

MAMMARY GLAND DEVELOPMENT IN DOGS AND CATS IN RELATION TO THEIR SEXUAL CYCLE

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Abstract

The mammary gland is a modified sweat gland specialized in milk secretion, whose development is synchronized with the sexual function, being directly influenced by the stages of the sexual cycle in both canids and felines. Knowledge of morphology and physiology is essential for a correct approach of the mammary diseases, an important pathology affecting two categories: the adult female and the newborns.

There are numerous controversies in the literature regarding the terminology and duration of the dog and cat sexual cycle, leading to the existent variability of the information. However, for the bitch it is unanimously accepted that most canine breeds have two estrous periods per year, usually in spring and autumn. This period can be systematically divided into proestrus, estrus, luteal phase and anestrus. In this species, the proestrus and estrus periods are long and necessarily accompanied by a long luteal phase. These are then followed by anestrus, which is not influenced by the season, unlike the cat. The queen is a seasonally polyestrous animal, which in the absence of mating goes into estrus every 2-3 weeks throughout the breeding season. She will have repeated cycles in the breeding season, which will only be interrupted by gestation, pseudo-gestation or various ailments.

The aim of this review is to describe the estrus cycle in dogs and cats, and its connection with mammary gland development, a gland with unique growth due to the fact that the final stage of development is reached in the adult female only during gestation.

Key words: mammary gland, dogs, cats, estrus cycle

The mammary gland is an accessory gland of the genital tract, an extensively modified sweat gland specialized for milk secretion, whose morpho-physiology is directly influenced by the stages of the sexual cycle in both cats and dogs (Drugociu & Drugociu, 2015; Raskin & Meyer, 2016).

Some of the international literature classifies bitches as monoestric animals, a term that defines the existence of only one estrus period per sexual cycle. Compared to mammals like horses, cattle and pigs, that have estrus cycles every three weeks during the breeding season, unless they remain pregnant, canines are different (Pretzer, S. D., 2008).

The cat is a polyestrous animal that usually has 2 estrous periods per year. However, under modern conditions of maintenance, cycles can occur all year round. Under normal circumstances, the anestrus extends over a period of 3-4 months in winter, when the daylight is short (Rosca, 2005).

A thorough knowledge of the reproductive physiology of the female cat and dog is necessary

to enhance success of diagnostic or therapeutic purposes, and also for assisted reproductive technologies. The aim of this article is to provide an overview of dogs and cats sexual cyclicity in relation to the mammary gland, in order to improve the veterinarians approach of the reproductive pathologies.

MATERIAL AND METHOD

An electronic search for English and Romanian publications was conducted in Web of Science, Scopus, PubMed and Google Scholar databases, using terms such as estrus, oestrus, cycle, mammary gland, reproductive, dog, cat, bitch and queen, and the articles and book chapters were analyzed.

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RESULTS AND DISCUSSIONS

Mammary gland development in canids and felines

Canids and felines have several pairs of mammary glands, arranged in two symmetrical, bilateral rows extending from the ventral region of the thorax to the inguinal area (Raskin & Meyer, 2016).

The mammary gland in the bitch is usually represented by 5 pairs of mammae, arranged in 2 thoracic or pectoral, 2 abdominal and 1 inguinal pair. Sometimes 6 or 4 more pairs may be found. (Drugociu & Drugociu, 2015; Silver, 1966) Unlike the bitch, the cat has 4 pairs of mammary glands: 2 pectoral and 2 abdominal, with the nipples traversed by 4-6 papillary ducts. (Drugociu & Drugociu, 2015)

The ratio between the secretory and stromal components of the mammary gland shows major variations depending on the stage of the sexual cycle (Rehm et al., 2007). In each gestation, duct proliferation, differentiation of secretory acini, secretion of milk by them, and involution of the secretory component of the gland at the end of lactation, with preservation of the ducts (Sorenmo et al., 2011). By puberty, the mammary gland grows in accordance with the body's development and the ducts develop in a circular pattern at a short distance from the nipple. Vascularisation at this level is similar to that of the skin (Silver, 1966).

At birth, the animal's mammary gland has only the large ducts formed. These extend only a short distance from the nipple into the subcutaneous mesenchymal tissue. With puberty and secretion of oestrogens, cell proliferation of the ductal end is activated. Ductal development and the formation of lobulo-alveolar units will occur under the influence of increased progesterone levels during diestrus and gestation. A third stage is undergone under the influence of gestational prolactin when it will cause differentiation of secretory alveolar cells so that at parturition the mammary gland is characterized by a secretory lobulo-alveolar ductal structure (Donald J. Meuten, 2017).

