Artificial Insemination in Queens in the Clinical Practice Setting: Protocols and challenges

Performing Artificial Insemination in Queens in a Practice Setting: Protocols and challenges

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Abstract

Practical relevance

Despite substantial advances in feline-assisted reproduction having been recently reported, use of these procedures in cats is limited and routine application of assisted reproductive techniques is still far from being a reality in veterinary clinics. Nevertheless, there is an increasing demand from domestic cat breeders for artificial insemination (AI) techniques that are already commonly used in dogs. For tomcats and queens of high breeding value, in which natural breeding is not possible for various reasons, AI could offer a solution.

Clinical challenges

AI in cats is more difficult than in other species – both in terms of semen collection/handling and oestrous cycle management in the queen given, for example, that ovulation must be induced.

Aim

For practitioners interested in cat reproduction and wishing to perform AI in queens, there are challenges to be overcome, and greater understanding of techniques and procedures is pivotal. This review aims to contribute to improved knowledge by providing an overview of AI protocols, encompassing choice of breeding animals, procedures for semen collection, oestrus and ovulation induction, AI techniques and equipment.

Equipment

Dependent on the animals involved in the AI procedure and the specific technique chosen, essential equipment may include an artificial vagina, electroejaculator, endoscope (sialendoscope) and special catheters for transcervical insemination. Other instrumentation and materials needed are usually readily available in a veterinary facility.

Evidence base

The information and any advice/reccomendations reported in this review are drawn from specific feline research and reviews published in scientific peer reviewed journals, animal reproduction books or in national and international Congresses by the Authors or other researchers. Author experience was also taken into consideration in the choice of protocols or procedures proposed in this review.

Keywords: Artificial insemination, techniques, oestrus management, sperm collection

SERIES OUTLINE

This article forms part of a series of evidence-based reviews on feline reproduction and reproductive problems, written by key opinion leaders. An outline of the series is available at: bit.ly/JFMSreproduction

Introduction

Although artificial insemination (AI) in the cat was first described in the 1970s[1] and, during the following five decades, substantial advances in feline-assisted reproduction have been reported, its use in cats is limited, particularly in comparison with dogs where this practice is very common. AI in cats is more difficult than in other species not only in terms of semen collection and handling, but also due to oestrous cycle management in the queen, given, for example, that ovulation must be induced.[2] Nevertheless, the demand for semen collection, evaluation and subsequent use by AI is growing as a way to preserve important or valuable genetic material,[3] as a means of disease control (eg, respiratory infections) and in order to bypass psychological or physical breeding problems. In

addition, the domestic cat is used as a model in the study of endangered wild felids and also some human diseases, such as obesity and diabetes.

Choice and evaluation of breeding animals

In the authors' opinion, AI should be considered only for those animals in which natural mating is not practicable; in particular, for problems associated with copulation (fearfulness, inexperience, distraction, mate preference), painful pathologies such as orthopaedic or buccal disorders, and genital disorders in the tom) and/or queen (eg, phimosis, persistent penile frenulum or acquired pathologies). Even if a tom has a history and/or evidence of infertility (eg, poor semen quality due to an acquired condition), natural mating should be encouraged where possible. Reproductive performance is enhanced by the multiple matings that are a feature of normal feline reproduction, and the natural induction of ovulation stimulated by coitus.

As for natural breeding, the choice of tom and queen for AI should be based on a prebreeding examination. Before the reproductive component of the examination, a complete history (including vaccination history, viral diseases) and a thorough general physical examination should be performed to assess for any non-reproductive abnormality. The recommended minimum database includes a complete blood count and serum chemistry, urinalysis, serology for feline leukaemia virus, feline immunodeficiency virus and feline infectious peritonitis virus, and blood typing to ensure compatible matings.[4] As discussed in accompanying reviews in this series on breeding and pregnancy management, neonatology and fading kitten syndrome (bit.ly/JFMSreproduction), a kitten with type A or AB blood born to a mother with type B blood is at risk of developing neonatal isoerythrolysis after maternal colostrum is ingested, with sequlae including pigmenturia, icterus, anaemia, tail tip necrosis and sudden death.[5]

Before moving on to examination of the reproductive system, pertinent aspects of the reproductive and management history, and physical examination findings should be evaluated and recorded.[5] Examination of the reproductive tract in the queen should include inspection of the vulva and transabdominal palpation of the uterus to exclude abnormal uterine enlargement. Ovarian morphology can be evaluated using two-dimensional ultrasound (see accompanying review on infertility in queens at bit.ly/JFMSreproduction), but this technique cannot provide information about organ function, such as vascular perfusion.[6]

A thorough reproductive examination in the tom should consist of manual palpation of the testicles, assessing for position, size, texture and symmetry, followed by ultrasound to assess any irregularities within the testicular parenchyma. Unfortunately, there is scant information in the literature concerning imaging of the normal feline testes;[7–9] although age-related histological changes in the testes have been reported, there is no description of diagnostic ultrasound.[10] The penis should be evaluated for any discoloration, discharge and the presence of spines. Exteriorisation of the penis may be difficult without sedation, so this portion of the examination might best be accomplished just before sperm collection when the tom is sedated or anaesthetised[3].

