SOME PHYSIOLOGICAL FEATURES OF THE BITCH

Wafir M. Saleh & Ahmmad Shamkhi

Department of surgery and obstetrics, College of Vet. Medicine, University of Baghdad/Iraq
Department of Anatomy and Histology College of Vet. Medicine, University of Baghdad/Iraq

ABSTRACT

Sound knowledge of the bitch reproductive cycle is essential, pubertal age, kind of estrus with its duration, gestation period and puppies care, all these events and further more are so important for the owner or canine interest. Dogs and due to their friendship manner to the closely mammals which is the human being makes this relation as courtship. Man takes his dog as the more helper creature as home share, guarding his own properties, sheep, tracing, experimental animals and so on. For these reasons it is so important to have some Knowledge concerning the physiological features of these mammals.

KEYWORDS: bitch, dog, estrus, Anestrus, proestrus, sanguineous discharge, pseudo pregnancy.

INTRODUCTION

Domestic dogs are Monestrous, typically non-seasonal, polytocous, spontaneous ovulators, have a slightly longer spontaneous luteal phase, the resulting inter-estrus intervals of 5–12 months are variable among bitches (commonly 6–7 months) ranging from highly variable to regular (Jeffcott and Lindsay, 1989). Non-pregnant bitches have a prolonged luteal phase with a more protracted decline in serum progesterone than in pregnancy because bitch has no uterine luteolytic mechanism. The obligate anestrus of 8–40 weeks is terminated by poorly understood interactions of environment (e.g. pheromones, possibly photoperiod) and a potential endogenous circannual cycle in sensitivities of hypothalamic dopaminergic, serotonergic and/or opioid pathways (Christie and Bell, 1971). Bitch will only allow the male to mate during a restricted time coinciding with ovulation, due to prolonged periods of estrus female might need to be bred several times to augment the chances of conception (Russell, 2008). Bitch seems to has one cycle per year but some breed are so fertile that adapted herself to have two cycle per year, for that, bitch is adapted herself to have 2-3 cycle each 1.5-2 years (Claudia, 2009).

Generally estrus cycle is divided to 4-phases, each is controlled by pituitary hormonal induction and ovarian functions, these phases according to Champlin, et al. (1973) are: Proestrus phase: The first stage in the estrous cycle immediately before estrus, characterized by development of both the endometrium and ovarian follicles. Bitch has a true pro-estrus characterized by the presence of vulvar edema, swelling and a sanguineous discharge. Some fastidious bitches show no evidence of discharge as they are continually cleaning the perineum. The bitch is attractive to males but will not accept the male (Kutzler, 2007): Estrus phase: The second stage in the estrous cycle immediately before metestruus characterized by receptivity to a male and to mating, often referred to as "heat" or "in heat". Pheromones may also be secreted only at this stage of her cycle. Bitch will accept the male and adopts the breeding stance. The vulva becomes less edematous and the vulvar discharge becomes clearer, less sanguineous and less copious. The duration of pro-estrus and estrus combined is about 18 days, i.e. 9 days each, however, this can be very variable, some bitches showing very little sign of pro-estrus before they will accept and stand for the dog and others producing a copious sanguineous discharge during true estrus. Ovulation usually occurs 1 or 2 days after the onset of estrus, although, using laparoscopy, it has been observed that some follicles continue to ovulate up to 14 days later (Wildt et al., 1977). Metestrus phase (diestrus): The third stage characterized by sexual inactivity and the formation of the corpus luteum, and an increase in the blood concentration of progesterone. This stage starts when the bitch ceases to accept the dog; however, there is dispute about its duration. Some consider that it ends when the corpora lutea have regressed at 70–80 days whilst others measure it in relation to the time taken for repair of the endometrium, 130–140 days (Jöchle, and Andersen, 1977). Anestrus: Not a stage in the estrous cycle, but a prolonged period of sexual rest where the reproductive system is quiescent. The average interval between successive estrus periods is 7 months, but it is variable, breed may have an effect. For example, for Rough Collies breed it is 37 weeks and for the German shepherd 26 weeks other breeds that were studied had mean intervals between these two periods, mating does not influence the interval (Christie and Bell, 1971).

