Review Report

# Estrous cycle manipulation in cats

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## Abstract

For decades, interest in reproductive physiology of the domestic cat has largely been driven by its importance as a model for wild felids and human biomedical research. As such, several assisted reproductive technologies have been established in cats. Despite the growing need for feline theriogenology, the application of these tools in clinical practice is extremely limited. We discuss: 1. reproductive physiology of the queen and her unique challenges; 2. estrus induction (photoperiod, social interaction, and pharmacologic [gonadotropins, GnRH agonists]); 3. natural cycle monitoring (blood hormones, fecal hormone metabolites, behavior, vaginal cytology, transabdominal ultrasonography) and ovulation induction (manual stimulation, pharmacologic [gonadotropins, GnRH agonists]); 4. estrus suppression (photoperiod, melatonin, GnRH agonists, progestins); and 5. permanent nonsurgical contraception (immunocontraception, gene therapy). This review will summarize published reports on estrous cycle manipulation in felids, both wild and domestic; notable differences between cats and dogs are highlighted and comments based on the authors' personal experiences and preferences for application are included.

**Keywords:** Felids, estrous cycle, estrus induction, ovulation induction, natural cycle, estrus suppression, contraception

## Introduction

In veterinary medical research, the number of publications featuring dogs outnumbers cats ~ 3:1.[1] Cats also remain understudied in theriogenology.[2] Perhaps, in part, because the domestic cat is viewed as an extremely fecund species. When one considers the estimated 80 million unowned, outdoor cats that live in the USA,[3] it may appear incongruous to focus on assisted reproduction in cats. However, there is a growing body of literature that demonstrates that infertility is a major issue in cats.

Early embryonic collections following natural matings in domestic short hairs produced good-quality embryos only from ~ 73% (38/52) of queens;[4] the remaining cats either failed to ovulate (~ 8%) or had degenerating embryos (~ 8%), unfertilized oocytes (~ 10%), or no oocytes/embryos (~ 2%).[4] Most data in purebred cats are derived from case studies[5,6] and self-reported questionnaires,[7-10] with 15-42% of queens failing to conceive after natural mating.

Despite an empiric lack of interest in feline theriogenology, the basic reproductive biology of the cat has been well-studied. This was largely driven by its importance as a model for wild cats.[11] It is speculated that modern felids originated from a common ancestor ~ 11 million years ago and as such, their reproductive physiology has remained well-conserved across cat species.[12] The domestic cat has also proven to be an important model organism for biomedical research. The genomic organization of the cat is highly similar to humans[13] and cats possess ~ 250 naturally-occurring genetic disorders with analogous pathologies to human diseases.[14] Assisted reproductive technologies (ARTs), such as in vitro fertilization (IVF), embryo transfer (ET), and artificial insemination (AI), have therefore been developed in the domestic cat to aid in the conservation of wild felids,[15] to propagate naturally occurring genetic disease models,[16] and to produce genetically modified animals.[17]

Although many ARTs are well-established in the cat, these tools are rarely utilized in general practice (especially compared to dogs) and, for most veterinarians, their clinical experience with cat reproduction begins and ends with neutering. With both the number and professionalism of cat breeders rising, there is an increased (and as of yet unmet) demand for the application of domestic cat ART in veterinary medicine. This review considers: 1. the reproductive physiology of the queen and her unique challenges; 2. estrus induction; 3. natural cycle ovulation induction; 4. estrus suppression; and 5. permanent nonsurgical sterilization in the queen.

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## Reproductive physiology of the queen

The queen is seasonally polyestrous; the breeding season begins when daylight length increases and anestrus occurs after a reduction in daylight hours.[18,19] Cats in equatorial zones may breed continuously, whereas cats at the polar circles only cycle for ~ 6 months.[20] In the northern hemisphere, the breeding season usually begins around February and ends by September.[21] Temperature also has a role, as queens in warmer climates may continue to cycle as late as November before entering anestrus.[22] Furthermore, periods of extreme heat and/or humidity can increase the interestrus interval.[23] The breed also influences photoperiod sensitivity as long-haired breeds tend to have a shorter, more-defined breeding season than short-haired breeds.[24] For cats housed exclusively indoors without exposure to natural light, alterations in the daily photoperiod can be utilized to manipulate the queen's estrous cycle.[25-27] Different protocols are reviewed in **Photoperiod**.

The queen is categorized as an induced ovulator,[28] with the act of copulation serving as the canonical stimulus for release of gonadotropin releasing hormone (GnRH) and the subsequent surge of luteinizing hormone (LH) from the anterior pituitary gland. Amplitude of the LH surge is directly correlated with the number of copulations; 8-12 copulations over a 4-hour period produced peak concentrations.[29] LH concentrations were significantly lower with only 4 copulations over the same interval, and were even lower with a single breeding. In the aforementioned study, 50% of estrual queens ovulated after 1 copulation, whereas every queen ovulated after > 4 copulations. Ovulation was determined by the amount of LH released, not by differences in the follicles' responses to similar LH concentrations.[30] Furthermore, ovulation appears to be an all-or-none event, as the number of mature follicles present during the preovulatory period correspond to the number of corpora lutea after mating in the queen. Because the queen requires an external stimulus to ovulate, one must consider ovulation induction when designing estrus manipulation protocols without natural mating (e.g. AI and ET).

It should be easy to manipulate a queen's estrous cycle because, in the absence of mating, the queen would ostensibly lack corpora lutea and not require luteal control protocols. However, spontaneous ovulation is a well-documented phenomenon in cats.[31-35] Reports vary greatly in the percentage of females that spontaneously ovulate (35-87%). The rate of each queen also varies widely among reports; some females rarely spontaneously ovulate, whereas others may consistently ovulate without copulatory stimuli. Interestingly, wild felids also demonstrate a spectrum of ovulation patterns, with some being almost exclusively induced-ovulators and others exhibiting a high rate of spontaneous ovulation.[36,37] To date, felids are the only taxon reported to exhibit both spontaneous ovulation in some individuals and exclusively induced ovulation in others.[38]

In equids and ruminants, prostaglandins are often employed during a luteal phase to regress the mature corpus luteum (CL) and return the female to estrus within a predictable interval.[39] Unfortunately, the feline CL is refractory to prostaglandin $F_{2\alpha}$ treatment.[40,41] GnRH antagonists that induce luteolysis in dogs[42] are also ineffective in cats.[43] Dopamine agonist cabergoline has been successfully used with[41,44] or without[41,45,46] a prostaglandin to induce abortion in cats. Cabergoline regresses the CL via its inhibitory effect on prolactin secretion. The use of cabergoline in estrus induction protocols has not been reported, likely due to its long treatment period (5-15 days) and undefined interval for CL regression. Although aglepristone has also been used to successfully induce abortion in queens, it is important to note that the mechanism of action differs; aglepristone is a progesterone receptor antagonist and cannot regress the feline CL.[47]

## Estrus induction

There are a variety of indications for estrus induction in queens. As a long-day breeder, cats have a period of anestrus during short days and estrus manipulation is required if out of season breeding is desired. Cats can experience primary anestrus (delayed puberty) or secondary anestrus (abnormally long interestrus intervals in an adult queen that previously displayed cyclicity).[48] Management practices (e.g. photoperiod, social interaction) should be reviewed before initiating pharmacological intervention and prepubertal queens should not be induced with exogenous hormones because they are more likely to develop a high number of cystic follicles.[48] Spontaneous ovulation should be ruled out before diagnosing secondary anestrus. Finally, estrus induction is often utilized for cycle synchronization in ARTs, such as AI, collection of in vivo-matured oocytes for IVF, and recipient-preparation for ET.

