

## Reproductive Biology 101 – Kutzler

This lecture has been designed to provide a primer on canine and feline reproduction for both scientists unfamiliar with this topic as well as lay people. It will cover sexual differentiation, reproductive anatomy, puberty, reproductive physiology of both male and female cats and dogs, and, finally, pregnancy physiology. Breeding management, parturition, dystocia, causes of infertility, and estrous cycle manipulation are not covered in this review.

Commonly used abbreviations in canine and feline reproductive biology:

- CL: corpus luteum or corpora lutea; develops on the ovary at the site of each ovulation and secretes progesterone (a steroid hormone)
- FSH: follicle stimulating hormone; protein hormone secreted by gonadotrope cells within the anterior pituitary
- GnRH: gonadotropin releasing hormone; peptide hormone secreted by the hypothalamus
- IEI: interestrous interval; period of time from the onset of proestrus until the onset of the subsequent proestrus (note: interestrous is an adjective and interestrus is a noun)
- LH: luteinizing hormone; protein hormone secreted by gonadotrope cells within the anterior pituitary

### Sexual differentiation

Normal sexual differentiation is dependent upon the successful completion of three consecutive events: establishment of chromosomal sex, gonadal sex and phenotypic sex. Chromosomal sex (normally XX or XY) is established at fertilization and maintained by mitotic division after that. Sexual differentiation is completed by 46 days of gestation, where gestation lengths are 65 days for dogs and 64 days for cats. In the early embryo, the gonads are undifferentiated. Gonadectomy of XX or XY embryos before gonadal differentiation results in the development of a female phenotype, leading to the conclusion that the basic embryonic plan is female. A gene located on the Y-chromosome (Sry gene) encodes a testis-determining protein that results in testis formation and establishes the male gonadal sex. Sry acts as a master switch turning on several genes that are located on other chromosomes, thus inducing a cascade of gene products that are necessary for testicular development. In the absence of the Sry gene, the default pathway to female gonadal sex is initiated and an ovary develops.

Following gonadal differentiation, development of the internal and external genitalia occurs. In the early embryo, both sets (Wolffian and Müllerian) of internal tubular structures develop initially. Two testicular secretions (Müllerian-inhibiting factor and testosterone) are responsible for masculinizing the tubular reproductive tract. Müllerian-inhibiting factor (MIF), a glycoprotein produced by Sertoli cells, causes the Müllerian (female) duct system (uterine tubes, uterus, cervix and cranial vagina) to regress. Testosterone, a steroid hormone produced by Leydig cells, promotes the formation of the vas deferens and epididymides from the Wolffian ducts. Testosterone is metabolized to dihydrotestosterone (DHT) within the cells of the urogenital sinus, the genital tubercle and the genital swellings to result in the formation of the prostate and urethra, the penis and the scrotum, respectively. Androgen-dependent masculinization is mediated through the binding of testosterone or DHT to the androgen receptor protein, the product of a gene on the X-chromosome.

Canine and feline male external genitalia originate from an undifferentiated urogenital tubercle (which forms at the level of the embryonic membrane that forms the cavity into which both the intestinal and urogenital tracts empty). As a direct result of the influence of DHT, the prepuce develops from a circular plate of ectoderm creating a hole at the level of the tip of the penis, and the junction between the preputial and penile mucosa (balanopreputial fold) dissolves at a later stage, also as a direct result of the action of androgens. In the absence of DHT (as in the normal female), the vagina, vestibule and vulva develop. Determination of gender in newborn dogs and cats may be difficult because the difference at this age is a slightly longer anogenital distance in males (13-15 mm) versus females (7-8 mm).

### Reproductive anatomy

The female reproductive anatomy in the bitch and queen has some similarities as well as some important distinctions (Shively MJ et al 1985). In both species, the vulva is located directly below the anus. The vestibule is the aspect of the vagina closest to the vulva. The clitoris and clitoral fossa are on the caudal ventral surface of the vestibule. The urethral orifice lies on the midline floor of the vestibule, near the vaginal-vestibular junction. The angle of the vestibule is approximately 60°. The size of the vagina in the mature queen is approximately 2 cm long x 4 mm wide, whereas in the bitch, the length of the vagina varies by body size. In the bitch, the vagina is relatively long and is horizontal from the cervix to the urethral orifice. As viewed with a vaginoscope, the dorsal median postcervical fold lies just behind the cervix in the most

interior part of the vagina. This fold gives the appearance of being the cervical opening and is sometimes referred to as a “pseudocervix.” The caudal median postcervical fold lies toward the tail from the cervix in front of the vagina and gives the appearance of being the cervical opening when viewed with a vaginoscope. In the bitch, the cervix is located in front of the pubic symphysis. The external cervical opening actually points downward at approximately a 45° angle.

Both bitches and queens have a Y-shaped uterus. The uterine body lies between the colon and the urinary bladder. The uterine horns extend in front of the uterine body and the tip of each horn is attached to the ovary by a short firm ligament. The uterine body can be palpated through the abdominal wall in cooperative, non-obese patients. The uterine horns are less easily palpated in normal, non-pregnant animals. The size of the uterus depends upon age, parity and stage of the estrous cycle. In the queen, the uterine body is approximately 2 cm long and the uterine horns are approximately 5 cm long. In both species, the diameter of the uterine body should be less than 1.5 cm diameter unless they are pregnant or pathologic (e.g., cystic endometrial hyperplasia, pyometra). The uterine tubes (improperly referred to as oviducts) are not normally palpable through the abdomen. The uterine tubes course in a circular manner on the ovarian bursa.