Signs of estrus

The first indication that pro-estrus approaching is the onset of slight swelling of the vulvar lips. This generally precedes the commencement of bleeding by several days; labial swelling is progressive during the pro-estrus period. Bleeding attains its maximum early in pro -estrus and continues at this level into the early part of true estrus. During the greater part of pro-estrus the bitch, although attractive to the dog, takes no interest in his attentions, she will not stand for him and generally attacks him if he attempts to mount her (Fig. 1).
Physiological features of the bitch

A day or so before the end of pro-estrus, her attitude changes, and she shows signs of courtship towards the male. These comprise sudden darting movements which end in a crouching attitude with her limbs tense and her face alert, she barks invitingly, but as the dog approaches she moves suddenly again. She will not yet allow him to mount, with the onset of estrus the invitation to coitus is obvious, she stands in the mating position with her tail slightly raised or held to one side, remains still while the male mounts and copulates, by end stages of coapulatory tie (15 to 25 minutes), she becomes restless and irritable and her attempt to free herself may cause the male considerable physical embarrassment. After the first 2 days of estrus, sexual desire gradually recedes, but with the continued persuasion of the male she will accept coitus until the end of the period, bleeding, although reduced in amount, generally continues well into estrus. More often, discharge becomes yellow as estrus proceeds, vulvar swelling and tumefaction are greatest at the onset of the stage of acceptance. During the course of estrus the enlarged labia become softer in consistency, some labial swelling continues into the first part of the metestrus phase.

Concannon, (2010) described the bitch ovarian activities in concerned to the estrus phases.

Endocrine changes during the estrus cycle

The main feature which distinguishes bitch from other species is the prolonged luteal phase, illustrated by the persistence of high progesterone levels in the peripheral blood. It is noticeable that progesterone levels start to rise before ovulation has occurred, which confirms the morphological evidence of preovulatory Latinization of mature follicles 60–70 hours before ovulation (Concannon et al., 1977). This preovulatory rise in progesterone may provide the stimulus for the bitch to accept the male (Concannon et al., 1975). In addition, it can also be used as a method to determine the timing of artificial insemination in that it should not be delayed long after concentrations > 2–3 ng/ml are observed in peripheral plasma (Jeffcoate and Lindsay, 1989) Estrogens rise rapidly just before the onset of standing estrus, and are rapidly followed by the LH peak, which lasts much longer than that of other species; ovulation occurs 24–96 hours after this (Phemister et al., 1973; Wildt et al., 1977). FSH concentrations at estrus reach a peak, coincident with that of LH. Prolactin appears to have a negative correlation with progesterone; thus, as progesterone levels fall towards the end of met estrus or pregnancy, Prolactin increases; it is the major autotrophic hormone in this species.

FIGURE 1: Swollen bitch vulva in pro-estrus phase, white arrow pointed a sanguineous discharge, bitch in estrus (Cheryl, 2011)