## Photoperiod

As discussed in the **Reproductive physiology of the queen**, the cat is a long-day seasonal breeder and is highly responsive to changes in photoperiod. Increasing exposure to light thus represents one of the easiest and most efficient techniques to induce cyclicity.

A light:dark cycle of 8:16 hours was sufficient to cease cyclicity immediately;[26] plasma estradiol concentrations were dramatically reduced, with concentrations significantly lower than interestrus concentrations in females exposed to 14 hours of light per day. When females were again exposed to 14 hours of light, cyclicity resumed 12-26 days later (mean 16.3 days). A much longer anestrus was observed in a different study[27] where queens took an average of 44.6 days for estrus after returning to 14 hours of light exposure. Resumption of estrus occurred significantly sooner (mean 15.6 days) by providing 1 hour of light during the dark period.

A light:dark cycle of 12:12 hours (similar to equatorial conditions) created the most productive year-round cyclicity (as defined by percent of successful breedings per week).[20] The same study reported that if a shortened and more-defined breeding season is desired, 2 months at 9:15 hours light:dark followed by 14:10 hours light:dark significantly increased the number of litters born 6 months after the change to 14 hours of light. Similarly, if a queen has been maintained under constant artificial lighting conditions for years, reducing the light exposure to 8 hours per day for 2-3 months will allow her to experience a period of anestrus and may improve fertility.[49]

Cats exposed to 24 hours of light will still demonstrate cyclicity, but the rate of estrus is decreased (only a mean 0.8 periods of estrus were observed the first month, and ~ 1 estrus/month was observed the following 2 months).[26] Plasma estradiol concentrations demonstrated prolonged periods of proestrus (up to 7 days), suggesting initial follicular development was temporarily suppressed. Thus, it is not the authors' recommendation to maintain queens under constant light exposure.


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Finally, cats maintained in a home setting are often exposed to both natural and artificial light. Because the artificial lighting in a home setting is not constant, it may not result in predictable ovarian cycles. Anecdotally, most intact home-housed cats do not cycle during the short daylight period.

### Social interactions

Interactions with conspecifics have the potential to influence the estrous cycle of the queen. In the aforementioned photoperiod study,[27] queens that returned to long-day light conditions (14 hours) after a period of short-day light exposure (8 hours) took an average 44.6 days to exhibit estrus.[27] Introduction of other queens in estrus at the change of the photoperiod allowed the queens to resume cyclicity significantly sooner (22.3 days), likely through the influence of estradiol and pheromones.[50,51] Similarly, it is also advised to house prepubertal females with cycling queens to help stimulate the onset of puberty.[51] Conversely, inter-female aggression has been cited as a cause for females to not show overt signs of estrus, particularly for timid cats that are lower in the social hierarchy.[48]

Exposure to an intact male has also been documented to affect cyclicity in cats. Similar to exposure to an estrual female at the onset of long-day light conditions, introduction of a male shortened the interval to cyclicity resumption, with the same number of days (22.3) until the first estrus was observed.[27] The presence of a male can help accentuate signs of estrus[51] and even the noncopulatory presence of a tom can increase the rate of spontaneous ovulation.[35]

### Pharmacologic estrus induction

The goal of a pharmacologic estrus induction is to recapitulate the natural cascade of the hypothalamic-pituitary-ovarian axis, so drugs that mimic the actions of GnRH, follicle stimulating hormone (FSH), and/or LH are utilized. Initial studies to induce folliculogenesis in the cat focused on serial injections of porcine-derived FSH. Five daily injections were successful in initiating follicular development; however, ovarian hyperstimulation was observed[52-54] and the authors reported logistical challenges in giving multiple injections, particularly when this protocol was used in wild felids.[55-58]

Subsequent studies have focused on equine chorionic gonadotropin (eCG) which is longer acting and only requires a single injection.[59,60] In cats, eCG is primary used for its folliculogenic activity; however, high dosages or serial treatment can induce ovulation.[53] More commonly, human chorionic gonadotropin (hCG) is used as the luteotrophic agent to induce ovulation in feline ovarian stimulation protocols.[61,62] However, hCG also demonstrated folliculogenic activity in the cat, including the capacity to stimulate growth and maturation of smaller (< 2 mm) antral follicles.[60] Thus, although eCG is predominantly folliculogenic and hCG luteotrophic, each exhibits duality in the cat and can mimic the other's principle action.

Historically, the most common protocol to induce estrus and ovulation prior to a timed ART procedure was to give 100 IU intramuscular eCG, followed by 75 IU intramuscular hCG 80-85 hours later.[63] The timing of the procedure depends on which ART is employed. Queens will ovulate ~ 30 hours after hCG treatment,[61] so, oocytes are collected for IVF 25-27 hours (i.e. preovulatory) after hCG treatment[63] and AIs are conducted 31-33 hours (i.e. postovulatory) after hCG treatment.[15,59]

One drawback with eCG/hCG is that both are large glycoproteins that persist in circulation for 4-5 days in cats and can induce formation of antigonadotropin antibodies, causing the female to become refractory to future treatment.[60,64,65] Additionally, due to its folliculogenic activity in cats, hCG can promote undesirable secondary follicular growth and ovulations.[66] These ancillary follicles and secondary CLs disrupt the postovulatory endocrine environment and potentially have a negative impact on embryo survival following AI or ET.[67] Alternatively, pLH has a very short half-life and remains in circulation for just hours after injection.[68] A protocol using 100 IU intramuscular eCG and 1,000 IU intramuscular pLH with an 85 hour interval between treatments was highly effective for inducing ovulation in ET recipients without significant formation of secondary ovarian structures,[69] and producing high pregnancy percentages with both ET and AI procedures in cats.[15] To the authors' knowledge, there are only 2 sources of pLH in the USA (Novatein Biosciences and Prospec Bio) and the current price per cat dose ($331-$475) make it cost-prohibitive in most applications.[70]

Anesthesia prior to ovulation may have a detrimental effect on ovulation. Queens treated with eCG and hCG that were anesthetized immediately before ovulation demonstrated a low rate of ovulation and a reduced pregnancy rate (14%) following intrauterine AI, compared to eCG/hCG-treated females that were anesthetized immediately after ovulation (50% pregnancy rate).[61] Alternatively, ovulation-induction with hCG on days 2-4 of a natural cycle demonstrated a higher level of success in the queens anesthetized for AI prior to ovulation (56%) versus queens anesthetized after ovulation (21%).[71] Thus, the compromising effect of anesthesia on ovulation may be limited to queens exogenously treated for estrus induction.

