ESTROUS CYCLE REGULATION, ESTROUS INDUCTION AND PREGNANCY TERMINATION IN THE QUEEN

J.VERSTEGEN, DVM, MSC, PHD, DECAR, K. ONCLIN, DVM, PHD, DECAR
VETERINARY COLLEGE OF THE UNIVERSITY OF LIÈGE DEPARTMENT OF ANIMAL CLINICAL SCIENCES, SMALL ANIMAL THERIOGENOLOGY.
Bd Colonster 20 B44, B4000 Liège Belgium email: J.Verstegen@ulg.ac.be

Seasonality and estrous cycle

In the absence of pregnancy or pseudopregnancy, the cat will show repeated estrous cycles every 2 or 3 weeks in Spring, Summer and Autumn. In breeding colonies with controlled light schedules and in household cats with evening lighting, cats can become non-seasonal breeders and show estrus during winter. It is believed that photoperiods influence the reproductive processes via the pineal gland and its main hormone, melatonin, in a way similar to that observed in the mare.

Behavioral cycle:

In terms of behavior, the cycle of the queen can be divided into the heat and the non-heat periods.
The heat period can be divided in proestrus and estrus. The behavioral proestrus (1-4 days) and estrus periods (2 – 19 days) last together in average between 3 and 10 days. Whereas estrus is characterized by male acceptance, the first days (from 1 to 4) of behavioral heat, associated with calling but not the acceptance of the male, can be termed proestrus. Proestrus *per se* is clinically difficult to detect because some cats express estrous behavior and accept the mating without this preliminary transition period. Follicular growth begins during the proestrus, but plasma estradiol concentration is still low and insufficient to allow full behavioral expression of heat.
Estrus begins with the queen allowing the mating process and ends with the first refusal. It is characterized by maximal synthesis and effects of the follicular estrogens.
Queens, as well as males, can easily block and hide any reproductive behavior following particular environmental or social circumstances. It is not rare to have queens showing no signs of estrus when in home and, as soon as they are away from the owner's eyes, presenting dramatic rollings and callings. Males may ignore or attack females if they are in an hostile or unknown environment. On the other hand, some really affectionated queens can present heat signs not really different from their usual behavior, this making the heat detection difficult in the absence of male. Although controversial and certainly difficult to assess in conditions other than controlled breeding colonies, it appears that estrus is shortened by mating.

Interestrus and anestrus.

In the absence of matings and / or spontaneous ovulation, heat periods are observed at intervals of average 10-14 days (interval 0-20 days) during the whole reproductive season. This interval period without evidence of estrous is called interestrus. It corresponds to an apparent quiescence of the ovaries, which however, are already preparing for the next follicular growth of the next estrus. During the interestrus, the estrogen levels usually decline to basal values. In some cats however, sequential follicular growth waves may overlap, estrogen concentrations may not

decline and the queen may appears in constant estrus. This phenomenon is often falsely called « nymphomania » or prolonged estrus (cf. infra).

True ovarian quiescence is only found during anestrus, which, in contrast to interestrus, is a long period without sexual activity. Anestrus occurs under very short natural daylight days (winter) or when the cats are submitted to short artificial lightening periods of 4-6 hours/day.
Anestrus could be absent in animals submitted to constant long days, particularly in animals living indoors or animals living in breeding colonies with constant lightening.

Mating and induced ovulation

The cat was considered to be a reflex mediated induced ovulator, i.e. a coital stimulus induced a neural firing reflex stimulating the medio-basal hypothalamus to synthesize and liberate high levels of GnRH, which allow in turn and through the release of pituitary LH, the ovulation to take place. However, there are nowadays many evidences showing that cats may ovulate spontaneously, without any mating. In absence of spontaneous ovulation, which we don't know yet the responsible mechanisms interestingly, it has been demonstrated, even if it is still controversial to some aspects, that the ovulation rate seems to be directly related to the amplitude of the LH surge, which may be itself correlated to the number, interval and quality of the matings. The interval between the vaginal stimulations and ovulation is indirectly proportional to the number of matings and to the endocrine status at the time of copulation. Cats that have been in estrus for several days ovulate sooner after matings than cats that have just entered estrus. Although queens may accept the male during the second or third day of follicular growth (first and second day of estrus), some cats may only show a sufficient release of LH in response to copulation by the fourth of fifth day. Cats seem to require estrogen impregnation for several days before copulation can induce an LH surge sufficient to cause ovulation. This ovulation can therefore occur as early as 24 hours or as late as 52 hours after the induced LH surge. After ovulation, signs of heat disappear within 24 to 48 hours. Increase in plasma progesterone concentration typically follows ovulation. If ovulation does not occur, the follicles become atretic and estrogen concentration declines. Luteinization does not occur and newly recruited follicles may start to grow after a few days of interestrus. In the queen, unlike the bitch, events after ovulation will depend on whether or not the mating has been fertile. Indeed, 2 situations may be observed:

Ovulation without fertilization

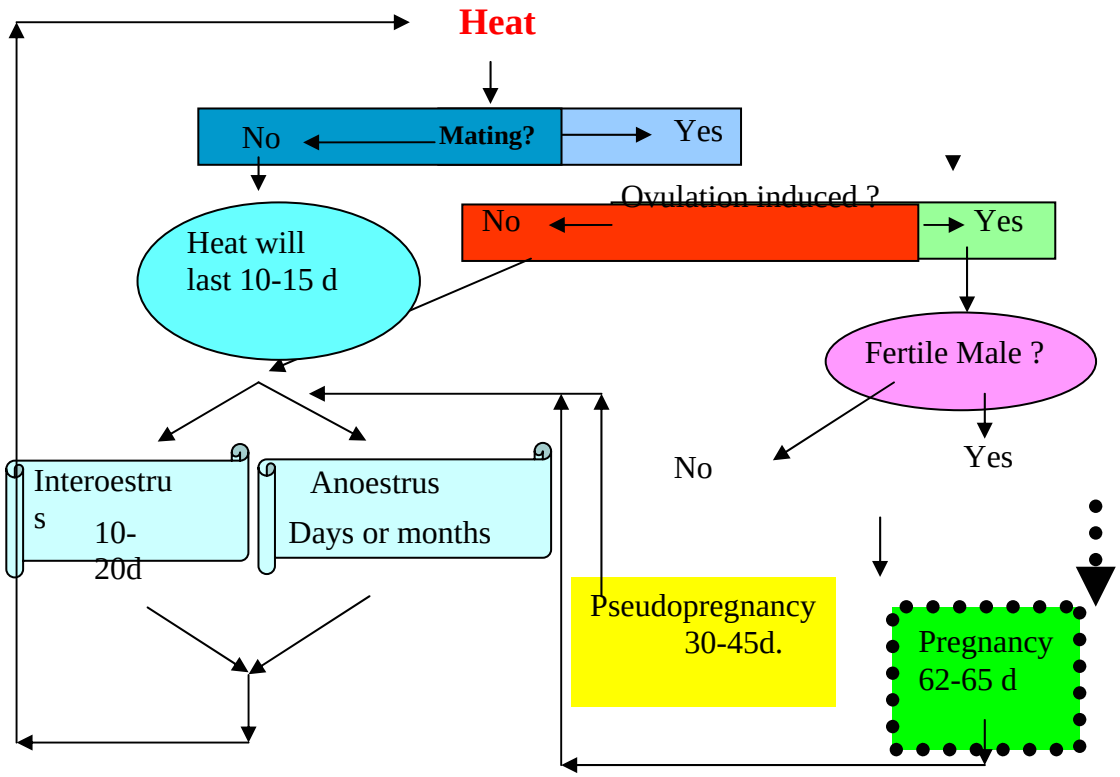
When oocytes are not fertilized after ovulation, corpora lutea develop and produce progesterone for a period of about 25 to 45 days. This luteal phase is shorter than the one associated with pregnancy and is therefore often called pseudopregnancy. In the cat, pseudopregnancy is not, as in dogs, associated with behavioral changes or lactation. No significant clinical signs are observed, but a prolonged period without estrous signs. At the end of this short luteal phase of pseudopregnancy, a brief period of interestrus will precede the next return to estrus, if it is still the breeding season, otherwise, anestrus will occur. In house living queens, induction of pseudopregnancy could be a safe way to stop the reproductive behavior for a while. This can be easily obtained by the injection of GnRH or hCG. Mechanical vaginal stimulations have sometimes been proposed, but on the one hand, it is not always easy to realize and on the other hand, it is, in contrary to medical induction, far to be consistent in terms of ovulation induction.

Ovulation and fertilization

If the queen is mated with a fertile male or successfully inseminated, mature ovulated secondary oocytes are fertilized in the oviducts where the embryos develop for 4-5 days. Embryos then migrate into the uterine horns where implantation occurs around days 12 to 16 post-mating.