Sexual cycle in canids

The bitch is a diestric animal (when referring to the number of estrous cycles per year), with an average length of sexual cycle of about 7 months, with many particularities (Runceanu et al., 2007). Because of this, the values found in the literature show major differences. However, the interval between two successive cycles should be constant in the same individual. (Roşca, 2005) The

intervals resulting from the overlapping different literature sources are: Proestrus (4-7-10 days), Estrus (5-9-14 days), Diestrus (1-2 months) and Anestrus (2-4-5 months) (England et al., 2010; Noakes et al., 2019; (Rehm et al., 2007).

In bitches, the proestrus and oestrus periods are long and always accompanied by a long luteal phase - diestrus (whether the bitch is pregnant or not) (England et al., 2010; P. Concannon, 2009).

Proestrus is the phase of intense follicular activity, with estrogenic dominance, but the female does not accept mating. Clinically, the beginning of this period is marked by the appearance of the first vaginal bleeding, and ends when the female will accept the male (Feldman and Nelson, 2004). Now, under the action of FSH and LH, follicular growth and development takes place, followed by secretion of estrogens from the follicular granulosa. These reach their maximum serum concentration at this stage, then they decrease.

Estrogens then induce a proliferation of epithelial cells, which is more obvious in the vaginal mucosa, changing its epithelium from cuboidal to a layered squamous epithelium that will become hyperplastic (most likely in order to prevent trauma to the vaginal mucosa during mounting) (Concannon, 2011). This is important because it allows the clinician to identify the stage of the cycle. Similar studies are also in queens (Zambelli, D., & Cunto, M., 2005). The earliest evidence of entry into the proestrus is the presence of erythrocytes among the superficial and basal epithelial cells sampled (most commonly using a moistened cotton swab) (England et al., 2010).

High LH and FSH concentration is crucial for follicular growth and development. However, as follicles mature, inhibin is secreted, a specific inhibitor of FSH, so towards the end of proestrus, FSH secretion becomes stationary. (England et al., 2010; Runceanu et al., 2007)

Estrus is characterised by an increased concentration of oestrogens, which decline before ovulation. Clinically, the beginning of this period is marked by the female's acceptance of reproduction, and ends when she no longer accepts it (Feldman and Nelson, 2004). At this stage, there is congestion and swelling of the external genitalia, secretion of a characteristic fluid (smelly, bloody mucus at the beginning of estrus that becomes clear towards the end of estrus). The female becomes restless, constantly searching for the male, and in its proximity takes the characteristic position for mounting (Noakes et al., 2019).

Ovulation in the bitch is spontaneous, occurring at the end of the heat cycle, and is stimulated by a spike in LH secretion - which occurs after a maximum concentration of estrogen

is reached. Ovulation occurs 2 days after the LH peak. Of interest is the fact that in bitch, the LH peak is of prolonged duration - 24-48 h, leading to a pre-ovulatory luteinization of the follicles and an increase in serum progesterone concentration before ovulation, so that at the time of ovulation, the concentration of progesterone is close to 5.4 ng/ml, changes that are essential for the bitch's sexual behaviour (England et al., 2010) Thus, in the bitch both oestrus and diestrus are of progesterone dominance. (Feldman and Nelson, 2004)

In bitches, unlike other species, the ovules are not fertilisable immediately after ovulation, but need to mature within 1-5 days, depending on the individual (Ciupe et al., 2020) This is due to the

fact that unlike other species in which ovulation of a secondary oocyte (of order II) occurs, in the bitch ovulation of a primary oocyte (of order I) takes place, which will have to mature, becoming a secondary oocyte capable of being fertilised after 96 to 108 hours. This secondary oocyte usually remains viable for 24-48 hours (day 5-6 after LH peak) Figure 1. Most of the time, an equal number of luteal bodies can be seen on the two ovaries. This is important because it has been observed that often the number of fetuses in a uterine horn is not always identical to the number of corpora lutea on the corresponding ovary. Thus, embryo migration into the contralateral uterine horn is a common occurrence. (Noakes et al., 2019; Pretzer, 2008; Reynaud et al., 2020)