Oestrus management and ovulation induction

AI can be performed in queens in natural or induced oestrus, with induction of ovulation on the second or third day of oestrus (assessed on the basis of oestrus behaviour and vaginal cytology); this corresponds to ovulation induced by coitus, which is normally within 24–48 h.[11,12] Natural oestrus is preferable because treatment with some exogenous gonadotropins, a regimen typically used in AI, has been reported to produce an inappropriate maternal endocrine response.[13] Oestrous cyclicity can be induced in normal queens in anoestrus by artificially modifying the photoperiod with a supplemental lighting programme or by various therapeutic protocols described in the literature.[13-22]

Follicular development and ovulation are induced in queens by treatment with exogenous gonadotropins. Administration of equine chorionic gonadotropin (eCG) is widely used due to its follicle-stimulating hormone (FSH)-like action, which triggers ovarian follicular activity. Commonly used protocols consist of intramuscular (IM) administration of 100–150 IU of eCG, followed by 75–100 IU of human chorionic gonadotropin (hCG) IM 80–90 h later,[13,14-20] and produce a high number of follicles and related corpora lutea. However, eCG treatment has some disadvantages, such as the necessity to repeat the injections provided by some protocols (which can cause immune reactions) and induction of ovarian superstimulation or superovulation (Figure 1). Also, eCG has luteinising hormone (LH)-like activity, which can induce development of follicular cysts or premature luteinisation.[23]

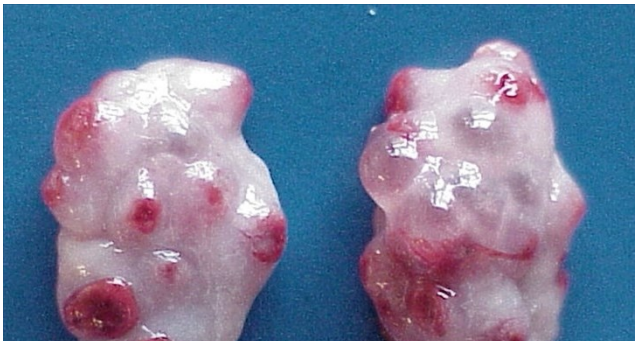

Figure. 1. Ovaries of a queen after an eCG/hCG stimulation protocol. The queen was spayed about 60 hours after hCG administration. Superstimulation is evidenced by the presence of several functional structures (follicles, corpora haemorrhagica) in both gonads. Thick and thin arrows indicate respectively some of follicles and corpora haemorragica on the ovaries.

Direct stimulation of pituitary activity with gonadotropin-releasing hormone (GnRH) has been investigated using different approaches.[24,25] Oestrus induction and pregnancy have been obtained with continuous administration or release of a GnRH analogue (lutrelin, deslorelin, leuprolide) via a subcutaneous osmotic mini-pump or implant.[25,26] Among the GnRH agonists, deslorelin is classified as a superagonist and it has a receptor affinity that is 200 times superior to that of endogenous GnRH.[27,28] It is available commercially as a slow-release subcutaneous implant (4.7 and 9.4 mg, Suprelorin; Virbac) and its administration initially induces an acute stimulatory phase that lasts for several days, characterised by a large increase in LH and FSH concentrations. Prolonged exposure to these molecules subsequently leads to downregulation of GnRH receptors on the gonadotrope cells and, in turn, reduced synthesis of LH and FSH.[29-31]

A positive response to an oestrus induction protocol using 4.7 deslorelin implants has been described in 13 queens by the current authors (Zambelli et al[32]). Oestrus was detected within an average of 5.0 ± 2.2 days after implant placement in all 13 queens, with a mean number of 4.8 ± 1.6 follicles per animal. Seven of 13 queens exhibited behavioural manifestations of oestrus; other studies, however, between queens in which estrus was successfully induced, have reported oestrus behaviour in only 2/14 and 1/10 subjects .[33,34] The stimulation induced by deslorelin is considered comparable with a physiological oestrus,[23] with follicle numbers within the normal range[24,35] and without the induction of cysts or other pathology (Figure 2).[32]