The biology of pregnancy has not been investigated in cats as deeply as it has been in other mammal species. Pregnancy can be accurately timed when reproduction has been allowed for one or 2 days only and ovulation induced by correctly managed mating (cf. supra). In controlled conditions, gestation length is 63-68 days, but may range from 62 to 72 days when mating is allowed for several days and the exact day of ovulation is not determined. Ovulation follows efficacious matings by a mean of 30 hours and fertilization of the produced secondary oocytes occurs in the oviducts. Blastocysts enter the uterus on days 5–6 and migrate from one horn to the other one for a few days before attachment (around day 14) and implantation (days 14-18). The placenta is of endothelio-chorial type and circumferential–zonary in morphology. Average litter size varies between breeds (from 2 to 5 kittens) and increases with parity (until 5 to 8) and then declines in old queens. Spontaneous abortion or resorption is nowadays well documented and may reach 5 to 30 %, it can include from 1 kitten to the whole litter. The litter size is always smaller in primiparous queens.

Parturition is always associated with a decline in plasma progesterone, but this decline can be observed as early as 5 to 10 days before whelping or as late as 5 to 10 days after parturition. Queens can remain pregnant sometimes for days, without any significant plasma progesterone



concentration, possibly because parturition depends on the local withdrawal of the small amount of progesterone produced by the placenta. The peri-partum luteolysis involves the production of prostaglandins, which are well known to induce abortion when administered during pregnancy in

queens. The pre-partum temperature drop observed in dogs, and correlated to the decline in progesterone, is not consistent in feline species.

At the end of the lactation period, after littering, if it is still the breeding season, a brief period of interestrus will follow until the next estrus; otherwise anestrus will occur. In lactating queens, this estrus period usually occurs about 10 to 15 days after weaning. However, in some cases, estrus may be observed in nursing females 10 to 15 days after littering. In this case, if she is not mated or if ovulation did not occur, the queen will return to estrus normally every 10 to 20 days. The first mating after kittening is often not fertile, due to incomplete uterine involution, but mating during the next estrus may be fertile and it is not rare to see a nursing queen, which is pregnant again before weaning. Spontaneous or induced follicular growth with estrous behavior and mating have been described during pregnancy, suggesting that the ovaries are responsive to gonadotrophins during gestation in this species, but confirming the centrally mediated inhibiting effects of progesterone on follicular growth. Due to the multiple matings observed during the heat period, superfecundation, characterized by fertilization of ova with semen from different males, and then whelping of kittens of different fathers, is often observed in natural non-controlled conditions.

Estrous induction

Induction of follicular growth and ovulation can easily be achieved in queens, but clear indication for estrous induction has to be demonstrated before the beginning of the treatment. Indeed, it is important to know that cats are highly responsive to estrus induction treatment and overstimulation, leading to superovulation or production of numerous anovulatory follicles with a cystic appearance, is far from being exceptional. Such a treatment, inappropriately performed, may easily lead to follicular cysts, early embryo resorption due to an abnormally high plasma estradiol concentration or definite infertility. This is particularly true in the highly responsive prepubertal or young adult animal.

Indications for estrous induction are delayed puberty, prolonged anestrus or synchronization of queens.

Estrous synchronization may be required in some occasions, particularly for embryo transfer or in association with all in vitro procreation techniques. This is particularly true and often realized in wild species of felids, but this technique will probably become more and more efficacious and will be available or useful in small animal reproduction cases of infertility or in view of the conservation of high value genetic materials.

Many treatments (table 1) have been proposed to induce estrus in cats and include the use of eCG (PMSG), FSH, hCG and GnRH. Many protocols are described with very high differences in the doses and schedules used, for example, doses of PMSG ranging from 100 to 1000 u.i. from once to once every day for 5 to 7 days have been proposed with various results in term of pregnancy. The authors' preferred treatment is: PMSG (100 – 150 i.u.) in a single bolus administered to anestrous cats, followed 4 to 5 days later by one injection of HCG (50-100 i.u.). This treatment leads to ovulation and pregnancy results similar to those observed with natural matings (over 80 %). It is important to note that repeated injections of the exogenous gonadotrophins (eCG or hCG) may lead to the production of antibodies against FSH and LH, which may cross react with the endogenous and exogenous hormones and lead to a subsequent decreased response to stimulation or subsequent infertility (neutralisation of the endogenous

gonadotrophins). For this reason, it is highly advisable not to repeat this type of treatment and to use it appropriately, that is only when really needed. GnRH has been recommended at a dosage of 1 µg/kg SC until signs of estrous behavior are noted or for a maximum of 10 days.

During estrus, ovulation can be induced mechanically by vaginal stimulations. Exogenous hormones can also be used: GnRH (5 to 25 µg/cat) or hCG (50 to 250 i.u/cat) may be administered IM or IV to induce ovulation and therefore terminate estrus. Ovulation generally occurs 24 to 36 hours after the injection and is followed by a 30 to 45 days pseudopregnancy if the animal is not mated or inseminated.