FIGURE 2: Hormonal status of different phases of bitch estrus cycle (Wright, 1982).
Vaginal smear
The cyclical changes which occur in vaginal cytology have been described in detail (Griffiths and Amoroso, 1939; Hancock and Rowlands, 1949; Schutte, 1967; Rozel, 1975). Vaginal smears stained with either a simple stain such as Leishman’s or a trichrome stain At the onset of pro-estrus, large numbers of erythrocytes are present; however, when true estrus occurs the number of erythrocytes is reduced, smear consists of superficial cell types from the stratified squamous epithelium, such as a nuclear cells, cells with pyknotic nuclei and large intermediate cells. Towards the end of estrus, numbers of polymorph nuclear neutrophil leucocytes appear in the smear, and these become the dominant cell type during metestrus. In anestrous nucleated basal and intermediate cells of the stratified squamous epithelium, together with a few neutrophils, forms the characteristic smear. The vaginal epithelium during anestrus is of the low, columnar, cuboidal type and comprises two or three layers only, during pro-estrus the epithelial cells change to the high, squamous, stratified type and persist in this form until the later stages of estrus, the stratum corneum and the layers immediately beneath it are lost by desquamation during the pro-estrus and estrus periods, leaving a low, squamous structure which becomes converted to columnar epithelium in 1–3 weeks after the end of heat. During metestrus (and pregnancy) the epithelium is of a higher columnar type than during anestrus. During pro-estrus, estrus and early metestrus the epithelium and lamina propria are infiltrated with large numbers of neutrophils which eventually escape into the vaginal lumen.

Endometrium
The endometrium shows considerable change during the estrus cycle. The endometrial glands in pro-estrus and estrus are loosely coiled with very obvious Lumina and deep epithelial lining. During met estrus the glands become larger, the Lumina smaller and the coiled parts in the basal layer of the endometrium more tortuous. As the bitch passes into anestrus, there is a reduction in the amount and degree of coiling of the glands. At about 98 days after the onset of estrus, i.e. in metestrus, there is evidence of desquamation of the endometrial epithelium; however, by about 120–130 days the epithelium has been restored by proliferation of cells from the crypts of the endometrial glands.

Changes in the ovaries
During anestrus the ovaries are oval and slightly flattened, bitch of medium size they measured approximately 1.4 cm from pole to pole and 0.8 cm from
the free border. No appreciable follicles can be seen, although on section the minute remnants of the CLs of the previous cycle may be seen as yellow or brown spots. In the young bitch, the surface of the ovaries is smooth and regular, but in the aged animal it is irregular and scarred. At the commencement of proestrus, developing follicles have already attained a diameter of 0.5 cm. They progressively enlarge, until at the time of ovulation their size varies from 0.6 to 1 cm, ovary is considerably enlarged, its size depending on the number of ripe follicles present. Owing to the thickness of the follicle wall it may be difficult to distinguish between follicles and CLs. Prior to ovulation, the surface of the follicle shows a slightly raised papule, pin-head-sized, and the epithelium covering it is brown, which contrasts with the flesh color of the remainder of the follicle. A remarkable feature of the ripening follicle of the bitch is the thickness of its wall, due to hypertrophy and folding of the granulosa cells, which can be seen on section with the naked eye as evidence of preovulatory luteinization. Ovulation is spontaneous and normally occurs 1 or 2 days after the onset of the period of acceptance. Most of the follicles rupture over a period of 48 hours (Wildt et al., 1977); oocyte is capable of being fertilized for up to 108 hours after ovulation (Tsutsui and Shimizu, 1975). CL at first contains a central cavity, but becomes filled by compact luteinized cells by the 10th day after ovulation, by which time the body has attained its full size (0.6–1 cm) and comprises greater mass of the ovary. As a rule an approximately equal number of CLs are found in each ovary, although occasionally there are wide differences that the numbers of fetuses in the respective cornua in pregnancy frequently differ from those of the CLs in the ovaries on the respective sides. Embryonic migration into the cornua on the opposite side would appear to be common. On section; the CL is yellowish pink; it remains unchanged in the non-pregnant bitch until about the 30th day after ovulation, then slowly atrophies.

**Ultrasound appearance of the ovaries**

Using a trans-abdominal approach via the flank, with the bitch standing and a 7.5 MHz real time linear array transducer, England and Allen (1989) were able to identify ovarian structures at the onset of proestrus; these were circular and anechoic and were obviously developing antral follicles. When the bitch was in estrus, they had increased in size, reaching a maximum of 4–13 mm in diameter on day 13 (day 0 being onset of proestrus). The walls of the follicles became thickened from day 10 onwards, presumably due to preovulatory Luteinization; there was no evidence of follicular collapse associated with ovulation. At 25–30 days after the onset of proestrus the ovaries were difficult to identify.