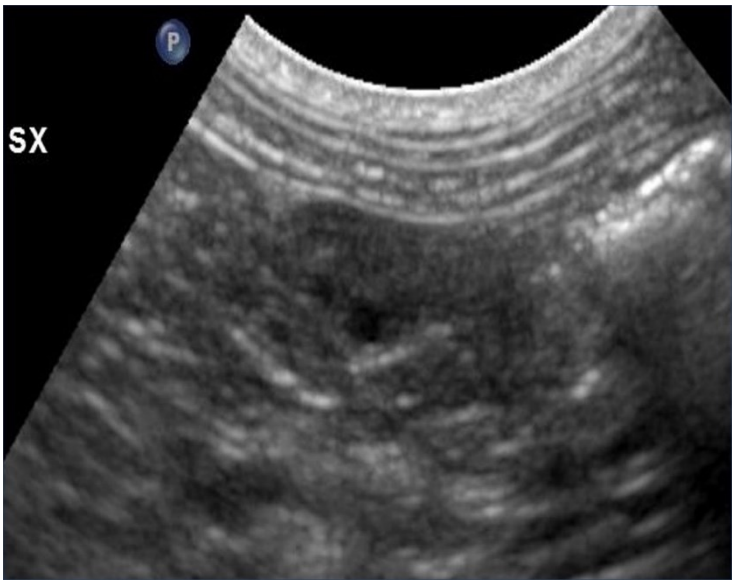

Figure. 2. Ultrasonographic assessment of the ovary (red arrows) of a queen after deslorelin stimulation. The number of follicles present is within the normal range. The white arrow indicate a follicle.

Three of the 13 queens reported in the aforementioned study by Zambelli and colleagues[32] were treated by AI following removal of the subcutaneous deslorelin implant. All became pregnant and gave birth to healthy litters.

In queens involved in AI programmes, ovulation is generally induced by administration of 100–250 IU hCG IM on days 2 and 3 of oestrus.[36-38] A study by Chatdarong et al[39] supports this protocol. Based on evaluation of parameters such as cervical patency, oestrus behaviour, vaginal cornification and estradiol-17β serum concentration, these authors recommended performing intravaginal AI on the second day of oestrus, even if, in queens with only a short period of cervical patency in late oestrus, a second insemination 2 days later is advisable. Ovulation in queens implanted with deslorelin has been obtained with a single dose of hCG, 100 IU IM, once the peak of oestrus was identified. It has been suggested that ovulation can be indirectly confirmed by serum progesterone assay, 5–6 days after hCG administration.[32]

Ovulation has also successfully been obtained in queens by administration of 1000 IU of porcine luteinising hormone (pLH) 85 h after eCG treatment to induce oestrus.[40] Vaginal stimulation (with a glass rod or sterile cotton swab) of queens in oestrus is additionally described as a method of inducing ovulation.[41]

The protocol for oestrus and ovulation induction currently used by the authors, based on the study by Zambelli et al,[32] is described in Box 1. For any AI programme, it is recommended that queens should preferably be in anoestrus or postoestrus (interoestrus) and showing pre-treatment basal levels of serum progesterone (≤2 ng/ml). This permits the best possible stimulatory effects to be achieved by the planned AI.

Box 1

Authors' protocol for oestrus/ovulation induction and AI

1. Perform vaginal cytology and serum progesterone assay to confirm anoestrus or postoestrus (interoestrus)
2. Administer deslorelin implant (4.7 mg)
3. At peak of oestrus administer 100 IU of hCG IM
4. First AI: 24 h after hCG and implant removal
5. Second AI: 48 h after hCG administration
6. 5–6 days after hCG administration: progesterone assay for confirmation of ovulation

Semen collection for AI

The priority for semen collection is to obtain good quality ejaculates with minimal stress for the animal. Sperm may be collected in clinical practice using different methods, with use of an artificial vagina (AV) and electroejaculation (EE) having been the first techniques described, in studies in the 1970s.[1,42-50] More recently, urethral catheterisation after pharmacological induction (UrCaPI) has been reported as a simple method of semen collection,[51-60] and is now one of most widely used semen collection techniques, both in domestic cats and wild felids.[61] While researchers frequently obtain sperm for experimental studies by squeezing or slicing epididymal tissue after routine orchiectomy, [62-64] these techniques are not used in clinical practice for AI procedures.

