Evaluation and insemination options for the subfertile queen
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Abstract
Causes of infertility in the queen are often multifactorial. Cattery management should be addressed prior to evaluation of individual animals. Factors such as housing/lighting, preventive care, and retroviral disease status of the colony should be evaluated. A complete physical examination is vital to rule in or out anatomical abnormalities leading to infertility. Transabdominal ultrasound is a useful tool for noninvasive evaluation of the reproductive tract, and vaginal cytology combined with hormone assay and behavior can be used to stage the estrous cycle. In queens with good breeding management, cystic endometrial hyperplasia is a common cause of infertility and is a major predisposing factor for pyometra. Recent research has demonstrated continued advances in assisted reproductive techniques in felids, including several options for the use of frozen semen.

Keywords: Queen, cat, infertility, pyometra, artificial insemination

Introduction
The female cat is a seasonal polyestrus induced ovulator, and appropriate breeding management is often the key to good fertility in a cattery. Minor changes in procedures may have an enormous impact on pregnancy rate and litter size. When presented with an infertile queen or generalized infertility in a cattery, a detailed history and observation of protocol is as essential as the complete physical examination. The queen is a long day breeder, therefore records of the animals should be evaluated to see if the infertility is seasonal. Maintaining the animals under artificial lighting for at least 14 hours per day reduces seasonal variation, but in this author’s experience, even cats maintained under this lighting system experience a decrease in pregnancy rates during the shorter days of the year. A queen will typically show estrus within one to two months after initiating an artificial lighting period.

The queen requires more than one mating to achieve enough stimulation for the luteinizing hormone (LH) surge necessary for ovulation. Ovulation occurs in approximately 50% of queens following one mating, but approaches 100% of queens allowed to mate four or more times. Observation of breeding episodes is helpful to evaluate the number of times the tom attempts to copulate and also evaluating the female for the classic “after reaction” that confirms copulation took place. If the after reaction does not occur, penetration of the penis into the vagina was unlikely.

A general and reproductive physical examination (with blood chemistries and endocrine testing when indicated) should be performed to rule out obvious causes of infertility dealing with physical limitations (vaginal stricture) or endocrine abnormalities. If physical abnormalities are present or if there is no other obvious cause for the infertility, a karyotype of one or both members of the breeding pair may be necessary to confirm the genetic makeup of the animal is normal.

Use of a high quality ultrasound unit will allow imaging of the uterus by starting at the bladder and moving cranially. The uterus should be evaluated for size, symmetry of the horns, the presence of cystic structures and fluid within the lumen. Ovaries can usually be detected immediately caudal to the kidneys and can be evaluated for the presence of large follicular cysts.

Management
Successful management of a breeding cattery requires knowledge of normal feline breeding behavior. General management practices to ensure cleanliness of facilities and animals, comfortable housing, and adequate ventilation are required. The vaccination status of the animals should be evaluated to ensure adequate disease control. While a closed colony is preferable with no new animals entering, such a policy may also limit the introduction of new genetics. A quarantine period with adequate testing procedures should be mandatory for all new additions.
When breeding naturally in a cattery, the queen should be taken to the tom, preferably when she is showing signs of estrus (heat). Signs include vocalization, rubbing on walls, and rolling on the floor. Additionally, vaginal cytology examination may be performed every two to three days. A moistened sterile cotton tipped applicator is inserted gently into the vaginal canal of the cat approximately 1cm. Vaginal cytology can be assessed for the percentage of cornified cells. In this author’s experience, the percentage of anuclear cornification during estrus is lower in the queen than in the bitch and many queens will be receptive to the male when the vaginal cytology is 70-75% cornified. Inducing ovulation by taking a vaginal cytology is a concern for some practitioners, but if the sample is taken gently, the risk is low because LH release requires multiple mating episodes over a short period of time. A single swab is unlikely to be enough stimulation to induce the LH surge.

If heat detection is not possible, the queen can be placed with the male for at least two weeks. The pair should be monitored closely for breeding activity or fighting (if the queen is not in estrus). If fighting occurs, separate the pair and retry in a few days, or until the vaginal cytology indicates estrus. Once the pair has been together for at least two weeks, serum progesterone concentration can be used to confirm ovulation (greater than 2 ng/ml). At this time, the queen may be returned to her housing awaiting a pregnancy examination. If the progesterone is less than 2 ng/ml, mating has likely not occurred, or has not occurred sufficiently to induce the LH surge and ovulation. The queen should be returned to the male and monitored for breeding activity. If ovulation still does not occur, inducing ovulation followed by artificial insemination may be necessary.