For gonadotropin treatment to be maximally effective in cats, a quiescent ovary at the time of treatment is needed.[11] The high rate of spontaneous ovulation in cats complicates one's ability to artificially control the ovaries because high circulating progesterone concentrations can reduce or even prevent the effectiveness of exogenous hormone treatment.[11] Regressing the CL, although a typical means of regulating the estrous cycle in other mammals, is ineffective in felids.[40]

To address this issue, 2 strategies may be employed. The first is to confirm that the queen is nonluteal before initiating treatment. This can be accomplished with vaginal cytology (refer to **Real-time estrous cycle monitoring**) and a serum progesterone assay (nonluteal is defined as < 2 ng/ml progesterone). The second strategy is to suppress estrus before initiating the hormone treatment protocol. A nonpregnant luteal phase in the cat lasts ~ 40 days,[72] so the goal is to achieve ovarian inhibition over the same time interval. This allows natural regression of any current CLs and prevention of any new spontaneous ovulations.

The synthetic progestin levonorgestrel has been used to successfully down-regulate the feline ovary.[43] Six silastic rod implants (36 mg levonorgestrel/rod) were placed for 39 days before gonadotropin stimulation and then removed 2 days prior to placement.[33] Queens with elevated estradiol at the time of treatment completed a normal surge before returning to baseline. However, levonorgestrel successfully inhibited the initiation of new estradiol surges throughout the treatment period and the ovaries were highly responsive to the gonadotropin treatment after a 2 day withdrawal period. Although

highly effective, the biggest drawback to this technique is its labor intensiveness, requiring two anesthesia events before the estrus induction can begin.

For this reason, the oral progestin altrenogest is more commonly employed. Similar to levonorgestrel treatment, females with increased estradiol at treatment initiation completed a normal surge before returning to baseline.[73] Unlike levonorgestrel, altrenogest-treated queens had new estradiol surges during treatment, albeit at a significantly lower rate, and none ovulated during treatment. Given that the domestic cat can have ovarian follicular activity during a luteal phase, it is not surprising to observe some activity during exogenous progestin treatment.[72] The authors investigated 3 doses of altrenogest, and concluded that the middle dose (0.088 mg/kg) was optimal for the cat as it produced normal baseline estrogen and progesterone concentrations and a more uniform return to follicular activity (10-16 days) versus either the low dose treatment (0.044 mg/kg; 2-12 days) or the high dose treatment (0.352 mg/kg; 9-35 days). Exogenous gonadotropin treatment 3-5 days after altrenogest withdrawal produced consistent follicular development and synchronous ovulation,[74] normal luteal function,[75] improvements in embryo development,[75] and high (83-86%) AI pregnancy rates.[75,76]

The latter strategy (ovarian suppression) is more commonly adopted because the former strategy (confirmation of a nonluteal phase) may include queens in the follicular stage of estrus that respond suboptimally to estrus induction protocols compared to queens in interestrus.[43] An inactive ovary contains primordial follicles and follicles in a gonadotropin-independent continuous growth phase resulting in a more uniform population of early antral follicles that are highly receptive to gonadotropin stimulation and creates a more uniform ovarian response.[43] Furthermore, eCG is often used due to its FSH-like activity in cats to stimulate follicular growth. However, eCG also has LH-like activity and can trigger ovulation if given to a cat that is already in estrus.[60] Typical timed AI protocols administer eCG ~ 5 days before AI.[15] In this example, the queen would ovulate several days early, and the oocytes would likely be too senescent at the time of AI to fertilize. Finally, there is some evidence that progestin exposure (either through exogenous treatment or endogenous progesterone from a spontaneous ovulation) primes the ovary to be more sensitive to the effects of gonadotropins.[43,74]

Direct stimulation of the pituitary with GnRH agonists has also been investigated. Treatment of a GnRH agonist initially causes an acute stimulatory phase that lasts for several days and is accompanied by a large increase in FSH and LH concentrations.[77,78] With prolonged exposure, GnRH receptors are down-regulated, FSH and LH production is reduced, and temporary infertility is induced. Deslorelin is a GnRH agonist with a biological potency 10-144 times higher than native GnRH.[79] Deslorelin is commercially available as a slow-release subcutaneous implant (Suprelorin®, Virbac). It is registered in the European Union (EU), Australia, and New Zealand for long-term suppression of adult male dogs (see **Estrus suppression**) and, as of June 2022, the 4.7 mg implant has been approved in the EU for use in male cats.[80,81] In the USA, Suprelorin F® is a Food and Drug Administration Indexed Product to manage adrenal disease in male and female ferrets.[80] Thus, any use of Suprelorin in the queen is considered extra-label.

Consistent results were reported using the 4.7 mg Suprelorin implants to induce estrus in queens.[82] The implants were placed in the umbilical area without sedation or general anesthesia. Queens were monitored for estrus via daily behavioral observation, daily vaginal cytology, and every other day transabdominal ultrasonography. Estrus was detected 5.0 ± 2.2 days after implant placement in 100% of the 13 queens tested. Seven females had behavioral estrus and an average of 4.8 ± 1.6 follicles were detected with ultrasonography. Once peak estrus was observed (as defined by vaginal cytology with 100% cornified cells on a clear background), 100 IU of intramuscular hCG was given. Peak estrus was identified 4-11 days after implant placement. Serum progesterone measured 5-6 days after hCG treatment confirmed ovulation in all queens. Three of the females were artificially inseminated twice; procedures were performed 24 and 48 hours after hCG treatment with fresh semen deposited transcervically into the uterine horns. The AIs were performed under general anesthesia, and the implant was removed during the first AI. All females became pregnant and gave birth to healthy kittens.

A separate study only reported successful estrus induction in 10% (2/20) of queens treated with a 4.7 mg deslorelin implant.[83] Ten females were treated 3 days after estrus began and another 10 females were treated 7 days after the end of estrus. Estrus was induced in 1 female from each treatment group. The 9 females that were treated after the end of estrus and failed to respond to estrus induction had serum progesterone concentrations > 1.5 ng/ml, indicating they were luteal at the time of treatment. On the contrary, the study with 100% estrus induction[82] only treated females in anestrus/interestrus, and the authors theorized the disparity of responses could be explained by the difference in stages of the estrous cycle during treatment. Indeed, it would appear much like the ability of the ovary to respond to gonadotropins, a quiescent ovary is more capable to respond to GnRH agonist treatment.

