, insemination should be done as close as possible to the maturation of these oocytes, that is, around 96 hours (4 days) after the LH peak, Therefore, the observation of estrus behavior in addition to Pap smears and serum progesterone levels are essential to achieve future pregnancy. During this time, semen deposition can be performed up to 3 times every 48 hours from the LH peak until the change from vaginal cytology to the diestrus phase (Moya *et al.,* 2021; Souza *et al*., 2023).

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