**Silent heat**

Silent heat is defined as ovarian activity with no associated vulvar swelling, serosanguinous vaginal discharge, or attractiveness of male dogs, while ovarian activity is occurring normally, no outward signs of “heat” are observed by owners or by other dogs, including intact males. Serum progesterone concentrations can be measured on a monthly basis to determine if functional ovaries are present. Levels greater than 2.0 ng/mL indicate the presence of functional luteal tissue (Johnston, 1991). Vaginal cytology can also be assessed on a regular basis to determine if percentages of superficial epithelial cells are increasing. Increasing percentages suggest influence of estrogen and functional ovarian tissue. Silent heats are not uncommon and can make determining if a young bitch is truly anestrous or not difficult (Löfstedt, and VanLeeuwen, 2002).

**Pseudo pregnancy**

Pseudo pregnancy was originally believed to be due to an intensification and prolongation of metestrus; however, a number of workers have demonstrated that there is no difference in the progesterone concentrations in the peripheral blood of bitches with or without signs of pseudo pregnancy. The most relevant features of the pathophysiology, clinical signs, diagnosis, preventions and treatments all are served to help those pets’ animals in overcoming this syndrome. Pseudo pregnancy typically is occurred in matured non-pregnant bitches about6-12 weeks followed the incidence of estrous (heat) and characterized by clinical (covertly) pseudo pregnancy in which nesting, body weight gain, mammary enlargement and excessive milk drop are the most relevant features, while subclinical (overly) pseudo pregnancy present when previous signs are less reliable. Prolactin, the key hormone in long-term canine estrous cycle, is the most important leutorophic factors from day 35 of the cycle; this hormone is stimulated by the progesterone falling down. Wafir and Ihsan (2016) described five methods were used to treat this syndrome and controlled the excessive secretion of prolactin hormone and cured the condition: Nutritional, Surgical, Natural, Hormonal and Supportive treatment method. Results showed that (effect index of those methods is related to the severity of the syndrome and bitch general condition), hormonal and Natural treatments gave same effective results with some incidence of sideupset. Nutritional method has a limited degree of affectivity. Surgical methods put affected animal in a sterile state but gave good limitations. Supportive method can be applied with a considerable result. The decline in progesterone appearing to coincide with the rise in prolactin acts as predisposing factor, if bitches undergo ovariohysterectomy when they are pseudo pregnant the condition can be intensified and prolonged, anti-prolactin drugs such as bromocriptine and cabergoline have been successfully used to cause remission of the signs of pseudo pregnancy(Butak, 2005).

**Estrus induction**

Estrus induction is used clinically in conjunction with routine breeding management, as when breeding opportunities are missed in following conception failure, or treatment for primary and secondary anestrus, many different schedules that have been used, were reviewed recently, but in any procedure that is adopted, timing of treatment is crucial for success, particularly if this is regarded not only as estrus induction, but also as ovulation and subsequent pregnancy. Furthermore, some of the current methods would appear to have significant merit for application in cases of prolonged anestrus and for enhancing fertility of research bitches in colonies of dogs maintained as animal-models of heritable or genetically-based diseases of interest in human or veterinary medicine. It is important to emphasize the term “fertile
estrus” and to consider using the term “fertile proestrus” because the stimulation of ovarian activity in a bitch can lead to any of the following responses, (Concannon et al., 1997), and those responses are; 1) a “false proestrus” that fails to progress into an estrus or estrus-like changes in behavior and/or reproductive tract morphology; 2) a “false estrus” that follows an induced proestrus and involves many, most or all of the behavioral and morphological changes associated with a normal fertile estrus but is non-ovulatory; 3) a “fertile estrus” that follows an induced proestrus and that has most or all of the characteristics of a normal, spontaneous estrus; and 4) in some cases, a “fertile quasi-estrus” or “fertile proestrus” that involves most or all of the endocrinological changes seen in normal proestrus and estrus as well as a fertile ovulation, but also involves a less than normal change in vaginal cytology, vulval swelling and/or receptive behavior, or even an absence of receptive behavior, with fertility only demonstrable by appropriately timed artificial insemination (Concannon, 1993). It is because of such variation in “responses” to many protocols that the bitch should be carefully monitored for clinical signs including vaginal cytology and changes in vulvar and vaginal morphology, as performed for any critical managed breeding, and also monitored for changes in circulating progesterone concentrations that can differentiate between a false and ovulatory estrus.