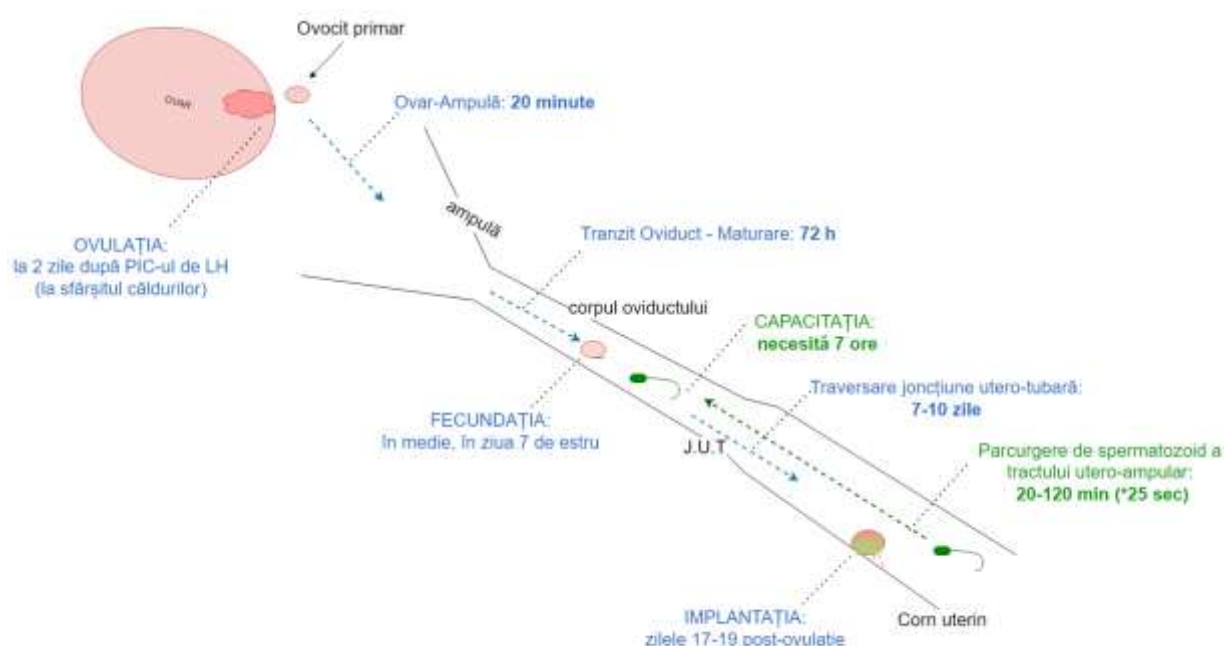


Figure 1 - Representative diagram in bitch, with key events on segments ovary-oviduct-uterine horn.

This delay between ovulation and fertilisation in bitches leads to relatively frequent over-fertilisation, which results in the fertilisation of eggs with semen from several males, producing offspring of different breeds. Therefore, in artificial insemination with refrigerated semen, the female is inseminated on the second day after ovulation, the lifespan of the spermatozoa covering days 2 and 3. In artificial insemination with frozen semen, as the thawed spermatozoa have a very short lifespan, insemination is carried out on day 3. (Ciupe et al., 2020; Pretzer, 2008)

In bitches, estrous behaviour is prolonged for about 7 days after ovulation. After ovulation, the luteal phase of the sexual cycle (diestrus) begins, when the concentration of progesterone continues to increase. In bitches, the term diestrus is preferred, precisely because of the particularities of the sexual cycle, namely the fact

that progesterone secretion starts as early as the oestrus phase (luteal follicles). Metestrus on the other hand, describes a period of luteal body activity as a distinct entity, not applicable in this situation. (Feldman and Nelson, 2004)

Progesterone modifies the characteristics of the cervical mucus (viscous), decreases muscle excitability, preparing the uterus for embryo implantation. The diestrus phase is considered to begin when oestrus signs have ceased, and ends when progesterone concentration returns to basal levels (<1.0 ng/ml). (Feldman and Nelson, 2004) During the early part of the luteal phase, progesterone concentration is the same in pregnant and non-pregnant females, unlike in other species such as cats. (England et al., 2010)

The luteal stage in the pregnant bitch lasts for about 66 days from ovulation to parturition, while in the non-pregnant bitch it lasts for about

63 days. In the second part of the luteal phase in pregnant bitches, the concentration of progesterone starts to decrease, while at the same time there is an increase in the concentration of prolactin. Inhibition of prolactin secretion may result in the termination of the luteal phase, or of gestation if it has been established. After parturition, newborn breastfeeding induces an increase in prolactin secretion, which lasts for about 6 weeks and gradually decreases as milk production diminishes until weaning (Chastant, 2023; England et al., 2010).

Anestrus is the period between the end of the luteal phase and the onset of the return to proestrus. The onset of this period is difficult to identify clinically in the un-estrus female because

there are no obvious changes between diestrus and anestrus. When the previous cycle was accompanied by gestation, the first part of anestrus includes lactation (Concannon, 2011; England et al., 2010; Feldman and Nelson, 2004).

