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

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(Article begins on next page)

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

3

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11

12 **Abstract**

13 Practical relevance

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

20

21 Clinical challenges

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

24

25 Aim

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

31

32 Equipment

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

37

38 Evidence base

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

44

45

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

47

48 SERIES OUTLINE

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

52

53

54

55

56 **Introduction**

57 Although artificial insemination (AI) in the cat was first described in the 1970s¹ and, during the
58 following five decades, substantial advances in feline-assisted reproduction have been reported, its
59 use in cats is limited, particularly in comparison with dogs where this practice is very common. AI in
60 cats is more difficult than in other species not only in terms of semen collection and handling, but
61 also due to oestrous cycle management in the queen, given, for example, that ovulation must be
62 induced.² Nevertheless, the demand for semen collection, evaluation and subsequent use by AI is
63 growing as a way to preserve important or valuable genetic material,³ as a means of disease control
64 (eg, respiratory infections) and in order to bypass psychological or physical breeding problems. In

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

67

68 **Choice and evaluation of breeding animals**

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

77

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

89

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

97

98 A thorough reproductive examination in the tom should consist of manual palpation of the testicles,
99 assessing for position, size, texture and symmetry, followed by ultrasound to assess any
100 irregularities within the testicular parenchyma. Unfortunately, there is scant information in the
101 literature concerning imaging of the normal feline testes;⁷⁻⁹ although age-related histological
102 changes in the testes have been reported, there is no description of diagnostic ultrasound.¹⁰ The
103 penis should be evaluated for any discoloration, discharge and the presence of spines.
104 Exteriorisation of the penis may be difficult without sedation, so this portion of the examination
105 might best be accomplished just before sperm collection when the tom is sedated or anaesthetised³.

106

107 **Oestrus management and ovulation induction**

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

116

117 Follicular development and ovulation are induced in queens by treatment with exogenous
118 gonadotropins. Administration of equine chorionic gonadotropin (eCG) is widely used due to its
119 follicle-stimulating hormone (FSH)-like action, which triggers ovarian follicular activity. Commonly
120 used protocols consist of intramuscular (IM) administration of 100–150 IU of eCG, followed by 75–
121 100 IU of human chorionic gonadotropin (hCG) IM 80–90 h later,^{13,14-20} and produce a high number
122 of follicles and related corpora lutea. However, eCG treatment has some disadvantages, such as the
123 necessity to repeat the injections provided by some protocols (which can cause immune reactions)
124 and induction of ovarian superstimulation or superovulation (Figure 1). Also, eCG has luteinising
125 hormone (LH)-like activity, which can induce development of follicular cysts or premature
126 luteinisation.²³

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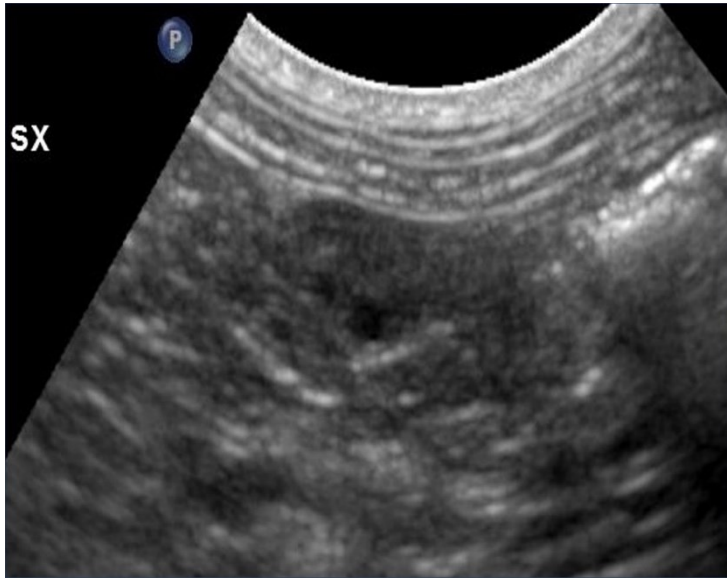


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Figure. 1. Ovaries of a queen after an eCG/hCG stimulation protocol. The queen was spayed about 60 hours after hCG administration. Superstimulation is evidenced by the presence of several functional structures (follicles, corpora haemorrhagica) in both gonads. Thick and thin arrows indicate respectively some of follicles and corpora haemorrhagica 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 al³²). 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



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

175

176 Three of the 13 queens reported in the aforementioned study by Zambelli and colleagues³² were
177 treated by AI following removal of the subcutaneous deslorelin implant. All became pregnant and
178 gave birth to healthy litters.