Although cats are induced ovulators, spontaneous ovulations sometimes occur. If a female continues to refuse a male, a serum progesterone concentration should be assessed to determine of luteal tissue is present. If the progesterone is greater than 2ng/ml, luteal tissue is present. In the non-pregnant queen, the luteal phase lasts approximately 45 days, and the queen should be monitored every one to two weeks utilizing vaginal cytology, behavior, or progesterone assay to determine when the luteal phase has ended and routine breeding management instituted at that time. Pregnant queens should be placed in a quiet area, alone if possible, with a covered box for nesting and queening. A stressed queen is more likely to cannibalize her young so every attempt should be made to reduce stress around the time of queening.

Causes of infertility

Causes of infertility in the queen are often divided into broad categories but this discussion will focus on those that fail to produce a pregnancy despite appropriate breeding management. In a natural breeding situation, queens that show persistent receptivity to the male are likely failing to ovulate. This may result from lack of adequate stimulation to produce the LH surge or a primary ovarian dysfunction such as ovarian follicular cysts. Evaluation of the breeding records, number of witnessed matings, and presence or absence of the “after reaction” of the queen following the coitus will assist in assessing if adequate stimulation has occurred. If ovulation is not occurring due to inadequate copulatory stimulation ovulation may be induced pharmacologically (see below) and followed by either natural mating or artificial insemination. The formation of ovarian follicular cysts can be a frequent occurrence in the queen and incidence increases with age. Presence of follicular cysts is best confirmed by transabdominal ultrasonography. Cysts are often hormonally functional, producing high concentrations of estrogens. Treatment options include attempts to induce luteinization of the cyst(s) by administration of either 500 IU human chorionic gonadotropin (hCG) or 25µg gonadotropin releasing hormone (GnRH) intramuscularly. Surgical resection has also been reported with a return to fertility.

In this author’s experience, the most common primary uterine problem in the queen is cystic endometrial hyperplasia (CEH) with or without concurrent pyometra. In the author’s research cattery, approximately 75% of queens diagnosed with infertility (failure to produce offspring following appropriately managed breeding(s)) had histological evidence of CEH within their uterus. Clinical signs in this CEH affected group of queens were first noted at three years of age (decreased litter size or failure to produce kittens consistently). Intact female cats over the age of five years have been shown to be at risk for clinical disease associated with CEH/pyometra. Diagnosis can be suspected based on history of reduced fertility and by the ultrasonographic appearance of the uterus in severe cases. A uterine biopsy
provides definitive diagnosis and may be required for less severe cases; typically, these are obtained surgically. If CEH is severe, prognosis for fertility is guarded and ovariohysterectomy is recommended. Pyometra often occurs secondary to CEH and, when severe, necessitates ovariohysterectomy (preferred treatment). In the author’s cattery, pyometra has been observed in young queens (less than three years of age). In these cases the uterus appeared grossly otherwise healthy, lacking clinical signs of underlying CEH. The queens were not systemically ill and the only clinical sign was a purulent vaginal discharge. Treatment with enrofloxacin (5mg/kg intramuscularly or orally twice daily) and natural prostaglandin (0.25 mg/kg dinoprost tromethamine subcutaneously or intramuscularly twice daily) was followed by a return to fertility in over half the cases treated. The uterus should be monitored daily by gentle palpation and transabdominal ultrasound to assess amount of fluid within the lumen and overall uterine size during treatment. Prostaglandin therapy should be continued until fluid accumulation within the uterus is resolved and the uterus has returned to normal size. Breeding may be attempted approximately four weeks after successful treatment. In a small number of the treated queens, recurrence of the condition was observed during the next luteal phase. In these cases the histopathology demonstrated CEH which likely favored recurrence. A technique for nonsurgical uterine lavage as a treatment for uterine infection has been described in the large non-domestic, larger feline species, but would be much more difficult to perform in the smaller domestic queen.

Ovulation induction and artificial insemination

Ovulation induction

Queens are induced ovulators, thus ovulation induction protocols can be critical to success. One group has shown consistent results inducing estrus and ovulation using a combination of equine chorionic gonadotropin (eCG) and hCG in a timed artificial insemination protocol. Treatment is initiated in nonestral, nonluteal queens as determined by observing for behavioral estrus and serum progesterone less than 1 ng/ml. Queens received an initial injection of eCG (100 IU IM) followed by an injection of hCG (75 IU IM) 85 hours later. Insemination is performed 31-33 hours after the hCG injection and ovulation is expected between 25-30 hours after hCG. Using this protocol, 100% of queens ovulated with a 75% pregnancy rate (6/8 queens) when inseminated with either laparoscopic intrauterine or laparoscopic oviductal inseminations. An alternate method is to administer hCG intravenously twice daily on days two through four of estrus. The ovulation rate using this protocol was 95.6% (43/45 queens). In this study, queens were inseminated at 15, 20, and 30 hours following the last hCG injection. Ovulation is expected to occur between 25-27 hours after administration of hCG.