Sexual cycle in cats

The cat is a seasonal polyestrous animal, which in the absence of mating enters oestrus every 2-3 weeks throughout the breeding season. In the northern hemisphere this season usually starts in January (as daylight increases) and continues until September. In breeding cats there are usually only 2 estrous periods (and therefore 2 possible gestations) per season. The age of first cycle onset, or puberty, depends not only on physical maturity but also on the season (Table 1).

Table 1

Age of puberty in cats

Date of birth	Sexual maturity	The first estrus	*** If they reach the specific weight of 2,3-2,5 kg
Cats born in JUNE	reach SM in month JANUARY of the following year	and will have their first estrus at 6 MONTHS OLD.	
Cats born in MARCH	reach SM during the anestrus season (OCTOBER - DECEMBER)	they won't have an estrous cycle until January or February of next year, therefore they will have the first oestrus at 10 MONTHS OLD.	

*SM – sexual maturity

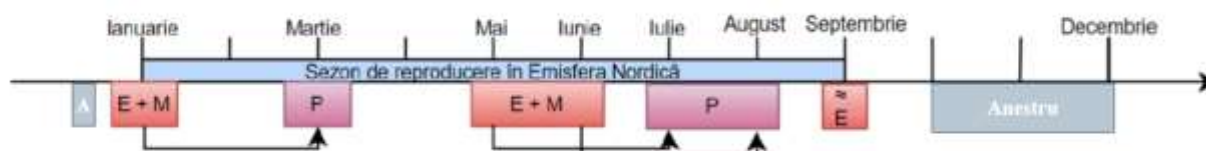


Figure 2 - The estrous season of the cats from the Northern Hemisphere. E - estrus, M - mating, P – parturition

The stages of the cat's sexual cycle are proestrus, estrus, (metestrus = interestrus), diestrus (a short period of sexual inactivity). However, since the cat has a induced ovulation, in the absence of mating luteal phase will not follow, so estrus will be followed by a period of sexual inactivity (interestru), which differs endocrinologically from the luteal phase - after interestru the cat enters estrus again. The luteal phase, however, occurs when ovulation occurs and the female remains, or does not become, pregnant. The cycle repeats itself before the end of the reproductive period, after which the last metestrus of the reproductive period is followed

by a period of longer sexual inactivity, i.e. anestrus. So, cats have three varieties of sexual cycle *Figure 3* (England et al., 2010; Reynaud et al., 2020; Johnson, 2022).

Therefore, if after ovulation, fertilisation of oocytes does not occur, or for other reasons pregnancy does not occur, the female will enter pseudopregnancy for about 45 days. Ovulation is therefore followed by the formation of the corpus luteum both in and out of gestation. If gestation does not set in, CL reaches peak progesterone activity in 10-15 days, then begins to decline, reaching baseline values in days 30-35. Behavioural changes and mammary gland

enlargement in pseudopregnancy are not observed in the cat as they are in the bitch, but only slight swelling of the mammary gland can be observed (Arikan et al., 2009).

Follicular growth and development occur in the proestrus due to FSH and LH secretion. During this period the cat intensifies its rubbing of the head by objects or owners, often interpreted as affectionate behaviour. On the other hand, the

proestrus may continue without the cat showing any change in behaviour. It is, however, the period when the male is attracted to unreceptive females (Rosca, 2005).

During oestrus the oestrogen concentration is increased and clinically, specific meowing is noticed and the cat accepts the male. Up to 5-7 matings can occur within 1-2 hours if the couple is not disturbed (England et al., 2010).