**Gonadotrophin administration**

The earliest studies utilized serial administrations of proestrus-inducing FSH or FSH-like gonadotropin preparations including PMSG for 10 or more days (with or without later administration of an ovulation inducing dose of an LH-like hormone such as hCG. Gonadotropin administrations have varied in source, dosage, and biopotency, as well as in pattern and frequency of administration, and efficacy has ranged from 0 to 30%, and repeatability has been a problem. PMSG administration at doses of 20 i.u./kg/day for 10 days often causes hyper-secretion of estrogen and has the potential to cause uterine dysfunction and/or uterine disease. Improved pregnancy rates (50%) occurred when PMSG (20 i.u./kg/d) was administered for only 5 days and immediately followed by hCG (500 i.u./dog once on day 50, the latter as a proestrus-enhancing treatment that apparently further stimulates ovarian follicle development such that the induced proestrus progresses and spontaneously culminates in an estrus in which ovulation occurs d spontaneously (Arnold et al., 1989). The hCG may have provided a stimulus similar to that provided by increased endogenous LH pulses during natural cycles. Another approach has been to use an LH-like hormone preparation to induce a proestrus i.e. human menopausal gonadotropin (HMG) with a 1:1 ratio of LH to FSH biopotencies, and to then allow the induced proestrus to proceed spontaneously to an estrus in which the ovulation rate appears to be about 90% and the fertility rate about 50%. This use of HMG (2-4 IU/kg/d x 9 d) with its relatively high “LH-content” has been suggested to better substitute for the natural pro-preproestrus increase in LH seen in the normal ovarian cycle (Wanke et al., 1997). During the week prior to natural proestrus, the increase in plasma LH pulse frequency is more obvious, is of greater relative magnitude, and involves a greater percent increase in mean concentrations than any corresponding increase in FSH secretion. Fertile estrus has also been induced by frequent injections of purified LH.

**Estrogen pre-treatments**

Estrogen preparations have been administered as a means to “prime” the bitch for subsequent serial administrations of proestrus-inducing gonadotropin preparations including PMSG or FSH with or without later administration of an ovulation inducing hormone such as hCG. The improved efficacy of gonadotropin administration following estrogen treatments is not well documented, but could be the result of two or more known effects of estradiol. Individual administrations of estrogen may cause transient suppressions of LH and FSH secretion via classical negative feedback on the hypothalamus and pituitary and be followed in each case by a rebound release of LH and FSH in turn facilitated by a priming effect of the prior estrogen on GnRH secretion and/or pituitary sensitivity to GnRH. Any such stimulatory fluctuations in LH and/or FSH although undocumented would likely alter the follicular status of the ovary at the time of subsequent gonadotropin administration. Alternatively or additionally, estrogen might act directly at the level of the ovarian follicles to stimulate antral follicle development to more advanced stages (Bouchard et al., 1993). Short term estrogen treatment alone in some bitches can produce an induced proestrus that persists and is subsequently followed immediately by a fertile estrus, without any gonadotropin administration. The success rate is variable and appears to have been greatest in bitches that have been observed, claimed or assumed to have experienced a prolonged or persistent anestrus, or to be in late anestrus, prior to estrogen treatment, the greatest success rate administered the estrogen in late anestrus i.e. after 3-4 months into an induced-anestrus in bitches with a luteal phase was terminated by prostaglandin-F administration. The protocol used diethylstilbestrol, 5mg tablets orally, daily for 6-9 days, i.e. until 2 days into an induced proestrus (Concannon, 2010).