Deslorelin implants have also been utilized to down-regulate ovarian activity prior to eCG/hCG stimulation; 10 queens were treated with 4.7 mg deslorelin implants for 90 days.[84] Following a 10 day withdrawal period after implant removal, cats were treated with eCG/hCG and spayed 3 days later. The authors recovered ovulated oocytes via oviductal flushing and confirmed viability with propidium iodide dye exclusion, but no further assessments were made for oocyte quality or competence. It is of note that on average, females had 13.1 ± 5.5 CLs and 8.5 ± 5.5 follicles, indicating that deslorelin pretreatment did not prevent gonadotropin-induced ovarian hyperstimulation.

The combined use of eCG and GnRH has been explored as an alternative protocol for ET recipient synchronization in the cat. Anestrual queens were treated with 100 IU intramuscular eCG followed 80 hours later with 1 or 2 (12-hour treatment interval) subcutaneous injections of 25 µg GnRH agonist gonadorelin.[69] Only 1 female (out of 5) in each group ovulated. The 2 ovulatory females had significantly higher serum LH concentrations compared to anovulatory cats, suggesting that an insufficient pituitary release of LH was responsible for ovulation failure.

Finally, there is a single report on the use of serial intramuscular naloxone treatments (0.04 mg/kg daily for 4 days) in conjunction with a single intramuscular hCG (1,000 IU) treatment


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to induce estrus and ovulation in the cat via antagonization of the hypothalamic GnRH opioid block.[85] Eight of the 9 treated females ovulated (based on increase in serum progesterone); hCG treatment appeared to be necessary for ovulation induction, as none of the females (n = 4) treated only with naloxone ovulated.

## Natural estrus ovulation induction

One of the largest drawbacks to estrus induction is that it often relies on exogenous gonadotropins to stimulate follicular growth, oocyte maturation, and ovulation. In other mammals, gonadotropin treatment can affect the normal follicular, oviductal, or uterine environment leading to poor quality oocytes or reduced implantation rates.[86,87] In cats, exogenous gonadotropins can hyperstimulate the ovary and create an abnormal endocrine environment. Compared to naturally cyclic females, gonadotropin-treated queens produce a higher number of total follicles (5 in a natural state[72] versus > 10 with gonadotropin treatment),[60] a higher number of unovulated follicles[54] (ovulation is an all or nothing phenomenon in the naturally-mated queen),[30] and more follicular cysts.[53] Additionally, gonadotropin-treatment in felids has been associated with the production of antigonadotropin antibodies, disruption of oviductal embryo transportation, and reduced embryo quality.[32,60,64-66,88]

Timed estrus induction protocols are often paired with a period of ovarian suppression beforehand. Although some data suggest progestins may have a positive role in priming the ovary to favorably respond to gonadotropin treatment,[43,73] the entirety of effects exogenous progestins can have on the uterine environment is still unknown. Progesterone and its receptors in the uterus have a major role in both maintaining pregnancy and in the progression of disease, creating a delicate balance that is not entirely understood, even in human medicine.[89] As a species that demonstrates both induced and spontaneous ovulation, it is difficult to conclude whether progesterone presence prior to an estrus phase is advantageous, detrimental, or has no effect on fertility.

Ovulation induction following a natural estrus represents a viable alternative to estrus induction and has the potential to reduce or eliminate the need for exogenous gonadotropins. In humans, exogenous ovarian stimulation is associated with a higher rate of pregnancy loss before pregnancy can be clinically detected and a reduced implantation rate compared to natural cycle conception rates.[90] Natural cycles are aimed at achieving physiological concentrations of estradiol and progesterone, and ovulation can be induced by natural mating behavior, manual stimulation, or exogenous hormone therapy. Irrespective of the specific technique used for ovulation induction, reliable and accurate detection of estrus is obligatory for success.

## Real-time estrous cycle monitoring

Estrus is characterized by a rapid increase in estradiol, from a baseline plasma concentrations of ~ 15 pg/ml to > 20 pg/ml as the ovarian follicles grow into distinct, vesicular structures > 2 mm in diameter.[72] Serum estradiol could be considered for estrus monitoring, but there is a ~ 1-2 week turnaround time in commercial reference laboratories (e.g. 7-16 days at IDEXX) and, to the best of the authors' knowledge, there are no commercially available in-house estradiol assays validated in the cat. The long turnaround paired with the inherent difficulty in serial blood sampling feline patients currently precludes the use of serum estradiol as a useful tool to monitor the estrous cycle in real-time.

Following natural mating, LH surges within minutes, ovulation occurs 24-32 hours later, and progesterone increases 1-2 days after ovulation.[72,91] Because cats have no preovulatory surge in progesterone, serum concentrations cannot inform the ideal breeding window as it is used in domestic dogs. However, serum progesterone can be useful to confirm ovulation. The authors recommend waiting a minimum of 5 days after the ovulation-inducing event to perform a serum progesterone test.

Feces are the major route of excretion for both estradiol and progesterone metabolites in domestic cats.[36,92] Excreted fecal hormone metabolites accurately reflect hormonal patterns in the blood, considering the appropriate time delay (12-24 hours) for metabolite passage from the blood into the feces. Unfortunately, the gut transit delay plus an additional ~ 48 hours for sample shipment, processing, and assaying renders this technology unsuitable for real-time natural cycle monitoring. However, fecal hormone analysis remains a valuable noninvasive tool for retrospective longitudinal hormone monitoring in domestic and nondomestic felids.

Unlike their canine counterparts, felids do not display vulvar swelling or vaginal bleeding during the estrous cycle.[21] Because of the minimal overt outward changes, behavior has been the mainstay for monitoring felid estrous cycles. Stroking of the flanks and perineal region by a handler may be used to elicit treading of the hind feet and lordosis (bent forelegs with hind quarters elevated and lateral tail deviation).[93] Other behaviors that may be associated with estrus in the queen include rolling, intense vocalization, frequent urination, and increased restlessness. However, there is a great deal of individual variation in what behaviors are expressed. Queens that are particularly affectionate can exhibit estrous behaviors, including lordosis, during times of anestrus. Additionally, behavioral estrus can lag behind physiologic estrus, with only 8% of cats demonstrating estrous behaviors on day 1 (as defined by > 20 pg/ml plasma estradiol), whereas 80% of cats show such behavior on day 4.[93]