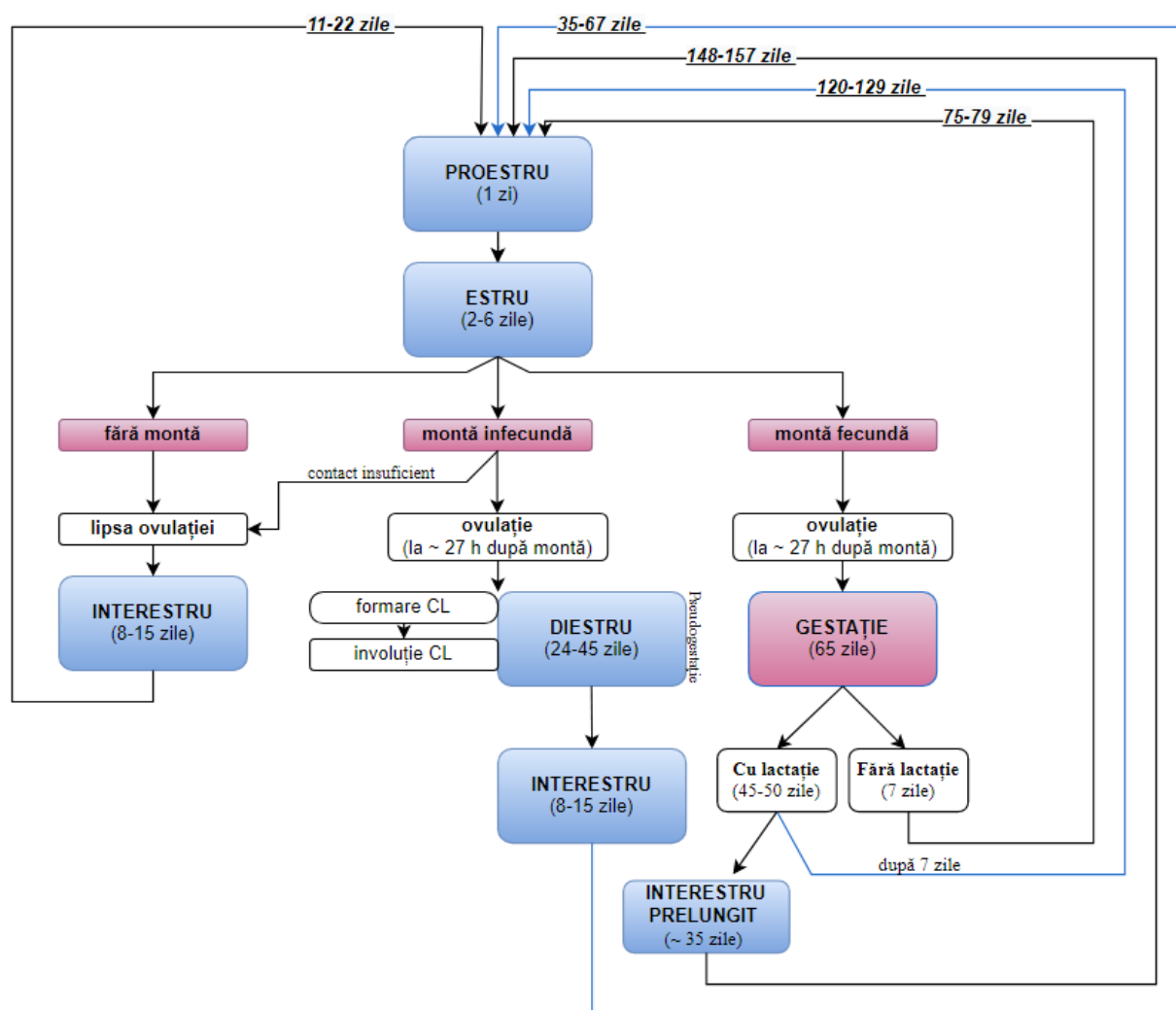


Figure 3 - Stages of the cat reproductive cycle

Ovulation occurs as a result of mechanical stimulation of the vaginal walls (Vansandt, 2022). It is reported that about 50% of cats ovulate after a single copulation, while for the rest the release of sufficient LH for ovulation is achieved when about 4 copulations occur in 2-4 hours. Ovulation will take place about 27 hours after mating. After ovulation, oocytes can be fertilised immediately, as they are mature (England et al., 2010).

At the end of the estrus there are therefore 3 possible outcomes (Rosca, 2005):

1. Lack of mating: proestrus (0-1 days), oestrus (2-6 days), interestrus (8-15 days).

2. Infecund mating: proestrus, estrus, diestrus, interestrus.

3. Fecund mating: proestrus, oestrus, gestation.

Anestrus is the seasonal period of ovarian inactivity that usually occurs in winter (Noakes et al., 2019). Interestrus is the period between one oestrus and the next oestrus (8-15 days) in females that have not ovulated or had fertile breeding (England et al., 2010).

The data collected in this review will be beneficial for research on the reproductive pathologies, artificial insemination, in dogs and

cats, but also for the therapeutical approach of the diseases.

CONCLUSIONS

In order to truly recognize what is abnormal in reproductive diseases, medical practitioners must have an understanding of what is considered normal for the canine and feline estrous cycle.

The particularity of the cat's sexual cycle comes from the fact that the cat has induced ovulation. During copulation, receptors located in the vulva will stimulate the release of LH from the anterior pituitary, triggering ovulation.

There are at least 3 particularities in the sexual cycle of canids: each cycle lasts a minimum of five months, the diestrus period is similar in pregnant and non-pregnant bitch, and the anestrus period occurs regardless of pregnancy status, being characterized by a prolonged period of ovarian inactivity.

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