Both ovaries are located just behind each kidney, suspended by a suspensory ligament. The ovaries are normally not palpable abdominally or rectally. An ovarian bursa covers each ovary. The ovarian bursas usually contain fat, making the visualization of the ovarian surface difficult. The size of the ovaries depends upon what structures are present. During estrus, 3-7 follicles develop per ovary, which become corpora lutea following ovulation. Polyovular follicles, which are follicles that contain more than one oocyte, are common in both queens and bitches. Unlike other mammals, folliculogenesis is not complete until approximately 120 days after birth (Andersen AC & Simpson ME 1973, Barber MR et al 2001, Blackmore DG et al 2004, Durrant BS et al 1998, Tesoriero JV 1981). Senile ovarian atrophy, the equivalent of menopause in women, does not occur in normal bitches or queens.

In litter-bearing species, the mammary glands are typically arranged in two rows that are bilaterally symmetrical and extend from the ventral thoracic to the inguinal region. The parenchyma, or secretory tissue, is present only during pregnancy, pseudopregnancy, during lactation, and for 40-50 days after weaning. The teats indicate the position of the glands. The number of teats varies from 8 to 12, with generally 5 mammary gland complexes on each side of the midline in the bitch and 4 mammary gland complexes on each side of the midline in the queen. The number of external openings on a teat varies from 8 to 20 ducts per teat. The openings are located on the blunt end of the teat in an irregular pattern.

The male reproductive anatomy in the dog and tom also has some similarities as well as some important distinctions. The testes are located within the abdomen at birth and descend into the scrotum around 10 days of age. Although information regarding the hormonal control of testicular descent as late as 4-6 months of age exists, it is reasonable to expect both testes to be palpable within the scrotum by 6-8 weeks of age. In the dog, the testes lie horizontally in the scrotum, whereas in the tom, the testes are perineal in location, just below the anus. The testes should be symmetric in shape and size. The normal shape of a testis is oval and it should have a smooth surface. Measuring the total scrotal width (TSW) with calipers will provide a result that is highly correlated to daily sperm output (Olar TT et al 1983).

The tom has both a prostate gland and paired bulbourethral glands. However, the prostate gland is the only accessory sex gland in the dog. In the dog, the prostate gland may be palpated by digital rectal examination. The prostate is normally bilobed, symmetrical, non-painful to palpation, no lumps or bumps, and has a homogenous consistency (firm, not fluctuant or fluid-filled). As in men, the prostate normally enlarges with age (benign prostatic hyperplasia), but this enlargement should be symmetric. In the tom, the bulbourethral glands are located just in front of and on either side of the anus.

The canine penis contains a bone, called the os penis. The os penis should extend to within 1-2 cm from the tip of the glans penis. The penis is best evaluated in the erect state with the prepuce reflected caudal to bulbus glandis. The bulbus glandis becomes very large and bilobed when the penis is fully erect. The penile mucosa should have a smooth surface that is interrupted by engorged blood vessels. This is in contrast to the postpubertal intact feline penis, which has a band about 4 mm wide and consisting of ~120-150 backward pointing spines encircling the glans, forming 6-8 vaguely circular rows (Aronson LR & Cooper ML 1967). Those at the proximal end are ~0.7 mm long and those at the distal end are ~0.1 mm long. The spines consist of a connective tissue core, covered by an epithelial layer of which the outer surface is heavily cornified, bearing striking resemblance to the spiny papillae on the cat's tongue. The spines completely regress 13-24 weeks after castration. It is important to mention that penile spines are not necessary to induce ovulation in queens after mating (Aronson LR & Cooper ML 1967). The smooth penis of the castrate will still cause ovulation. However, the spiny penis may be more effective than the smooth penis during a typically brief intromission period because an intense level of stimulation is needed to initiate ovulation (Aronson LR & Cooper ML 1967).

## Sexual maturity

Puberty in both the female dog and cat is defined as the occurrence of the first estrus (onset of receptive behavior). In males, puberty is defined as the maturation of the hypothalamic-pituitary-testicular axis and normal sperm production that is capable of producing offspring. In toms, sexual maturity has occurred when the penile spines have reached their full size. In male dogs, territorial marking behavior (“leg lifting”) occurs at the time of puberty. Most puppies and kittens attain puberty between 8 and 19 months of age, with a range of 3.5 to 24 months for both males and females (Wildt DE et al 1981). Environmental factors can affect the onset of puberty. Most female cats born early in the season or exposed when young either to tomcats or cycling females or to increasing amounts of light show the first signs of estrus before similar individuals born later or not exposed to these factors. As with queens, litter mates or bitches housed together may show synchronicity of the onset of puberty. However, with the exception of a few breeds (e.g., Basenji), the onset of puberty in domestic bitches is not influenced by season. Onset of puberty is also influenced by body condition and breed. In cats, body weight must be greater than 2.3 kg. Oriental breeds (Siamese, Burmese) have an earlier onset of puberty, whereas long-haired breeds and Manx have a later onset of puberty (11-21 months). Smaller breed dogs generally have an earlier onset of puberty than larger breeds.