<u>Artificial vagina</u>

This technique is used in catteries and research colonies, where the animals are properly trained and semen is collected frequently for AI, sperm preservation or other purposes. By contrast, for a single collection in an untrained cat in the clinical practice setting, this is usually a fruitless method. The principal advantages of using an AV are the low cost of the equipment, and no need for restraint for the cat; in addition, a single technician can perform the collection. Training to the use of an AV is possible in many cats, and can generally be completed after 2 weeks of gentle handling during exposure to a queen in oestrus.[47] In preparation for the collection, the male is permitted to approach to the female as he would naturally, to encourage maximum sexual arousal. At the point when he mounts the queen, the operator's gloved hand slips the AV over the penis and, after few coital thrusts, the ejaculate is collected. If the cat is well trained, it may be possible to collect the semen in the absence of a teaser queen. It is generally possible to repeat the procedure after an interval of about 10 mins. A rate of three collections per week does not impact sperm volume and concentration, but daily collection has been shown to cause a rapid drop in these parameters from day 4. [47]

Electroejaculation

This technique may potentially be used in any male that can be safely anaesthetised[65] and offers advantages over use of an AV in the context of a previously untrained or aggressive male or in the absence of a teaser queen. As such, this was previously considered the method of choice for elective sperm collection in cats. The main disadvantages are the necessity to anaesthetise the tom, and the cost of the instrumentation. Anyway, EE safety has been reported in a study showing no significant histological and endoscopic lesions in the rectum induced by the procedure.[66]

Various anaesthetic protocols and drugs have been proposed for cats undergoing EE, with ketamine being the most commonly used agent, either administered alone (20 mg/kg IM) or in combination with medetomidine (5 mg/kg IM and 80 µg/kg IM, respectively). The effect of medetomidine on the quality of electroejaculated sperm has been reported in a comparative study with ketamine.[67] Medetomidine permits collection of an ejaculate characterised by a higher number of sperm than after ketamine administration, and provides good pharmacological restraint and adequate analgesia. Moreover, while the passage of some sperm into the urinary bladder is normal during ejaculation in the cat, medetomidine does not increase the percentage of sperm flowing retrogradely into the bladder.[50] An anaesthetic protocol that includes 30–40 µg/kg IM of

dexmedetomidine combined with 3–5mg/kg ketamine IM has been reported for successful semen collection by EE,[3] but further data about the use of this drug for EE in cats (eg, successful rate, quality of semen collected, ecc.) have not been published.

In cats undergoing EE, the stimulus voltage and number of electrical stimuli appear to influence the number of sperm collected, but not the volume of the ejaculates.[46,68]

For sperm collection by EE, an electroejaculator connected to a rectal probe (1 cm diameter, 12 cm long) is required, equipped with two or three longitudinal stainless-steel electrodes. After inducing anaesthesia, it is first necessary to ensure the rectum is empty of faeces (which would decrease conductivity of the probe) and to clean the glans penis using saline-moistened gauze. The lubricated probe, with electrodes oriented ventrally, is gently introduced into the rectum to a depth of 7–8 cm and pushed ventrally to ensure good contact with the rectal mucosa and the pelvic plexus, which is located dorsally to the membranous urethra, between the prostate and bulbourethral gland. The prepuce is retracted to expose the the glans and a sterile Eppendorf tube is gently positioned onto the penis before starting the procedure (Figure 3).

Different electrical protocols have been reported in the literature and the one proposed by Howard et al in 1990[47] is the most commonly used in practice (Box 2). It is recommended that Eppendorf tubes are changed after each set of stimuli, because the higher voltages can induce urine emission, leading to contamination of samples. The male responds to electrical stimulation with a rigid and symmetrical extension of both hindlimbs. Assessment of this extension is very important. Absent, weak or asymmetrical movement suggests poor contact between the electrodes and rectal mucosa due to incorrect positioning of the rectal probe or the presence of faecal material.

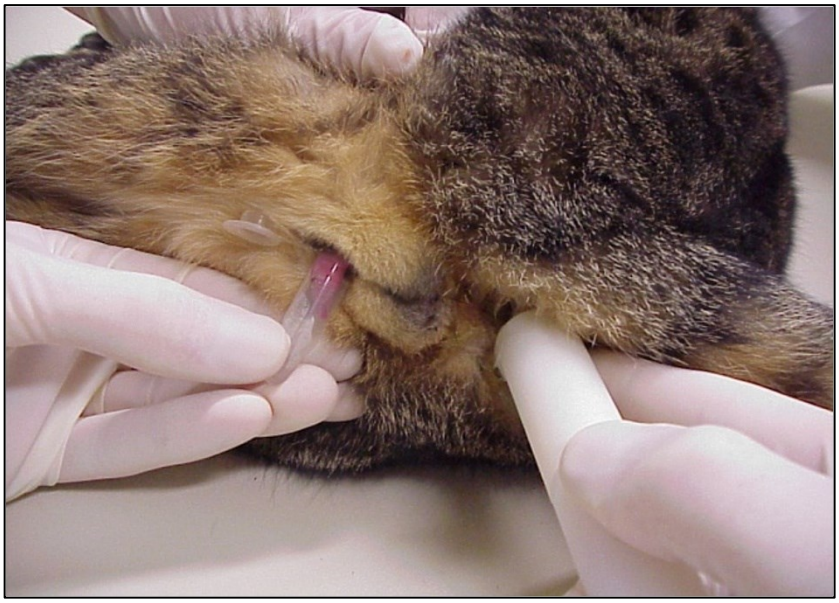

Figure 3. Semen collection by electroejaculation A *lubricated probe has been inserted 7-8 cm into the rectum and a sterile Eppendorf vial has been gently positioned onto the penis.*