For these reasons, it is the authors' recommendation to pair behavioral observation with vaginal cytology. Although not as commonly used as in dogs, vaginal cytology can help accurately determine estrus, especially if performed with regular (ideally daily) sampling. To distinguish other similar periods of the reproductive cycle, 2 or 3 consecutive vaginal cytology samples should be assessed for the proportion of basal, parabasal, intermediate, and superficial epithelial cells, as well as assessing for background polymorphonuclear cells, bacteria, and mucus.[94-96] Up to $^1/_3$ of females may have signs of estrus before cornified cells are noticed on vaginal cytology;[48] instead, clearing of the vaginal smear background (absence of cellular debris) is the most sensitive and earliest indicator of follicular activity, and occurs ~ in $^1/_3$ of cats during proestrus.[93] Vaginal cytology with clearing of the background, a reduction of cellular debris, and a proportion of superficial cells > 80% is indicative of estrus in both domestic cats and African lions.[94-96] Given that the cat is an induced ovulator, with vaginal stimuli from the tom during coitus being the canonical inducing agent, it is important to note that vaginal cytology examination alone did not increase the risk for ovulation induction.[96]

Ultrasonography is another tool that can aid in estrous cycle monitoring. Although transabdominal ovarian ultrasonography has been described to monitor ovarian follicular growth during estrus, it is not commonly employed in cats.[97] In the authors' experience, follicles are routinely identified as round anechoic structures, but corpora lutea (CL) are not easily visualized. In a study performed with queens in a trap-neuter-release program, only 55% (11/20) of CLs found on retrospective histopathology were identified via transabdominal ultrasonography.[98] The CLs that were readily identified were hyperechoic, large, and/or deformed along the ovarian margins. Identification was more challenging and/or not possible when they were iso- or hypoechoic to the ovary. Because timing of a natural cycle would primarily be based on follicular growth, ultrasonography can be beneficial. Verification of ovulation with ultrasonography could provide more immediate feedback, but this information can be achieved by other means (e.g. progesterone monitoring) if the CL(s) is/are not readily identified.

## Induction of ovulation

### Manual stimulation

During natural mating, there are 2 overarching factors that determine whether a female will ovulate after copulation: number of stimuli and timing relative to day of estrus. Single copulations can induce ovulation in a subset of females (21-50%), whereas multiple copulations (3-12) during a 4 hour period in a single day of estrus resulted in higher ovulation rates (83-100%).[29,30,99,100]

Although multiple copulations appear to be consistently superior across studies, there are various reports of the relative day of estrus and mating intervals used. In the first paper to describe manual ovulation induction, queens were stimulated with a glass rod during their first signs of estrus and ovulated 9 out of 12 times.[101] In more recent feline manual induction protocols, a series of 5 vaginal stimulations at 30 minute intervals during peak estrus or maximum follicular diameter induced ovulation in 72 (8/11) to 75% (9/12) of queens.[2,97] Peak estrus is variable among breeding ovulation studies, with most indicating on days 3-5 of estrus.[4,29,100] Breeding before the third day of estrus can reduce LH secretion and increase the chance of ovulation failure,[102] comparable to a report[97] that detected maximum follicular diameter on 3.8 ± 0.3 days.

### Pharmacologic ovulation induction

Early studies reported a wide range of hCG doses (50-500 IU) used to induce ovulation during days 1-2 of natural estrus, given either the day before or on the day of AI.[52,103] More recently, several studies have been performed using intravenous hCG treatment on days 2-4 of natural estrus, with either 2 100 IU injections given 24 hours apart or a single 250 IU injection.[71,104,105] Ovulation rate with either protocol was relatively high in all studies (82.4-95.6%), and these protocols were used in conjunction with vaginal or uterine AIs at 15, 20, and/or 30 hours after hCG treatment to successfully produce live offspring. Subsequently half-life and bioavailability of hCG was determine and that were similar between intramuscular and intravenous treatments[60]; however, to the authors' knowledge, no studies have subsequently been performed to assess ovulation rates with intramuscular hCG utilizing the aforementioned treatment protocol. Due to its potential to cause neutralizing immunoglobulins and undesirable secondary follicular growth, it is not the authors' recommendation to use hCG in natural cycle ovulation induction protocols.

Because of its small size (9 amino acids), GnRH is not detected by the immune system and therefore can be used repeatedly without the development of antiGnRH antibodies.[106] Nor is it associated with ovarian hyperstimulation, likely due to its differing mechanism of action, or more specifically, that it targets the pituitary, which creates another level of opportunity for feedback inhibition.[107] Treatment with a single intramuscular injection of 25 µg gonadorelin (GnRH agonist) resulted in a sharp increase in serum LH for queens in estrus or anestrus and ovulation was observed in 100% (4/4) of estrual queens.[108] A single 25 µg intramuscular injection of gonadorelin given on day 2 or 3 of natural estrus produced a comparable number of ovulations (4.1 ± 0.8) to a single intramuscular injection of 250 IU hCG given during the same time period (4.0 ± 0.9).[54] Subcutaneous treatment of 25 µg gonadorelin with repeated vaginal stimulations resulted in a 100% (7/7) ovulatory rate on day 3 of estrus.[96] A more recent study utilized a single intramuscular injection of 50 µg gonadorelin on day 2-4 of behavioral estrus, with successful ovulation occurring in 84% of treated females.[106]

The authors have used 2 treatments of gonadorelin 12 hours apart on days 3-4 of natural estrus (defined by vaginal cytology and/or behavior), with successful ovulation induction (defined by laparoscopic ovarian examination and/or fecal progesterone metabolite analysis) in the domestic cat (25 µg), ocelot (50 µg), Amur leopard (100 µg), and jaguar (100 µg). A single intramuscular injection of GnRH agonist buserelin (~ 50 times more potent than native GnRH[79] and not currently available in the USA) on day 4-6 of natural estrus in the Asiatic golden cat (3 µg) and lion (20 µg) resulted in ovulation, and in conjunction with AI, produced live offspring.[109,110]

The llama is an induced ovulator that relies on ovulation-inducing factors (OIFs) in the seminal plasma, rather than the physical act of copulation, to trigger ovulation. Beta nerve growth factor (Beta-NGF) has been identified as the potent OIF in llamas, capable of eliciting ovulation either through intrauterine infusion or intramuscular injection.[111,112] Beta-NGF has since been detected in the seminal plasma of a variety of other induced and spontaneous ovulator species, although cats have not specifically been investigated.[113] Sixty-seven percent (4/6) of cats treated on day 2 of natural estrus ovulated in response to subcutaneous cat seminal plasma, versus 0% (0/6) of cats treated with intramuscular cat seminal plasma, and 17% (1/6) of cats treated with subcutaneous purified llama Beta-NGF.[114] This suggests that cat seminal plasma may contain OIFs that help support ovulation in the queen. Although not currently available for clinical use, the identification and isolation of cat OIF molecules could offer a new avenue for ovulation induction in the queen.

Finally, as reported in the **Estrus induction** section, pLH has commonly been employed to stimulate ovulation after eCG-mediated estrus induction during timed ART procedures. To the authors' knowledge, there are no reports that have utilized pLH to induce ovulation from a natural cycle, but it could be considered as an alternative to hCG or GnRH treatment.