## Male canine and feline reproductive physiology

Gonadotropin-releasing hormone (GnRH) is secreted by the hypothalamus. The release of GnRH regulates all aspects of the sperm production. Bursts of GnRH induce pulsatile releases of LH from the pituitary. LH acts on the testicular interstitial cells (Leydig cells) to induce the pulsatile release of testosterone. GnRH also induces the release of follicle-stimulating hormone (FSH) from the pituitary, which promotes the early development of spermatozoa as well as the development and maintenance of the sustentacular cells (Sertoli cells). The Sertoli cells support the later development of the spermatozoa. They also produce inhibin, which inhibits FSH release from the pituitary.

In intact dogs, the resting plasma testosterone values may range from 0.4 to 6.0 ng/ml over a 24-hour period, closely following peripheral LH fluctuations. No diurnal rhythm has been reported. Testosterone has many functions, including promoting spermatogenesis and maintaining the functions of the efferent ducts, epididymides, and vas deferens. Testosterone also functions in the maintenance of libido and the growth and maintenance of the prostate gland. Testosterone causes a negative feedback on the release of GnRH and LH from the hypothalamus and pituitary. Therefore, any exogenous testosterone will reduce LH secretion, resulting in decreased Leydig cell secretion of testosterone and the eventual disruption of normal spermatogenesis.

Spermatogenesis is defined as the time required for one spermatogonia to become a mature spermatozoa in the testis. In the dog, approximately 54.4 days is required for spermatogenesis. This period may be altered by environmental or toxic factors. Epididymal sperm transit time is also necessary for spermatozoa to be capable of fertilization. The movement of the sperm cytoplasmic droplet from a proximal to a distal position and the acquisition of motility occurs during epididymal transit. This duration of epididymal sperm transit in the domestic dog is 14 days. Therefore, the rule of thumb estimate for the duration of spermatogenesis and epididymal transport is 62 days (2 months). Semen collection in the dog is performed using manual stimulation (Kutzler MA 2005). In the tom, semen can be collected by electroejaculation or toms can be trained to artificial vagina. The components of a feline artificial vagina include an assembled collection tube, a Pasteur pipette bulb, a 6 x 50 mm glass test tube, and a 100 ml polyethylene bottle, which serves as a warm water jacket (44-46° C) to control temperature.

## Estrous cycle physiology: bitch

Domestic bitches are nonseasonally monoestrous. As a result of this unique reproductive physiology, bitches spontaneously ovulate only once or twice per year and ovulation can occur at any time of the year. However, there are a few exceptions, such as the Tibetan Mastiff and the Basenji (Fuller JL 1956). The interestrous interval is the time from the onset of proestrus to the subsequent onset of proestrus, and includes proestrus, estrus, diestrus and an obligate anestrus. Proestrus is diagnosed clinically by the onset of vulvar edema and/or serosanguinous discharge, whereas estrus is defined as the onset of either behavioral signs (willingness to allow mating) or vaginal epithelial exfoliative cytology (>90% cornification).

The interestrous interval averages 31 weeks (Rowlands IW 1950, Christie DW & Bell ET 1971) with a typical range of 16 to 56 weeks (Christie DW & Bell ET 1971). In some bitches, estrus is much less frequent or outward (visible) signs of estrus are minimal to non-existent, giving the appearance of a prolonged interestrous interval (persistent anestrus). The variation in interestrous interval length owes itself to differences in the duration of anestrus. The duration of anestrus differs between and within dog breeds, indicating a genetic basis for anestrus length (Okkens AC & Kooistra HS 2006). It is important to mention that histological changes similar to involution in the bitch's endometrium are not complete until

135 days after the most recent estrous, regardless of whether the bitch was pregnant or not (Anderson AC & Simpson ME 1973). However, bitches with an induced estrus less than 4 months from the onset of the previous proestrus may still become pregnant (Volkman D et al 2004).

In the bitch, progression from early to late anestrus is characterized by a higher amplitude and larger number of hypothalamic gonadotropin-releasing hormone (GnRH) pulses (Tani H et al 1996), an increase in pituitary sensitivity to GnRH (Van Hafften B et al 1994), and an increase in ovarian responsiveness to LH and FSH (Van Hafften B et al 1994, Jeffcoate IA 1993). Serum FSH concentrations are increased throughout much of canine anestrus while LH concentrations are low, except near the end of anestrus (Concannon PW 1993). An increase in basal plasma FSH concentration is critical for initiation of folliculogenesis in dogs (Kooistra HS et al 1999, Onclin K et al 2001). FSH induces expression of LH receptors in the ovarian granulosa cells (Okkens AC & Kooistra HS 2006). Following initial follicle recruitment, LH is progressively able to replace FSH in the support of follicular maturation (Monniaux D et al 1997). In fact, supraphysiologic doses of LH alone administered to bitches in anestrus will induce follicle growth and proestrus (Concannon PW 1993, Verstegen J et al 1997). There is convincing anecdotal evidence that a “dormitory effect” occurs in canids, such that co-housing bitches in mid to late anestrus with proestrous or estrous bitches will shorten the duration of anestrus by 30 days or more in anestrus bitches (Concannon PW 1993). It is assumed that this phenomenon of “natural” estrus induction is mediated by pheromones but the mechanism by which an increase in gonadotropin secretion is mediated is not known.