Box 2

Electrical protocol for electroejaculation*

A TOTAL OF 80 STIMULI DIVIDED INTO THREE SETS

1st Set: 10 stimuli at 2V
10 stimuli at 3V
10 stimuli at 4V

2–3 mins of rest

2nd Set: 10 stimuli at 3V
10 stimuli at 4V
10 stimuli at 5V

2–3 mins of rest

3rd Set 10 stimuli at 4V
10 stimuli at 5V

*From Howard et al (1990)[47]

Semen collection by Urethral Catheterization after Pharmacological Induction (UrCaPI)

The sperm collection technique of UrCaPI, described in the past couple of decades,[51] overcomes some of the most important disadvantages encountered with the AV and EE techniques. UrCaPI does not require any specific equipment (artificial vagina or electroejaculator) or a trained cat (necessary for AV collection) and can be performed in any country without restrictions. Although semen collected with UrCaPI show macroscopic and microscopic differences from other semen collection technique (see below), it can be cryopreserved[57] and used for in vitro fertilisation[57] or

AI,[46] similar to sperm collected with an AV or EE. This technique represents an important tool in clinical practice, offering a cost-effective and simple means of collecting an ejaculate. A peculiarity of this technique is that it produces small-volume ejaculate samples with a very high concentration of spermatozoa.[40] This can be considered an advantage for artificial fertilisation and in vitro procedures, but it requires very careful sample handling to avoid damage to the spermatozoa. Dependent on the volume and concentration of sperm collected, the sample may be diluted with a small amount of tris-glucose-citrate (TGC) or Tyrode's albumin lactate pyruvate (TALP) medium,[57,69] which will also enable sample evaluation without utilising an excessive proportion of the ejaculate.

The reported dose of medetomidine for this technique is 130 µg/kg IM. This stimulates contraction of the vas deferens, with consequent release of sperm cells into the urethra, without inducing ejaculation.[51] Despite many anaesthetists being reluctant to use this dose, it has been proven to be safe and well tolerated.[52] As always before anaesthesia, a cardiological examination is recommended to exclude individuals with cardiovascular disease. Results of sperm collection after administration of high doses of medetomidine (120 µg/kg) or dexmedetomidine (60 µg/kg) and low doses of the two drugs (50 µg/kg and 25 µg/kg, respectively) have been compared;[56] it was observed that high doses of both drugs allow the collection of good quality sperm, while low doses were in general unsatisfactory in terms of both sperm collection and sedation level. In addition to these protocols, other doses of medetomidine (such as 80 or 100 µg/kg) and dexmedetomidine (such as 5 or 25 µg/kg associated to other anesthetic agent to reach an adequate level of sedation) are reported in the literature for successful semen collection by UrCaPI.[55,59,60]

Once adequate sedation has been achieved, an open-ended cat urinary catheter is introduced gently and slowly into the urethra to a total depth of 9 cm, while the prepuce is pulled caudally to distend the urethra; note that by not exceeding this depth, the tip of the catheter is prevented from reaching the bladder and collecting urine. Sperm collects inside the catheter by capillary action (Figure 4) and is transferred to an Eppendorf tube using an insulin syringe.

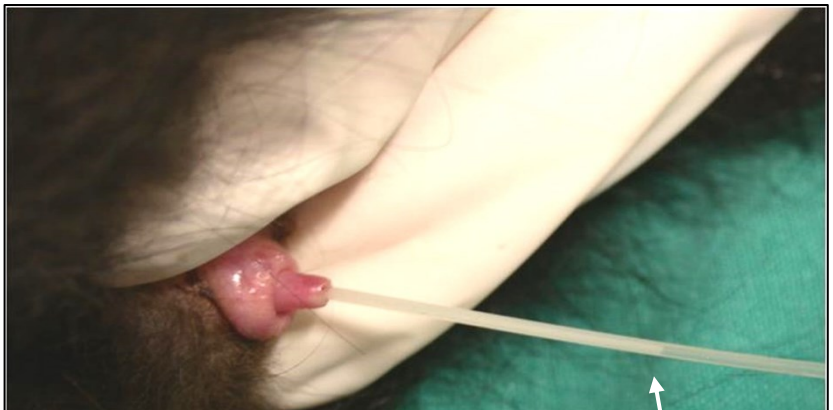

Figure 4. Semen collection by urethral catheterisation after pharmacological induction. Semen enters the catheter (arrow) by capillary action. In this image the catheter has been pulled caudally to show semen collected.