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## Estrus suppression

As discussed above, a quiescent ovary is required for estrus induction to be maximally effective. Ovarian suppression protocols that are specifically designed for treatment prior to gonadotropin are reviewed in the **Pharmacologic estrus induction** subsection. This section will address the other reasons safe and reliable estrus suppression is needed in cats.

Breeders often request estrus suppression in queens to temporarily delay breeding without compromising future fertility.[115] Even in cats not intended for breeding, owners may be reluctant to choose surgical neutering due to concerns about preexisting conditions or surgical complications.[116] Ovarian remnant syndrome is a well-documented surgical complication in cats, and in some cases, it may be difficult or impossible to identify and remove the residual ovarian tissue, whereas other means of reproductive control need to be considered.[117,118] Finally, cat overpopulation (i.e. unowned, outdoor 'community cats') is a global concern. The surgical model of trap-neuter-return (TNR) has remained the gold standard for humanely reducing community cat populations. Although the rise in subsidized spay-neuters has helped to significantly reduce the rate of shelter euthanasia in USA, TNR is limited by access to resources and veterinarians.[119] The COVID-19 pandemic amplified the shortage, creating a deficit of over 2.7 million spay/neuters for companion animals in USA.[120] This shortage is further magnified in developing countries with limited economic resources and some countries do not legally permit surgical neutering. Nonsurgical options for long-term (or permanent) contraception could help augment traditional TNR programs.

## Photoperiod

Queens are highly responsive to changes in photoperiod; a light:dark cycle of 8:16 hours is sufficient to immediately cease cyclity.[26] The time it takes to resume cyclicity after a return to long-day light conditions (14:10 light: dark hours) varies by study (12-46 days), and the interval can be shortened by providing 1 hour of light during the dark period, cohabitation with estrual females, or introduction of a tom.[26,27]

## Melatonin

Photoperiod exerts its effect on the queen's cyclicity via the retino-hypothalamic pathway to the suprachiasmatic nuclei that in turn regulates melatonin synthesis by the pineal gland.[121] Melatonin is a neuromodulatory substance that inhibits hypothalamic secretion of GnRH.[122] In the cat, melatonin synthesis peaks during the night and serum concentration is ~ 15-fold higher than during the day.[123] Thus, exogenous melatonin treatment is a logical target for estrus suppression.

Daily oral melatonin (30 mg/cat), given 3 hours before lights-off, is effective in suppressing estrus.[123] Serum melatonin concentrations peaked ~ 1 hour after treatment and remained significantly elevated above endogenous night-time concentrations for at least 8 hours. Three of the 6 treated females demonstrated a period of ovarian follicular activity early in treatment (based on fecal estradiol metabolite analysis), but all follicular activity stopped after day 25 of treatment. Following 35 days of melatonin treatment, females took 21-40 days (33 ± 2.8) to resume cyclicity.

The authors also studied the use of melatonin as an ovarian down-regulation strategy prior to gonadotropin stimulation and AI. Although 30 days of treatment prior to AI was successful in down-regulating the ovaries and still allowed the queen to respond to eCG/hCG treatment (given either at the end of melatonin treatment or after a 2 day withdrawal period), the authors did not recommend the use of melatonin as a pregonadotropin suppression protocol because it only marginally reduced ancillary follicle development and had no impact on quantity or quality of embryos produced from AI.

Some cats may be refractory to daily oral melatonin treatment. Therefore, long-term release subcutaneous melatonin implants have also been investigated. Most of the studies have utilized an 18 mg implant marketed for use in sheep (Melovine®, CEVA). Similar to oral melatonin treatment, complete suppression may require a period of time and estrus can occur soon after treatment. Therefore, estrous stage should be considered at the time of implant placement. Approximately 80% of queens implanted during estrus will have estrus behavior shortly after implantation.[124] Conversely, queens implanted in late interestrus demonstrate estrus in ~ 35% of cases[122,124-126] and queens implanted during anestrus[127] or early interestrus[125] did not come into estrus. The estrus that follows a melatonin implant may be fertile, as 1 queen in estrus after treatment was allowed to breed and produced a live litter.[122] Typical duration of estrus suppression for queens implanted during interestrus is 1-3 months,[122,124-126,128] but larger ranges (21-277 days; ~ 0.7-9.2 months) have been reported.[129]

An important consideration of the Melovine® implant is that the product is designed for sheep, and each pack contains 25 implants to be loaded sequentially to ewes with a single applicator.[81] This would not be considered appropriate by feline practitioners and thus, alternative application schemes, such as insertion through a skin incision,[122] should be performed. Similarly, preservation of sterility for the remaining implants needs to be addressed.

## GnRH agonists

Although GnRH agonist treatment initially causes an acute stimulatory phase,[77,78] prolonged exposure leads to desensitization of the GnRH receptors, reducing production and/or release of FSH and LH, inducing a state of infertily.[130] Most studies investigating the use of deslorelin in the queen have been performed with a 4.7 mg deslorelin implant (Suprelorlin®), therefore this review will focus on those data. In cats, the implant is typically inserted through a needle subcutaneously into either the subscapular[83,84] or umbilical area,[82] with the latter being preferred for easy removal of the implant.[81]

In male dogs treated with the 4.7 mg implant, serum deslorelin concentrations peaked during the first week after treatment and then gradually decreased, reaching undetectable concentrations around day 80.[130] No comparable pharmacokinetic studies have been performed in the cat. Clinical data from a study[82] suggests an initial peak of deslorelin during the first week of treatment likely occurs in the queen as well. The duration of efficacy, however, is much longer and more variable in queens compared to bitches. In one study, duration of efficacy ranged between 483-1,025 days (~ 16-34 months), with 1 female still clinically suppressed at the study's conclusion (1,102 days, ~ 37 months).[83] Other studies have reported efficacy to last 4-14 months,[129,131] and 18-26 months.[132,133] One of the latter mentioned studies treated 14 cats with 9.5 mg

deslorelin implants and 1 (7%) had no suppression of ovarian activity.[133] It is unknown whether this large variability in effect duration is due to individual variation of susceptibility to deslorelin, individual variation in the desensitization mechanism, degree of vascularization at the insertion site, or other undescribed factors.[80]

During the acute stimulatory or 'flare-up phase', estradiol concentrations surge and can lead to a behavioral estrus within a few days of implant insertion. The rate of estrus induction varies by study protocol and is largely influenced by the queen's estrous stage, with interestrus females being the most reliable estrus presenters.[82-84,133] Ovulation may also occur. In one report, 40% (4/10) of treated cats ovulated (determined with weekly blood progesterone analyses) during the flare-up phase.[84] It is important to note that these estrus events can be fertile, as high pregnancy rates were achieved using deslorelin to induce estrus for AI.[82] In this case, the implants were removed at the time of the procedure. However, pregnancy can be maintained in deslorelin-treated queens that are mated during the flare-up phase[134] or ~ 7-9 days before implant placement.[81,135] In one case report of a suspected mismating 8-9 days before implant placement, the queen delivered 4 healthy kittens, but had no maternal interest and had inadequate lactation.[135] Prolactin was not measured, so the specific mechanism of deslorelin-induced hypogalactia could not be identified. Following parturition, the queen entered anestrus and did not have another estrus until 498 days after treatment.