Pulsatile secretions of FSH and LH occur in mature bitches during anestrus. These two gonadotropins presumably have the role of recruiting cohorts of follicles for subsequent development and maturation. Another indication of follicular activity during anestrus has been the detection of estradiol-17 $\beta$  peaks in anestrus bitches. These peaks occur in the absence of externally visible or clinically apparent estrogenic stimulation. They may be important in the stimulation of estrogen receptor production in target tissues for the ensuing proestrus and estrus (Olson PN et al 1982). Factors regulating the duration of anestrus within individual female dogs are not perfectly clear, but the termination of anestrus in bitches is associated with increased serum concentration or pulse frequency of LH (Concannon PW 1993). Approximately 30 days before the onset of proestrus, a slight but significant increase in serum estradiol-17 $\beta$  concentration occurs in the bitch (Jeffcoate IA 1993). In addition, levels of mRNA encoding estrogen receptors  $\alpha$  and  $\beta$  in the hypothalamus, pituitary and ovaries increase from late anestrus to proestrus in bitches (Hatoya S et al 2003). These observations suggest that estradiol-17 $\beta$  may be involved in priming the hypothalamic-pituitary-ovarian axis in order to initiate an increased rate of pulsatile LH release.

Concentrations of FSH are high immediately prior to the onset of proestrus, but they decline during proestrus, and are at minimal concentrations in late proestrus. There is a simultaneous increase in plasma estradiol-17 $\beta$  levels and, to a smaller extent, estrone levels. Estradiol-17 $\beta$  concentrations peak in late proestrus and then rapidly decrease. Progesterone concentrations remain very low during this period. Estradiol-17 $\beta$  concentrations that stimulate the LH surge from the pituitary continue to decrease at the onset of estrus. The preovulatory rise in LH lasts for 24-72 hours. Progesterone begins to increase before ovulation, in conjunction with the onset of the LH surge, due to preovulatory luteinization of the follicles. A serum progesterone concentration that is double the baseline level during proestrus and in the range of 1.5 to 2.5 ng/ml coincides with the day of the onset of the LH surge.

Ovulation is spontaneous, is not dependent upon breeding, and occurs ~44 hours (36-50 hours) after the onset of the LH surge. All oocytes are ovulated at approximately the same time. In the bitch, a primary oocyte is ovulated, which is in contrast to most mammals that ovulate secondary oocytes. Oocyte meiosis is completed in the uterine tube. Canine oocytes require 2-3 days to complete meiosis and extrude the first polar body. They may remain viable for 3 or more days after meiosis is complete. By 6 days past the onset of the LH surge, some oocytes show signs of degeneration. Canine spermatozoa have a relatively long life span within the genital tract of an estrous bitch, retaining their fertilizing capacity for 6 days or longer. This provides ample opportunities for multiple sires of a single litter (superfecundation).

Upon stimulation to the perineal area, the estrous bitch will deflect the tail (“flagging”) and may rotate her pelvis to facilitate intromission. She responds to lumbar pressure by standing firmly and arching her back slightly (“lordosis”). The onset of estrous behavior appears to result from the decrease in peripheral estradiol-17 $\beta$  concentrations and is facilitated by the coincident preovulatory rise in progesterone concentrations. In ovariectomized bitches, sexual attractivity (proestrous behavior) can be induced with exogenous administration of estradiol-17 $\beta$ . Ceasing estradiol-17 $\beta$  administration and beginning progesterone therapy can bring about typical estrous (“receptive”) behavior. This treatment will also induce an endogenous LH surge (Concannon PW 1983). In a significant percentage of cycles, the onset of the LH surge coincides with the beginning of behavioral estrus (Chakraborty PK et al 1980). However, onset of behavioral estrus can occur days before or after the onset of the LH surge. Some bitches may not exhibit estrous behavior at all, despite normal endocrine events and ovulation.

The onset of proestrus is characterized by vulvar swelling. The changes characteristic of proestrus are gradual and progressive, with vulvar turgidity reaching a maximum in late proestrus coincident with the estrogen peak. Grossly, the labia become turgid, enlarged and warm to the touch (the origin of the colloquial term “coming into heat”). Simultaneously or shortly thereafter, a serosanguineous vaginal discharge begins. Proestrus bleeding results from extravasation (the escaping) of red blood cells by diapedesis (migration of blood cells through intact capillary walls) within the endometrium. The bloody discharge may not be obvious in bitches that lick their genitalia fastidiously, but will be present intravaginally. During proestrus, the uterus and vagina undergo extensive changes. The uterus enlarges, even doubling in length during this period, and the blood vessels become engorged. The endometrium becomes edematous, and the uterine glands begin to hypertrophy. The cervix becomes clearly distinguishable upon deep abdominal palpation at about the level of the wings of the ileum, just in front of the pubic bone. During estrus, the vulva is usually reduced in size and less turgid than in proestrus. Serosanguineous discharge is usually decreased. Elongation of the uterine horns continues during proestrus; however, the limitation of the broad ligament causes the elongated uterine horns to become tortuous during estrus.

A valuable clinical method of monitoring the canine estrous cycle is the use of exfoliative vaginal cytology, as the changes in the cytology closely reflect endocrinological events in the bitch. Epithelial cells collected should be from the most interior portion of the vagina and may be collected with a saline-moistened cotton swab. Care should be taken to avoid obtaining vulvar epithelial cells and excessively rough handling, either of which can lead to misleading results due to superficial cornified epithelial cells on the vulva or excessive numbers of cornified epithelial cells deeper on the vaginal epithelium. The major cell types present in a vaginal smear are noncornified (parabasal and intermediate) and cornified (superficial and anuclear squamous) epithelial cells, as well as blood cells. Parabasal cells are round or oval and are the smallest vaginal epithelial cells. They have large heterochromatic (different shades of the same color) nuclei and resemble fried eggs. Intermediate cells are larger cells and the cytoplasm:nuclear ratio has increased in comparison to the size of parabasal cells. The superficial cells have small, dark staining (pyknotic) nuclei and an angular shape to the cytoplasm. Anuclear squamous cells stain deeply basophilic (dark blue), are the largest of the vaginal epithelial cells, and resemble corn flakes.