Even though a visually normal sample and if good quality sperm does not confirm that an individual is fertile,[70] macroscopic sperm evaluation is nonetheless fundamental to define its quality parameters and suitability for use for AI. The characteristics of normal semen differ dependent on the collection method used. As would be expected, the ejaculate collected with an AV is very similar to that deposited in the vagina during a natural mating. By contrast, EE, because of greater stimulation of the accessory glands, induces the ejaculation of a higher volume of sperm than is collected with an AV. In comparison, UrCaPI produces a smaller volume/higher concentration sample than the semen ejaculated with EE (Figure 5).

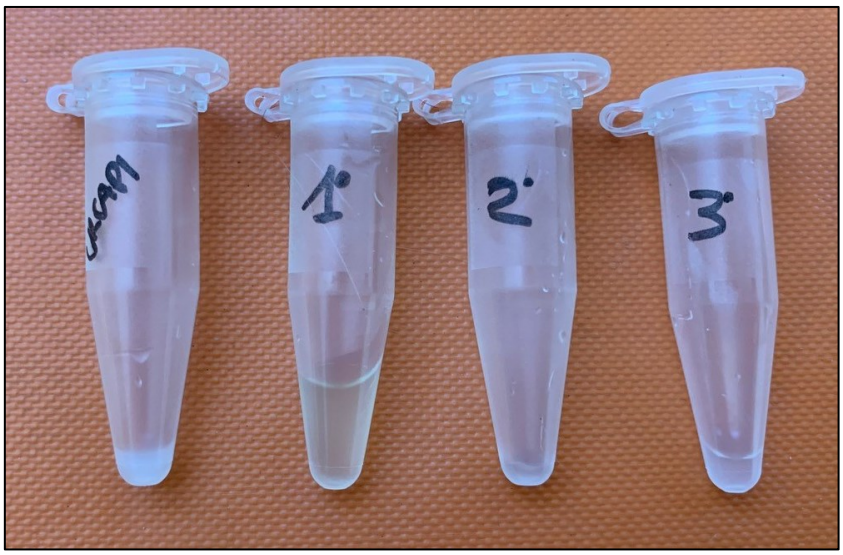

Figure 5. Semen collected (from left) by urethral catheterisation after pharmacological induction (UrCaPI) and electroejaculation after the first, second and third set of electrical stimuli

Sperm evaluation and insemination dose

A basic spermiogram should include evaluation of macroscopic parameters, such as appearance and volume, and microscopic parameters such as motility, morphology and viability. In specific circumstances, it may also be necessary to evaluate pH, osmolarity, sperm culture, membrane integrity and semen chemistry. In practice, however, particularly when feline semen is collected for AI using UrCaPI, the clinician often has insufficient sample volume for a complete examination. This represents an important limitation, as does the difficulty of maintaining constant environmental conditions (temperature, presence of oxygen, etc).

The recommended insemination dose (volume and total number of spermatozoa) varies dependent on semen type used (fresh or frozen/thawed) and site of sperm deposition during AI . Using fresh sperm, intravaginal insemination doses with $20x10^6$, $40x10^6$ and $80x10^6$ motile spermatozoa in a volume of 50–100 µl semen have been reported to produce conception rates of 6.6, 33.3 and 77.8%, respectively, in cats.[71] The higher insemination dose was comparable with the number of spermatozoa collected in two consecutive ejaculations;[72] and it seems reasonable to assume that cats copulate several times during oestrus probably as a means of maximising sperm dose.[71] A conception rate of 80% has been reported following surgical insemination of $8x10^6$ fresh sperm in a volume of 30 µl into one uterine horn.[73] The requirement for 10 times the number of sperm to achieve high fertilisation rates with intravaginal ($80x10^6$) versus intrauterine ($8x10^6$) insemination in the cat is also reported for the dog.[74,75]

Conception after artificial intrauterine insemination (see below) using $2.4–19.2x10^6$ fresh sperm has also been reported.[76] In particular, for transcervical uterine insemination, a dose of 200 µl of fresh or frozen/thawed semen containing $10x10^6$ or $30x10^6$ motile sperm, respectively, was indicated and a conception rate of 100% was obtained in queens where transcervical catheterisation was possible.[77] In a separate study, sequential uterine endoscopic transcervical insemination was reported in three queens using 8.1-53.3 $x10^6$ of fresh motile spermatozoa in 18–32µl collected by UrCaPI; each queen was inseminated two times and the conception rate was 100%.[32]

Several authors reported different conception rates using various doses of fresh or frozen sperm after intravaginal or intrauterine deposition (Box 3).[44,73,76,78].