Another curious variability observed with deslorelin treatment is that a subset of females can have a period of estrus that is not connected to the end of the implant's action, as these episodes will be followed by another prolonged period of anestrus. A study reported that 1 female (n = 20; 5%) had 2 periods of estrual signs, 138 and 155 days after treatment.[83] In another study, 1 female (n = 14; 7%) exhibited estrous behavior to her caretaker 3.5 months after beginning treatment, but did not allow a tom to mount her.[133]

In a study that removed implants at 3, 6, or 9 months after placement, the authors concluded that ~ 3 weeks are needed during increasing photoperiod to resume cyclicity, and this requirement can increase up to 7 weeks if the photoperiod is decreasing.[134] The length of implant placement had no effect on the length of time needed to return to estrus. A return to fertility after termination of deslorelin treatment (via surgical removal of implant or cessation of implant effect) has been consistently demonstrated. Studies have confirmed the queen's capacity to ovulate,[84] return to normal cyclicity,[83] and produce normal litters.[83] The most common side effect reported is weight gain that is often reversible after implant removal/failure without dietary intervention.[129] Other side effects occurred far less frequently and include persistent estrus,[129,134] galactorrhea,[129,136] and implant site lesions.[129,133]

Data on the effects of long-term treatment are lacking. There is one case report of a queen treated at 1 year of age and then treated repeatedly every time she showed estrus (which occurred ~ every 2 years). When she presented with estrus at 8 years of age, the female was spayed and her reproductive tract was examined histologically. The ovaries were juvenile in appearance, containing numerous primordial and primary follicles. However, the uterus demonstrated marked endometrial hyperplasia, suggesting that repeated deslorelin stimulation and subsequent flair-up stages can have a negative effect on uterine health.[137]

Overall, deslorelin treatment is regarded as relatively safe in cats, with minimal side effects, a quick return to fertility, and a high rate of efficacy. Its largest drawback is the wide range in duration of effect. Owners should regularly monitor for signs of estrus and consider intermittent vaginal cytology to more precisely predict when an implant's effect is waning.

## Progestins

Progestins are synthetic derivatives of progesterone that bind to the progesterone receptor with a greater affinity than endogenous progesterone.[138] Progestins have the same biological effects as progesterone and have been used for a variety of clinical cases, such as dermatologic and behavioral disorders.[139] Their main veterinary application remains as control of the estrous cycle.[140]

The mechanism of action by which progestins facilitate estrus suppression is not fully known. One well-accepted pathway proposal is through negative feedback on the hypothalamus and pituitary, suppressing release of GnRH, FSH, and LH.[141,142] Progestins may also inhibit sperm transport by thickening cervical mucus and reducing uterine motility, as well as preventing implantation through endometrial alterations.[141,143,144]

Progestins use in queens has been associated with cystic endometrial hyperplasia-pyometra complex, fibroadenomatous mammary hyperplasia, mammary neoplasia, adrenocortical suppression, and diabetes mellitus.[141,145-154] These effects were magnified with long-term treatment, higher dose usage, or when the queens were older and/or had preexisting conditions. It is therefore the authors' recommendation to not consider progestin therapy as a strategy for long-term fertility control in the queen. However, there is a global shortage of access to spay/neuter programs, which was amplified by the COVID-19 pandemic.[120] This has prompted animal welfare advocates and organization, such as the Alliance for Contraception in Cats & Dogs (ACC&D), to suggest the strategic use of megestrol acetate (MA) in queens when spay services are available but delayed.[155,156]

Megestrol acetate (6-methyl-6-dehydro-17α-acetoxyprogesterone, MA) is a potent progestin, with activity estimated to be several times higher than endogenous progesterone.[138] It is commercially-available as an oral formulation in several European countries.[157] MA became commercially-available in the USA in 1975 as an FDA-approved veterinary drug for female dogs (Ovaban®, Intervet Schering-Plough); off-label use in the cat was not uncommon.[139] In 2008, an extra-label formulation of MA was developed by a private veterinarian and marketed to free-roaming cat colony caretakers (Feralstat).[156] The intention was to serve as an adjunct to TNR programs by preventing pregnancy in queens waiting to be spayed, although some caretakers elected to use this product in lieu of surgical sterilization. The package insert instructed weekly dosing at ~ 0.1-0.2 mg/kg MA, which was significantly lower than dosing regimens previously reported to be effective at pregnancy prevention. A veterinary consultant for ACC&D interviewed several Feralstat users, who reported satisfactory results (i.e. generally healthy colony and pregnancy prevention).[158] However, no prospective studies have been performed to assess the safety and efficacy of MA given in this dosing regimen.


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Efficacy of weekly treatment of 2.5 mg/cat for at least 30 weeks in 244 cats was assessed.[150] Twenty-one females demonstrated estrus during treatment. Two females that were pregnant prior to treatment initiation had abnormal pregnancies; no other pregnancies were reported. An increase in appetite was reported in 33.6% (89/244) of queens and weight gain was noted in 13% (32/244) of queens. One female (0.4%) that received MA for 3 years developed pyometra and mammary adenocarcinoma.

Assuming a 4 kg body weight, 2.5 mg/cat would equate to 0.625 mg/kg/week, which is considered a low dose in cats.[157] This is an important point as higher progestin dosages are correlated with a higher rate and/or increased severity of side effects. It is of note that most of the European-based formulations list 2.5 mg/week for a maximum of 30 weeks as the suggested dosing regimen. It is contraindicated to start MA treatment when a female is luteal, as adding a synthetic progestin to endogenous progesterone could be equivalent to high-dosage treatment, and it is currently recommended to only treat queens in anestrus or interestrus.[157,159]

The American College of Theriogenologists (ACT) does not support the use of progestins, including MA, for contraception in free-roaming cat populations. In their joint Position Statement with members of the Society for Theriogenology, ACT cites the potential for inaccurate dosing, inadvertent treatment of pregnant females or nontarget species, and the adverse health events associated with progestin use as reasons for discouraging its use in this manner.[160] It was stated that, "[progestins], including megestrol acetate, may be available to veterinarians for treatment of individually owned cats, but only within the strict confines of a veterinarian-client-patient relationship, including a veterinary prescription." ACC&D currently supports the use of MA in queens, "that can be individually treated at prescribed times with an accurate dose, and whose health can be monitored over time …as a stopgap measure to prevent pregnancy in female cats at risk of conceiving while awaiting spay surgery."[155] While historically advising against the use of MA in free-roaming cats, ACC&D stated that because of the COVID-19 pandemic, and the subsequent strain that it has put on spay/neuter programs, they do support the consideration of short-term, low dose MA treatment in situations where surgical spay is not an immediate option.