The earliest cytological indication of the onset of proestrus is the presence of red blood cells among the parabasal and small intermediate cells characteristic of anestrus. As proestrus advances, there is a progressive increase in the proportion of superficial cells. Blood elements remain abundant, and the background generally has a granular appearance. These changes are indicative of estrogen stimulation. During proestrus, the vaginal smear will have <90% cornified epithelial cells. The average duration of proestrus is 9 days, but may be as short as 2 days or as long as 22 days. As defined by vaginal smear, estrus is the time period when  $\geq 90\%$  of the epithelial cells are cornified. The transition from cytologic proestrus to estrus is not always clear. The average duration of estrus is 9 days, but may be as short as 4 days or as long as 21 days.

Shortly after the beginning of diestrus, the female should begin to refuse the male, although she may still be attractive to male dogs. On vaginal cytology, the first day of diestrus is marked by a sudden decrease in cornified epithelial cells to <50%, which coincidentally occurs approximately 8 days after the onset of the LH surge. Shortly after the onset of diestrus, no cornified epithelial cells are present in the vaginal smear. There may also be a sudden appearance of relatively large numbers of leukocytes, predominantly neutrophils. Neutrophils may be seen within the cytoplasm of parabasal or intermediate cells. Such cells are referred to as metestrous cells. The uterus develops remarkable tone. The tone and tortuosity of the uterus may result in false diagnosis of early pregnancy by deep abdominal palpation at this stage. Mammary development in the non-pregnant bitch is subtle, but is noticeable by careful palpation about 30 days after the onset of the LH surge. Mammary development reaches maximum proportions around 70 days after the onset of the LH surge and complete regression occurs by 120 days past the onset of the LH surge. Mammary development reflects the prolactin status of the bitch. Overt pseudopregnancy, with very high levels of prolactin, is an exaggerated version of a normal response.

Progesterone concentrations decrease rapidly 24 hours prior to parturition (<2 ng/ml) and remain low until the next proestrus. There is not a great deal of difference between the nonpregnant and pregnant diestrus period in the bitch. The pregnant diestrus period may have a shorter period of progesterone elevation when compared to the nonpregnant diestrus period, with a more abrupt decrease at the end of the progesterone period in pregnant bitches. Progesterone induces the endometrial differentiation and endometrial gland secretion, maintenance of endometrial integrity and attachment of the placenta, and suppression of the uterine contractility, particularly by preventing the uterotonic activity of estrogens that are no longer present in high amounts 5 days post-LH surge. For those reasons, any modifications in the estrogen:progesterone ratio or corpora lutea progesterone secretion will induce changes leading to abnormal glandular secretion, myometrial contractility critical to fertility, impaired implantation or abortion.

### **Estrous cycle physiology: queen**

Domestic queens are seasonally polyestrous. Queens are long-day breeders such that a prolonged anestrus (the occurrence of no estrous behavior) occurs during short-day length (September to January in the Northern Hemisphere) (Scott

PP & Lloyd-Jacobs MA 1959, Hurni H 1981). Reducing the daily lighting time to 8 hours can prolong seasonal anestrus (Michel C 1993). Fourteen hours of light administered during the non-breeding season will result in the return to cyclicity in  $15.6 \pm 0.5$  days (Michel C 1993). In queens, lactational anestrus also occurs, which persists until 2-3 weeks after weaning. In addition to anestrus, the feline estrous cycle is divided into four additional parts: proestrus, estrus, postestrus and diestrus. However, the different stages of the estrous cycle are not as clearly defined as they are in bitches. Proestrus is not commonly observed in queens and lasts for 0-2 days (Schille VM et al 1979, Stover DB & Sokolowski HT 1978). Estrus is defined by estrous (receptive) behavior secondary to increased circulating estradiol 17- $\beta$  concentrations (Gay VL et al 1982, Herron MA 1977, Micheal RP 1958, Micheal RP 1962, Mills JN et al 1979, Mowrer RT et al 1975, Schille VM et al 1979, Wildt DE et al 1981). Estrus lasts for an average of 6 days (range: 2-19 days). During estrus, elevated serum estradiol-17 $\beta$  concentrations result in an increase in vaginal epithelial cell layers, resulting in cornification (greater than 40-60% of the vaginal epithelial cells are cornified), which can be determined using exfoliative vaginal cytologic examinations. Cells are collected using a small cotton-tipped swab from the vestibule/vagina (~1/2 inch in front of the vulva).