BOX 3

Minimum sperm dose in relation to semen type and site of insemination

Semen	Site of insemination	Number of spermatozoa (10^6)	Conception rate (%)
Fresh	Intravaginal	80	77.8[71]
Fresh	Intrauterine	8	80[73]
Frozen/thawed	Intrauterine	50	57.1[78]

Artificial insemination techniques

Fertilisation in the cat is possible using both artificial intravaginal insemination (AIVI) and artificial intrauterine insemination (AIUI) techniques. As reported above, and based on investigations performed under similar experimental conditions, approximately 10 times more fresh sperm are necessary with AIVI than with AIUI to obtain an 80% conception rate in the cat.[71,73] Additionally, Tsutsui et al[78] demonstrated that approximately five times more frozen sperm were needed compared with fresh sperm to achieve a 57.1% conception rate with AIUI. Early reports detailed surgical procedures for intrauterine insemination, but techniques for transcervical insemination have subsequently been described, avoiding surgical risks and postsurgical complications. Laparoscopic oviductal AI has also been reported using low sperm numbers (2 million sperm per insemination dose), and resulting in high fertilisation and pregnancy rates;[79] to date, however, this remains an experimental procedure.

<u>Artificial intravaginal insemination</u>

The first pregnancies after AIVI, using fresh or frozen semen, were reported in the 1970s by Sojka et al[1] and Platz et al[44]. The procedures described are simple and the equipment needed is generally readily available. Sojka and colleagues[1] used a 20 gauge, 9 cm long needle with a bulb, connected to a 0.25 ml syringe. Much more recently, a 1.5 mm diameter, 9 cm long nylon probe connected to a 1.0 ml syringe was used for insemination by Tanaka et al.[71] During vaginal insemination, sperm is deposited in the anterior vagina or posterior cervix. It is usually recommended that the queen is under general anaesthesia or heavy sedation for the procedure.[1,44] She is then generally maintained

in dorsal recumbency with the hindquarters elevated for 15–20 mins post-insemination to minimise sperm backflow.[44,71]

Artificial intrauterine insemination

In 1992, conception was reported after surgical uterine deposition of sperm using laparoscopy.[76] This AIUI procedure was performed for research purposes and has not been applied in clinical practice. AIUI after laparotomy has also been described, involving infusion of semen into the uterine horn with the greatest number of ovarian follicles or ovulations.[74,78]

Various techniques for uterine insemination by cervical catheterisation have been proposed, and different catheters have been developed. Catheterisation has been performed blindly using a 2 mm glass speculum,[80] and also with the aid of a modified polypropylene urinary catheter (2.7– 2.8 mm) used as speculum,[81,82] to facilitate the insertion of a 3 French gauge (Fr; 1 mm) tomcat catheter through the cervix; these methods are unsuitable for animals with a narrower cranial vagina lumen (<2 mm).[83,84] In 2001, another procedure for uterine insemination by transcervical catheterisation was proposed by one of the present authors (Zambelli).[83] This method allowed the uterine lumen to be reached using a 3 Fr catheter, modified with the addition of a rounded tip needle (0.65 mm) at the cut end, inserted into the vagina and through the cervix guided by transrectal palpation.[83,84] Success rates for cervical catheterisation using this method depend on training and experience of the practitioner.[84]

Endoscopic transcervical catheterisation has more recently been described for the first time in cats (Zambelli and colleagues) for different purposes, including AI.[32] For this procedure, the queens were positioned in sternal recumbency with the pelvis slightly elevated using a cushion (Figure 6). A human semi-rigid sialendoscope (Karl Storz, Germany; 120 mm length, 1.1 mm diameter, 0° direction of view, one operative channel) was inserted through the vestibule and moved forward in the vagina until the dorsal fold and cervix were visualised. Transcervical catheterisation was performed using a modified version of the catheter designed by Zambelli et al (2004)[84]: a 3 Fr, 11 cm long tomcat urinary catheter with a 100 mm stainless steel rounded tip needle (0.65 mm diameter) inserted at its cut end (Figures 7 and 8). The steel needle connected to the catheter was inserted through the vaginal lumen alongside the endoscope and moved through the cervical ostium, under direct endoscopic observation, and the endoscope was then removed. Endoscopic

transcervical catheterisation was successful in 12 of 14 animals (success rate 85.71%).[32] In one queen, the cranial vagina was reported to be too narrow for insertion of the endoscope together with the catheter, while in another queen, cannulation did not succeed because of excessive inclination of the cervical axis and the narrowness of the genital tract.