** GnRH and GnRH-analogue administration**

The increased LH pulse frequency observed prior to spontaneous proestrus has been mimicked in bitches in early or mid anestrus using the intravenous administration of relatively large doses of native GnRH (0.2-0.5 ug/kg) every 90 minutes to provoke LH pulses at that rate (Concannon et al., 1997). The initially large LH and FSH pulses become progressively smaller during the GnRH-induced proestrus as they do in natural proestrus, and usually result in a normal proestrus base on observed changes in estradiol, vaginal cytology and external genitalia, and one which is often followed by a spontaneous LH surge and estrus onset before or immediately after the termination of 10-14 days of GnRH administration. Less frequent injections of large doses of a GnRH agonist have also been successful. Neither pulse rate nor pulsatility itself appears to be critical in provoking a positive ovarian response. In fact, constant GnRH agonist administration for 10-14 days via subcutaneous osmotic-pumps requiring only a single administration have also been observed to result in a high rates of proestrus induction, spontaneous ovulation, and pregnancy...
Physiological features of the bitch

(Concannon et al., 1993). The most successful doses of three GnRH super-agonists estimated to be 160—180 x(times) that of native GnRH ranged from 0.6-1.8 ug LUT /kg/d for lutrelin and approx. 1.0 -1.5 ug NAF/ kg/d for nafarelin or a nafarelin analog, based on recent studies (Concannon et al., unpublished). Pregnancy rates using lutrelin have been 70-100% in several trials; the material is not commercially available. Others have successfully used single subcutaneous injections of buserelin (mixed with silastic), a GnRH-analog with a potency of about 15-30 x (times), native GnRH (Cinone et al., 1996); the 50 ug/kg dose likely translates into some 4 to 5 ug BUS/kg/d when applied to beagle-sized dogs; in contrast buserelin at 1.5 mg/kg every 8 h failed to reliably induce proestrus (Rota et al., 2002). Kutzler et al.(2002) and Volkmann et al.(2005) have demonstrated the successful use of single subcutaneous or sub-mucosal (vulval) insertions of a whole or a half biodegradable capsule containing the GnRH super-agonist deslorelin; a commercial formulation marketed for ovulation-induction in estrus horses (Ovuplant). Deslorelin has a bio-potency about 150 x(times) GnRH action and the implants likely provided doses of 4-8 ug/kg/d in beagle-sized dogs based on manufacturer's claimed release rates.

Dopamine agonist administration

Several studies have reported the use of a dopamine agonist (DA) administered orally at doses sufficient to lower plasma prolactin as a means to terminate anestrus either prematurely in normal bitches or therapeutically in cases of prolonged or persistent anestrus (Cirit et al., 2007). The efficacy has been anecdotally estimated to be about 70%, and possibly higher in bitches with prolonged anestrus; the resulting proestrus, when induced, has occurred after a variable duration of treatment ranging from 8 to 40 days; the average appears to be about 20 days; duration appears to be dependent on the stage of anestrus, with longer treatment required in early anestrus. Whether the simultaneous reduction in prolactin is part of the mechanism of action or if the mechanism involves other or additional dopaminergic effects is not known (Ajitkumar, and Prasseda, 2010). However, efficacy appears to depend on dopamine responsiveness sufficient to also cause suppression of prolactin; bitches that fail to experience suppression of prolactin also fail to show a clinical proestrus response. Two DA treatments reported to be effective have included bromocriptine (Parlodiel) at 0.05.or 0.1 mg/kg, p.o., q.d. or bid, and cabergoline (Galostop) at 5 ug/kg, p.o. q.d.; administration is until an induced proestrus is pronounced for 2 days or until the onset of estrus (Zöldág et al., 2001).

REFERENCES