Queens are induced ovulators, requiring external stimulation (such as natural breeding) to stimulate the release of pituitary LH and ovulation of mature follicles (Concannon PW 1993, Concannon PW et al 1980, Chakraborty PK et al 1979, Gay VL & Johnson LM 1982, Johnson LM & Gay VL 1981, Shille VM et al 1983, Wildt DE et al 1980, Wildt DE et al 1981). However, queens are often sexually receptive prior to the time when ovulation can occur. Breeding early in estrus (prior to the third or fourth day of estrus) can result in an attenuated LH secretion and ovulatory failure (Goodrowe KL et al 1989). It is important to mention that as many as 30% of unpaired, unmated and unstimulated female domestic cats ovulate without external provocation (Graham LH et al 1996, Gundermuth DF et al 1997, Lawler DF et al 1993). Postestrus (or sometimes referred to as interestrus) occurs following estrus if ovulation does not occur and lasts for an average of  $9.0 \pm 7.6$  days throughout the breeding season, unless ovulation occurs (Shille VM et al 1979). If ovulation occurs during estrus, diestrus ensues. If ovulation occurs but pregnancy is not established, then diestrus is shortened (pseudopregnancy). The average length of pseudopregnancy in queens is  $50.3 \pm 2.7$  days (Wildt DE et al 1981). Similar to the bitch, feline corpora lutea are autonomous for the first 30 days after ovulation and require luteotropic support of LH and prolactin for further maintenance. However, if pregnancy is established and maintained, then the duration of diestrus is the same length as gestation (approximately 64 days). Feline placentation and methods of pregnancy diagnosis are similar to that of dogs.

During the breeding season, queens exhibit estrous behavior every 2-3 weeks, unless ovulation occurs. However, the occurrence of estrus and ovulation are not repetitive, cyclic or predictable. Estrous behaviors in queens include head rubbing (proestrous sign), crouching with forequarters pressed to the ground and back hyperextended, lordosis with tail deviation, treading with hind legs, rolling and vocalizing. The breeding act in felids is very fast in comparison to dogs. Briefly, the tom mounts the queen and grasps her neck, and intromission and ejaculation occur all within one minute. Following mating, the queen demonstrates an "after reaction" for 1-7 minutes, which is characterized by disoriented, "frantic" rolling, stretching, and genital licking. She may refuse the male for a period of time following this or be receptive for another mating immediately. If left to their own devices, most queens will copulate 20-36 times over a 36-hour period. Approximately 50% of queens will secrete adequate LH to induce ovulation after a single breeding (Wildt DE et al 1980). However, the magnitude of LH release increases with an increase in the number of breedings. For optimal breeding management, allowing the pair to mate three times per day at 4-hour intervals through estrus should be recommended (Cain JL 1998). Ovulation typically occurs 29-40 hours after the first breeding.

### **Canine and feline pregnancy physiology**

The continuous availability of progesterone is required for the initiation and maintenance of pregnancy in both the bitch and queen. Elevated progesterone concentrations ( $>2$  ng/ml) are necessary for the entire length of gestation. The corpora lutea (CL) are the only progesterone source in bitches and the main progesterone source in queens. In both species, ovariectomy at any time during pregnancy will result in resorption or abortion. The feline placenta secretes an insignificant amount of progesterone, whereas there is no evidence in bitches supporting placental progesterone secretion.

There are no placental or embryonic luteotropic secretions that have been demonstrated in the bitch or the queen. Luteinizing hormone and prolactin, both of pituitary origin, are luteotropic in dogs and necessary for the maintenance of corpora lutea in the bitch. Prolactin appears to be the main pituitary hormone sustaining corpora lutea steroidogenesis. Its removal by dopamine agonists or other mechanisms induces an inhibition of prolactin secretion, leading to a functional luteal arrest and luteolysis with a blockage of progesterone secretion and abortion as a consequence. Prolactin concentrations rise at parturition and are maintained at high levels by suckling pups. The luteotropic role of pituitary LH is still controversial (Onclin K et al 2000).

Relaxin is only detectable in pregnant bitches and queens and is the only specific pregnancy-associated protein. Relaxin is

of placental origin, which explains why serum relaxin concentrations are not detectable at any time in nonpregnant diestrous bitches or queens. During pregnancy, relaxin is elevated from about 21-24 days after the onset of the LH surge until the end of pregnancy and reaches peak levels (~5 ng/ml) around 40-50 days of gestation. Relaxin concentrations decrease after parturition, but remain detectable for at least 30 days during lactation (Steinetz BG et al 1989).

In the bitch, the apparent length of gestation depends on what event counting begins from. Counting from the first breeding date of multiple breedings, a normal gestation can range from the timing between mating and ovulation. Apparent gestation length in the bitch has a range from 57 to 72 days if calculated from the day of the first mating to parturition and averages 63 days. This large variation is due to the long behavioral estrus in the bitch, and the fact that canine sperm of some males can retain fertilizing ability for 6-7 days post-mating or longer. Pregnancy length can also be evaluated based on the onset day of cytologic diestrus. The duration of pregnancy appears to be between 54-60 days with a mean of 57 days from the onset of diestrus. The actual gestation length is based from the onset of the LH surge and is 65±1 days (i.e., 63±1 days from the day of ovulation) (Johnston SD et al 1996).

In bitches, ovulation of primary oocytes occurs ~44 hours (36-50 hours) after the onset of the LH surge. Oocyte maturation (extrusion of the first polar body) is complete 2-3 days post-ovulation and sperm can penetrate the zona pellucida prior to extrusion of the first polar body. Queens ovulate secondary (mature) oocytes capable of fertilization immediately following ovulation. Fertilization (extrusion of the second polar body) occurs in the uterine tube. Embryos are present within the uterine tube 7 days past the onset of the LH surge and late morulas and early blastocysts enter the uterus 10 days past the onset of the LH surge. Prior to implantation, the embryos float freely within the uterine lumen, nourished by "uterine milk" (histotroph). Embryo migration between the uterine horns does occur and blastocysts space themselves out evenly between horns until 16 days after the onset of the LH surge. Embryonic implantation occurs 22 days after the onset of the LH surge. Uterine swellings corresponding to the implantation sites can be observed ultrasonographically at this time. The bitch and the queen have an endotheliochorial, zonary (circumferential), modified deciduate placenta. The poles of the chorioallantoic membranes are transparent (Anderson JW 1969). In the bitch, marginal hematomas are present at the edges of the zonary bands, are formed from maternal blood and contain uteroverdin, which has a black-green color. The canine embryo does obtain some metabolites from these areas, most notably iron.