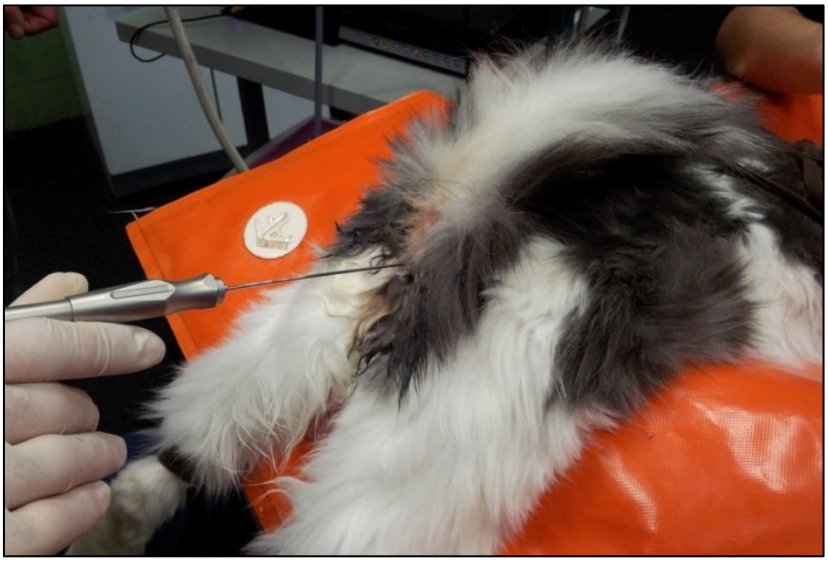

Figure 6. Queen positioned for endoscopic transcervical catheterisation. The endoscope is introduced at the level of the vulvar dorsal commissure, then, under direct endoscopic observation, the cervix is reached.

Figure 7. Sialendoscope and specially designed catheter used for endoscopic transcervical insemination

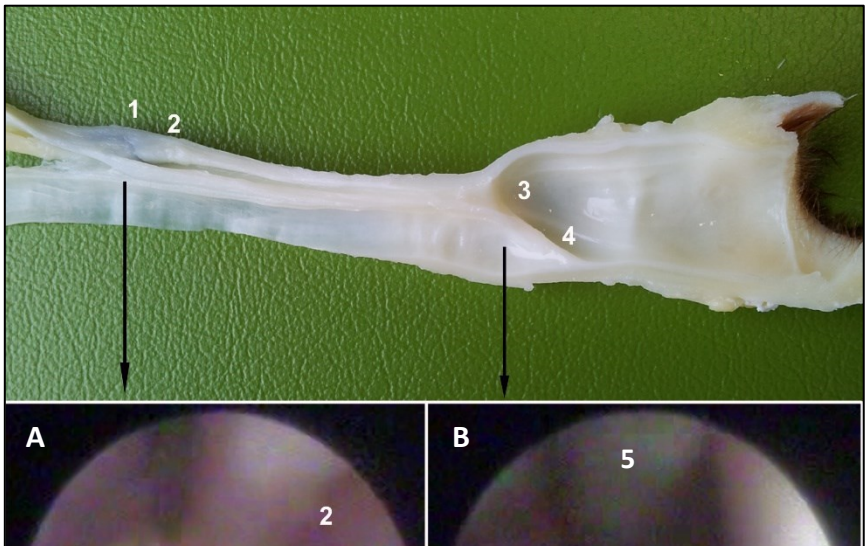

Figure 8. Endoscopic view of the cranial vagina (A) and vestibule (B) of a queen. The labels correspond to the anatomical landmarks shown in the gross specimen pictured above. 1 = cervix; 2 =vaginal dorsal medial fold; 3 = cranial vagina opening; 4 = urinary meatus; 5 =catheter used for endoscopic transcervical insemination

Endoscopic transcervical catheterisation represents a valid technique as it allows cervical cannulation in most queens, and can be quite easily performed by the endoscopy practitioner. It is a suitable method for AIUI, reducing the risks related to surgical uterine insemination.[77] Artificial intravaginal insemination and endoscopic transcervical catheterisation are to date the techniques mainly used when good quality and poor quality semen, respectively, has been collected.

Pull quotes:

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555 The priority for semen collection is to obtain good quality ejaculates with minimal stress for the
556 animal.
557 For a single semen collection in an untrained cat in the clinical practice setting, use of an artificial
558 vagina is usually a fruitless method.
559 When feline semen is collected for AI, generally and in particular using UrCaPI, the clinician often
560 has insufficient sample volume for a complete examination. This represents an important limitation.
561 The characteristics of normal semen differ dependent on the collection method used.
562 Approximately 10 times more fresh sperm are necessary with AIVI than with AIUI to obtain 80%
563 conception rates in the cat.
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