In conclusion, the authors do not recommend progestin-based contraception as a strategy for long-term fertility control. The use of MA may be considered in females where surgical sterilization is planned but delayed. Rigorous data on the safety and efficacy of low-dose MA treatment are lacking; careful patient selection and thorough clinical monitoring is warranted.

## Permanent contraception

Due to the cost and logistical demands of large-scale TNR programs, considerable effort has been placed towards the development of a single-dose, nonsurgical, low-cost alternative for permanent contraception in the cat. Early studies focused on the use of immunocontraceptive vaccines that control fertility by stimulating the production of antibodies against proteins that are essential for reproduction.

One such approach utilizes porcine zona pellucida (pZP) glycoproteins extracted from pig ovaries. Treatment in several mammalian species (e.g. horse,[161] rabbit,[162] dog,[163] elephant,[164] white-tailed deer,[165] and seal[166]) resulted in production of antibodies that bind to the surface of the oocyte, which block sperm penetration and subsequent fertilization. A vaccine formulation that incorporates pZP antigens into multilamellar liposomes (SpayVac™, ImmunoVaccine Technologies Inc) was investigated in the domestic cat, due to its ability to induce long-term contraception in other tested species.[167] All vaccinated kittens developed high antipZP antibody titers, but the treatment did not prevent cyclicity or pregnancy. Ovarian immunohistochemical analyses revealed that the antipZP antibodies produced by SpayVac-treated kittens did not recognize feline ZP (fZP). These results align with a study that demonstrated cat and pig zonae pellucidae expressed a very small number of shared antigenic determinants.[168] A subsequent study screened native soluble-isolated ZPs (SIZPs) isolated from 5 mammalian species: cows, cats, ferrets, dogs, and mink.[169] Treatments from all species resulted in antiSIZP antibody production. However, the antiSIZP antibodies had low cross-reactivity to fZP, as evidenced by low antifZP titers and lack of binding to feline ovaries.

The next immunocontraceptive investigated was GonaCon™, a GnRH vaccine developed by scientists at the United States Department of Agriculture-Animal and Plant Health Inspection Service Wildlife Service's National Wildlife Research Center (USDA-APHIS NWRC) for use in wildlife. Because GnRH is the 'master regulator' of reproduction, antibodies against hypothalamic GnRH prevents the normal hormone cascade required for sex-steroid production and gametogenesis.[170] GonaCon was originally developed for use in wild horses and white-tailed deer, but has since been applied to a variety of species, including the cat. After a single GonaCon injection, 93% of cats were infertile for the first year after vaccination, whereas 73, 53, 40, and 27% remained infertile for 2, 3, 4, and 5 years, respectively.[171]

Since GonaCon was initially produced by the NWRC, it has undergone several formulation changes.[172] Compared to the formulation tested[171] in 2011, the GonaCon formulation registered with the Environmental Protection Agency (EPA) in 2016 consisted of a different antigen-carrier protein and increased antigen concentration. Therefore, our laboratory investigated the safety and efficacy of this updated EPA-registered formulation.[173] All cats (n = 6) developed antiGnRH antibodies within 30 days after vaccination. The endpoint titer (1:1,024,000) was similar among all cats, and titers remained at that level throughout the duration of the study (4-6 months). Because the vaccine was tested on ovariohysterectomized cats, fertility could not be assessed. Therefore, a larger follow-up study in intact females was performed.[174] Sixty percent (12/20) of GonaCon treated females became pregnant within 4 months after breeding trial initiation. Two additional females became pregnant within 1 year after treatment, for a total of 70% (14/20) of queens that became pregnant following vaccination.

The poor contraceptive efficacy was not anticipated, based on the high rate of contraception in queens treated with the earlier GonaCon formulation[171] and high antiGnRH antibody titers observed with the current formulation in ovariohysterectomized queens.[173] Antibody titers were not performed in this study; therefore, batch to batch variation in vaccine production could not be ruled out. Individual vaccine response variation as well as differences in study population and design (the former study was performed with laboratory cats under

controlled, indoor conditions, whereas the latter was performed with cats adopted from shelters in an ambient-temperature facility with daily outdoor access) should also be considered. Irrespective of the cause for treatment failure, the overarching conclusion was that GonaCon cannot currently provide contraception for a sufficient proportion of the population to justify its use for control of free-roaming cats.

More recently, our laboratory reported a novel approach for long-term contraception in the cat utilizing anti-Müllerian hormone (AMH) that plays a critical role in ovarian folliculogenesis.[175] At high concentrations, AMH inhibits the recruitment of primordial follicles into the pool of growing follicles and decreases the FSH-responsiveness of growing follicles. An adeno-associated viral vector, delivered intramuscularly as a single injection, was used to overexpress AMH in adult female queens. Fecal hormone metabolite analysis was used to monitor progesterone and estrogen concentrations, and 2 breeding trials (4 months duration) were performed 1 and 2 years after treatment. All control cats produced kittens (3/3), but none of the treated cats became pregnant (0/6). Treated cats had a reduction in average progesterone concentrations, a reduction in the rate of spontaneous ovulation, and complete inhibition of coitus-induced ovulation. Furthermore, the cats' AMH concentrations remained elevated for 5+ years since initial treatment (unpublished data), indicating that gene therapy treatment may be able to provide contraception for the rest of the cats' lives. Further studies, large-scale production facilities, and FDA approval will be required before this product can be made commercially available.

In summary, a permanent, nonsurgical approach to sterilization would be a powerful tool for the humane control of free-roaming cat populations and could provide owned-cats with an alternative to surgical spay. Although considerable research has been conducted in this field, no permanent, nonsurgical sterilization products are commercially available. Despite success in many other mammalian species, immunocontraceptive approaches have not been effective in the cat. However, the application of gene therapy provides an exciting proof of concept and suggests the realization of nonsurgical sterilization in the domestic cat may be on the horizon.

## Conclusion

Many methods of estrus manipulation exist in felids depending on the goal of the treatment, but success rates vary widely among and within estrus induction, ovulation induction, and estrus suppression protocols. External stimuli such as light and social interactions play a major role in feline cyclicity and should be considered before pharmaceutical manipulation. Gonadotropins and GnRH agonists have successfully been used in cats for estrus and/or ovulation induction. Melatonin, GnRH agonists, and progestins can all suppress feline estrus. However, dose and duration of treatment should be considered on an individual basis. These tools are useful when working with the feline estrous cycle, but understanding each regimen's limitations is critical before making an appropriate selection.

## Conflict of interest

LV has served on the Alliance for Contraception in Cats & Dogs Ethical Review Board and is currently on the Scientific Advisory Board for the Michelson Found Animals Foundation Michelson Prize and Grants in Reproductive Biology Program. JB has no conflicts of interest to disclose.

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