In conclusion, this review has covered the basic elements of canine and feline reproductive anatomy, estrous cycle physiology, male reproductive physiology, and pregnancy, illustrating some of the differences unique to these specific species.

## References

- Anderson JW. 1969. Ultrastructure of the placenta and fetal membranes of the dog. I. The placental labyrinth. *Anat Rec* 165:15-35.
- Anderson AC, Simpson ME. 1973. The ovary and reproductive cycle of the dog (Beagle). Los Altos, Calif., Geron-X Inc.
- Aronson LR, Cooper ML. 1967. Penile spines of the domestic cat: their endocrine-behavior relations. *Anat Rec* 157:71-78.
- Blackmore DG, Baillie LR, Holt JE, Dierckx L, Aitken RJ, McLaughlin EA. 2004. Biosynthesis of the canine zona pellucida requires the intergrated participation of both oocytes and granulosa cells. *Biol Reprod* 71:661-668.
- Cain JL. 1998. Drugs used to treat reproductive disorders. *Vet Clin N Am Small Anim Pract* 28(2):395-410.
- Chakraborty PK, Wildt DE, Seager SWJ. 1982. Induction of estrus and ovulation in the cat and dog. *Vet Clin North Am Small Anim Pract* 12:85-91.
- Chakraborty PK, Panko WB, Fletcher WS. 1980. Serum hormone concentrations and their relationships to sexual behavior at the first and second estrous cycles of the Labrador bitch. *Biol Reprod* 22:227-232.
- Chakraborty PK, Wildt DE, Seager SWJ. 1979. Serum luteinizing hormone and ovulatory response to luteinizing hormone-releasing hormone in the estrous and anestrus domestic cat. *Lab Anim Sci* 29:338-344.
- Christie DW, Bell ET. 1971. Some observations on the seasonal incidence and frequency of oestrus in breeding bitches in Britain. *J Sm Anim Pract* 12:159-167.
- Concannon P, Hodgson B, Lein D. 1980. Reflex LH release in estrous cats following single and multiple copulations. *Biol Reprod* 23:111-117.

- Concannon PW. 1983. Reproductive physiology and endocrine patterns of the bitch. In Kirk RW (ed) *Current Veterinary Therapy VIII*. Philadelphia: WB Saunders, 886.
- Concannon PW. 1993. Biology of gonadotrophin secretion in adult and prepubertal female dogs. *J Reprod Fertil Suppl* 47:3-27.
- Durrant BS, Pratt NC, Russ KD, Bolamba D. 1998. Isolation and characterization of canine advanced preantral and early antral follicles. *Theriogenology* 49:917-932.
- Fuller JL. 1956. Photoperiodic control of estrus in the Basenji. *J Hered* 47:179-180.
- Gay VL, Johnson LM. 1982. Patterns of LH release in the ovariectomized, estrogen treated cat following sequential copulations or GnRH injections. *Biol Reprod Suppl* 26(1):51 (abstract).
- Goodrowe KL, Howard JG, Schmidt PM, Wildt DE. Reproductive biology of the domestic cat with special reference to endocrinology, sperm function and in-vitro fertilization. *J Reprod Fertil Suppl* 1989;39:73-90.
- Graham LH, Byers AP, Wildt DE, Armstrong DL, Brown JL. Natural versus chorionic gonadotropin-induced ovarian responses in the tiger assessed by fecal steroids. *Biol Reprod Suppl* 1996;54:114 (abstract).
- Gundermuth DF, Newton L, Daels P, Concannon P. 1997. Incidence of spontaneous ovulation in young, group-housed cats based on serum and faecal concentrations of progesterone. *J Reprod Fertil Suppl* 51:177-184.
- Hatoya S, Torii R, Kumagai D, Sugiura K, Kawate N, Tamada H, Sawada T, Inaba T. 2003. Expression of estrogen receptor  $\alpha$  and  $\beta$  genes in the mediobasal hypothalamus, pituitary and ovary during the canine estrous cycle. *Neuroscience Lett* 347:131-135
- Herron MA. 1977. Feline vaginal cytologic examination. *Fel Pract* 3:36-39.
- Hurni H. 1091. Day length and breeding in the domestic cat. *Lab Anim* 15:229-231.
- Jeffcoate IA. 1993. Endocrinology of anestrus bitches. *J Reprod Fertil Suppl* 47:69-76.
- Johnson LM, Gay VL. 1981. Luteinizing hormone in the cat. II. Mating-induced secretion. *Endocrinology* 109:247-252.
- Johnston SD, Root MV, Olson PSN. 1996. Canine pregnancy length from serum progesterone concentrations of 1-10 ng/ml. *Proc Symp Can Fel Reprod*, Sydney (abstract).
- Kooistra HS, Okkens AC, Bevers MM, Popp-Snijders C, Van Haaften B, Dieleman SJ, Schoemaker J. 1999. Concurrent pulsatile secretion of luteinizing hormone and follicle-stimulating hormone during different phases of the estrous cycle and anestrus in beagle bitches. *Biol Reprod* 60:65-71.
- Kutzler MA. 2005. Semen collection in the dog. *Theriogenology* 64(3):747-754.
- Lawler DF, Johnston SD, Hegstad RL, Keltner DG, Owens SF. 1993. Ovulation without cervical stimulation in domestic cats. *J Reprod Fertil Suppl* 47:57-61.
- Micheal RP. 1962. Estrogen-sensitive neurons and sexual behavior in female cats. *Science* 136:322-323.
- Micheal RP. 1958. Sexual behavior and the vaginal cycle in the cat. *Nature* 181:567-568.
- Michel C. 1993. Introduction of oestrus in cat by photoperiodic manipulations and social stimuli. *Lab Anim* 27:278-280.
- Mills JN, Valli VE, Lumsden JH. 1979. Cyclical changes of vaginal cytology in the cat. *Can Vet J* 20:95-101.
- Monniaux D, Huet C, Besnard N, Clément F, Mariana JC, Bosc M, Monget P. 1997. Follicular growth and ovarian dynamics in mammals. *J Reprod Fertil Suppl* 51:3-23.
- Mowrer RT, Conti PA, Rossow CF. 1975. Vaginal cytology: an approach to improvement of cat breeding. *Vet Med Small Anim Clin* 70:691-696.
- Okkens AC, Kooistra HS. 2006. Anoestrus in the dog: a fascinating story. *Reprod Dom Anim* 291-296.