Abortion induction

Veterinarians are less often asked to induce abortion in cats than in dogs. However, when it is necessary to induce an abortion, this can be achieved in the same way as it is in dogs. Estrogens can be used before implantation (before day 10 after mating). Estrogen treatment induces congestion, edema and alteration of the uterine milk, therefore preventing normal embryo migration and embryo implantation. In cats, fewer side effects are observed after estrogen treatment than in dogs. Bone marrow aplasia and uterine disease are rarely observed. Nevertheless, we consider that using estrogen is no longer advisable as new treatments with fewer side effects exist. Prostaglandins can be used. Doses of up to 500 µg/kg of natural analogues 2 to 3 times a day for 5 days may be given after pregnancy has been diagnosed. Side effects are less severe than in dogs. Generally, these side effects increase with the dose administered, but decrease with the repetition of treatments. Among the side effects, there are emesis and muscle jerking. No data exist concerning the use of synthetic analogues (like cloprostenol) alone in this species.

Prolactin inhibitors have been demonstrated to be potent abortion induction agents in cats as in dogs. Recently, we have demonstrated that after day 25 of pregnancy, abortion can be induced in cats using 3 injections of cloprostenol (5µg/kg) at 48h intervals associated with every day oral treatment with the prolactin inhibitor, cabergoline (5 µg/kg once a day for 8 days). Abortion is observed consecutively to a drop of progesterone below the threshold required to maintain pregnancy in this species. The main interest of this treatment is that it is not associated with any side effects. If abortion is induced before day 40, it is associated with fetuses resorption and no expulsion is detected. If realized later, expulsion of perfectly normal kittens may occur. Table 2 summaries the different abortion induction treatments available in cats.

Table 1: Described treatments to induce estrus in cats

Protocols	Dosages	Results	Comments
PMSG alone	100-1000 i.u. 5 to 7 days	Poor, large number of unovulated follicles	Wildt et al. 1978, Cline et al. 1980
PMSG + HCG	100-150 i.u. once a day 1 + 50-100 i.u. once a day 5 -7	Good, similar to natural estrus	Cline et al. 1980 Donoghue et al. 1997
FSH + hCG	2mg/d 4-5 days + 50-250 i.u.	Good	Dresser et al. 1987, Goodrowe et al. 1988

Table 2: Described treatments to induce abortion in cats

Protocols	Dosages	Results	Comments
Natural prostaglandins alone: dinoprost (Lutalyse®, Dinolytic®)	250-500 µg/kg 1 to 3 times a day for 5-7 days from pregnancy diagnosis	Good. Ideally this treatment has to be used before ossification, and is then associated with fetal resorption. Not to be used after day 50	Some side effects can be observed: prostration, emesis, salivation, muscle jerking
Synthetic prostaglandin alone	2.5-5 µg/kg 1 to 3 times a day for 5-7 days from pregnancy diagnosis	Good. Ideally this treatment has to be used before ossification, and is then associated with fetal resorption. Not to be used after day 50	Less side effects are be observed, than with natural PgF2alpha prostration, emesis of short duration
Estrogen: estradiol benzoate or cypionate	2 to 3 injections of 0.01 to 0.1 mg/kg every 2 days from mating. Not later than day 10.	Good but no longer advisable.	Estradiol benzoate is less toxic than cypionate. Side effects are less frequent in cats than in dogs Have to be given before a pregnancy diagnosis is made.
Prolactin inhibitors: bromocryptine - cabergoline	Cabergoline 5µg/kg/d for 7 days from pregnancy diagnosis. Bromocriptine 25 to 50 µg/kg 3 times /day for 1 week.	Contradictory results exist concerning the efficacy of this treatment before day 40. Seem to be efficacious after that period, but unfortunately abortion is then associated with fetal expulsion. Not to be given after day 55 because will then induce premature parturition instead of abortion.	Cabergoline seems to be more efficacious than the 2 other agents due to longer duration of action and a more important inhibitory effect on prolactin secretion. Side effects are weak or absent with cabergoline when compared to bromocryptine.
Association Cabergoline (Prolactin inhibitor) and cloprostenol (synthetic prostaglandin)	5 µg/kg/d for 7-10 days orally + 2.5 µg/kg SC. at days 1, 3 and 5. This treatment can be administered as soon as a pregnancy diagnosis can be performed : approximately day 25. .	Good When the treatment is applied before ossification (day 40-45) abortion occurs by resorption.	No side effects are observed. This is the authors' recommended treatment: efficacious, early in pregnancy, no side effects.