One concern with insemination protocols is the effect of anesthesia on ovulation. Howard, et al reported that queens inseminated (laparoscopic intrauterine) after ovulation produced more corpora lutea and embryos, and had a higher pregnancy rate than those inseminated prior to ovulation (50% vs 14.3% pregnancy rate). A mean of 6.6x10^6 motile sperm were used in both groups. Due to the concern that anesthesia may inhibit ovulation, many researchers elect to inseminate after ovulation has occurred (28-40 hours). However, others have not found an effect of anesthesia on ovulation rate with slightly higher doses of hCG.

Vaginal insemination

When frozen semen was used for vaginal insemination, the pregnancy rate was low (10.7% or 6/56 attempts). Vaginal insemination is therefore best reserved for cases in which fresh or good quality cooled transported semen is available. This procedure may be performed by inserting an open ended tomcath catheter approximately 1-2 cm into the vaginal vault in the non-anesthetized queen, depositing the semen, and elevating the hind quarters for approximately ten minutes to prevent back-flow. This technique is difficult in many queens due to a less than compliant nature and the violent after reaction associated with breeding/vaginal stimulation. Most researchers have better success using a deep vaginal insemination technique under general anesthesia. In the anesthetized queen, the vaginal vault is initially dilated with a 2mm diameter probe. The insemination pipette is then inserted 3-4 cm into the vaginal
canal, the semen is deposited and the hind quarters elevated after insemination. Ovulation induction is still required following transvaginal insemination regardless of whether or not anesthesia was used. The pregnancy rate following vaginal insemination was 77.8% when 80 x 10^6 fresh motile sperm were used. The pregnancy rate decreased to 33.3% using 40 x 10^6 motile spermatozoa and 6.6% with 20 x 10^6 motile spermatozoa. The use of at least 80 x 10^6 motile spermatozoa be used when performing a vaginal insemination is commonly recommended.7

Intrauterine insemination

The main advantage of intrauterine insemination in the queen is the reduction in insemination dose compared to vaginal insemination. In fact, a pregnancy rate of 80% was achieved using only 8 x 10^6 motile sperm (10% of the recommended vaginal dose) when inseminated surgically into one uterine horn and lower doses have also been successful. Intrauterine insemination is performed surgically with the queen under general anesthesia. The standard surgical method is to make a midline incision on the ventral abdomen. Both uterine horns are located and examined for signs of pathology prior to insemination. Following visual examination, one uterine horn is isolated. A 22-20ga intravenous catheter is inserted through the uterine wall (mid-horn) and into the lumen. The catheter is advanced off the needle into the uterine horn in the direction of the ovary. The needle is removed. Half of the semen (approximately 10µl) is infused through the catheter and into the uterine horn. Care should be taken not to contaminate the abdomen with the sperm sample. Once insemination is complete, the catheter should be removed and a gauze pad should be placed with direct pressure over the insertion site to control bleeding. The process is repeated using the remaining semen into the opposite uterine horn with a fresh catheter.

Alternatively, the insemination may be performed laparoscopically.6 The uterus is held adjacent to the body wall usingatraumatic forceps. An 18ga catheter is inserted through the ventral abdominal wall and into the uterine lumen as described above. Polyethylene tubing was then inserted through the catheter and into the uterine lumen. The semen was infused into the uterus through the tubing. When using very small amounts of semen, placing the sample in the distal end of the tubing will minimize loss of the inseminate.

Oviductal insemination

Recently, success has been described using very low numbers of sperm laparoscopically inseminated directly into the oviduct. This method is desirable in species or situations where the number of available sperm is very low. Swanson’s group has shown excellent success using as few as one million motile sperm. This technique requires the use of custom made forceps to grasp and secure the ovarian bursa. The bursa is then manipulated until the abdominal oviductal ostium is visualized. An 18ga intravenous catheter is inserted through the abdominal wall to serve as a port. A modified blunted 22ga needle with attached syringe is then inserted through the catheter and into the oviductal ostium (approximately 2 cm) and 5µl of semen (1x10^6 motile sperm) is infused into the oviductal lumen. One study found a pregnancy rate of 45% with a greater litter size when compared to laparoscopic intrauterine insemination (pregnancy rate of 18%) with the same insemination dose.6

Conclusion

In conclusion, breeding management of the subfertile queen may require veterinary intervention with one of the described artificial insemination techniques. Management of the male and female, ensuring adequate sperm for insemination and induction of ovulation is vital. However, in many cases, success can be found with persistence and practice.

References