- Olar TT, Amann RP, Pickett BW. 1983. Relationships among testicular size, daily production and output of spermatozoa, and extragonadal spermatozoal reserves of the dog. *Biol Reprod* 29(5):1114-1120.
- Olson PN, Mulnix JA, Nett TM. 1982. Concentrations of luteinizing hormone and follicle stimulating hormone in the serum of sexually intact and neutered dogs. *Am J Vet Res* 53:762-766.
- Onclin K, Lauwers F, Verstegen JP. 2001. FSH secretion patterns during pregnant and nonpregnant luteal periods and 24h secretion patterns in male and female dogs. *J Reprod Fertil Suppl* 57:15-21.
- Onclin K, Verstegen JP, Concannon PW. 2000. Time-related changes in canine luteal regulation: in vivo effects of LH on progesterone and prolactin during pregnancy. *J Reprod Fertil* 118:417-424.
- Rowlands IW. 1950. Some observations on the breeding of dogs. Proceedings from the Conference of the Society for the Study of Fertility (London) 2:40-55.
- Scott PP, Lloyd-Jacobs MA. 1959. Reduction in the anestrus period of laboratory cats by increased illumination. *Nature* 184:2022.
- Shille VM, Lundstrom KE, Stabenfeldt GL. 1979. Follicular function in the domestic cat as determined by estradiol-17 beta concentrations in plasma: relation to estrous behavior and cornification of exfoliated vaginal epithelium. *Biol Reprod* 21:953-963.
- Shille VM, Munro C, Walker Farmer S, Papkoff H, Stabenfeldt GH. 1983. Ovarian and endocrine responses in the cat after coitus. *J Reprod Fertil* 68:29-39.
- Shively MJ, Beaver BG. 1985. Dissection of the dog and cat. Ames, Iowa: Iowa State University Press.
- Steinetz BG, Goldsmith LT, Harvey HJ, Lust G. 1989. Serum relaxin and progesterone concentrations in pregnant, pseudopregnant, and ovariectomized, progestin-treated pregnant bitches: detection of relaxin as a marker of pregnancy. *Am J Vet Res* 50:68-71.
- Stover DG, Sokolowski JH. 1978. Estrous behavior of the domestic cat. *Fel Pract* 8:54-58.
- Tani H, Inaba T, Tamada H, Sawada T, Mori J, Torii R. 1996. Increasing gonadotropin-releasing hormone release by perfused hypothalamus from early to late anestrus in the beagle bitch. *Neurosci Lett* 207:1-4.
- Tesoriero JV. 1981. Early ultrastructural changes of developing oocytes in the dog. *J Morphol* 168:171-179.
- Van Haften B, Bevers MM, Van den Brom WE, Okkens AC, Van Sluijs FJ, Willemse AH, Dielemann SJ. 1994. Increasing sensitivity of the pituitary to GnRH from early to late anoestrus in the beagle bitch. *J Reprod Fertil* 101:221-225.
- Verstegen J, Onclin K, Silva L, Concannon P. 1997. Termination of obligate anestrus and induction of fertile ovarian cycles by administration of purified pig LH. *J Reprod Fertil* 111:35-40.
- Volkman DH, Kutzler MA, Wheeler R, Krekeler N. 2004. The use of deslorelin implants for the synchronization of estrus in diestrous bitches. Proceedings of the 5th International Symposium on Canine and Feline Reproduction (São Paulo, Brazil).
- Wildt DE, Chan SYW, Seager SWJ, Chakraborty PK. 1981. Ovarian activity, circulating hormones, and sexual behavior in the cat. I. Relationships during the coitus-induced luteal phase and the estrous period without mating. *Biol Reprod* 25:15-28.
- Wildt DE, Seager SWJ, Chakraborty PK. 1981. Behavioral, ovarian and endocrine relationships in the pubertal bitch. *J Anim Sci* 53:182-191.
- Wildt DE, Seager SWJ, Chakraborty PK. 1980. Effect of copulatory stimuli on incidence of ovulation and one serum luteinizing hormone in the cat. *Endocrinology* 107:1212-1217.