179

180 In queens involved in AI programmes, ovulation is generally induced by administration of 100–250
181 IU hCG IM on days 2 and 3 of oestrus.^{36–38} A study by Chatdarong et al³⁹ supports this protocol. Based
182 on evaluation of parameters such as cervical patency, oestrus behaviour, vaginal cornification and
183 estradiol-17 β serum concentration, these authors recommended performing intravaginal AI on the
184 second day of oestrus, even if, in queens with only a short period of cervical patency in late oestrus,
185 a second insemination 2 days later is advisable. Ovulation in queens implanted with deslorelin has
186 been obtained with a single dose of hCG, 100 IU IM, once the peak of oestrus was identified. It has
187 been suggested that ovulation can be indirectly confirmed by serum progesterone assay, 5–6 days
188 after hCG administration.³²

189

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

194

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

200

201 Box 1

Authors' protocol for oestrus/ovulation induction and AI

1. Perform vaginal cytology and serum progesterone assay to confirm anoestrus or post-oestrus (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

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Semen collection for AI

223 The priority for semen collection is to obtain good quality ejaculates with minimal stress for the
224 animal. Sperm may be collected in clinical practice using different methods, with use of an artificial
225 vagina (AV) and electroejaculation (EE) having been the first techniques described, in studies in the
226 1970s.^{1,42-50} More recently, urethral catheterisation after pharmacological induction (UrCaPI) has
227 been reported as a simple method of semen collection,⁵¹⁻⁶⁰ and is now one of most widely used
228 semen collection techniques, both in domestic cats and wild felids.⁶¹ While researchers frequently
229 obtain sperm for experimental studies by squeezing or slicing epididymal tissue after routine
230 orchietomy,⁶²⁻⁶⁴ these techniques are not used in clinical practice for AI procedures.

231

232 Artificial vagina

233 This technique is used in catteries and research colonies, where the animals are properly trained
234 and semen is collected frequently for AI, sperm preservation or other purposes. By contrast, for a
235 single collection in an untrained cat in the clinical practice setting, this is usually a fruitless method.
236 The principal advantages of using an AV are the low cost of the equipment, and no need for restraint
237 for the cat; in addition, a single technician can perform the collection. Training to the use of an AV
238 is possible in many cats, and can generally be completed after 2 weeks of gentle handling during
239 exposure to a queen in oestrus.⁴⁷ In preparation for the collection, the male is permitted to
240 approach to the female as he would naturally, to encourage maximum sexual arousal. At the point
241 when he mounts the queen, the operator's gloved hand slips the AV over the penis and, after few
242 coital thrusts, the ejaculate is collected. If the cat is well trained, it may be possible to collect the
243 semen in the absence of a teaser queen. It is generally possible to repeat the procedure after an
244 interval of about 10 mins. A rate of three collections per week does not impact sperm volume and
245 concentration, but daily collection has been shown to cause a rapid drop in these parameters from
246 day 4.⁴⁷

247

248 Electroejaculation

249 This technique may potentially be used in any male that can be safely anaesthetised⁶⁵ and offers
250 advantages over use of an AV in the context of a previously untrained or aggressive male or in the
251 absence of a teaser queen. As such, this was previously considered the method of choice for elective
252 sperm collection in cats. The main disadvantages are the necessity to anaesthetise the tom, and the
253 cost of the instrumentation. Anyway, EE safety has been reported in a study showing no significant
254 histological and endoscopic lesions in the rectum induced by the procedure.⁶⁶

255

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

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

268

269 In cats undergoing EE, the stimulus voltage and number of electrical stimuli appear to influence the
270 number of sperm collected, but not the volume of the ejaculates.^{46,68}

271

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

281

282 Different electrical protocols have been reported in the literature and the one proposed by Howard
283 et al in 1990⁴⁷ is the most commonly used in practice (Box 2). It is recommended that Eppendorf
284 tubes are changed after each set of stimuli, because the higher voltages can induce urine emission,
285 leading to contamination of samples. The male responds to electrical stimulation with a rigid and
286 symmetrical extension of both hindlimbs. Assessment of this extension is very important. Absent,
287 weak or asymmetrical movement suggests poor contact between the electrodes and rectal mucosa
288 due to incorrect positioning of the rectal probe or the presence of faecal material.

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

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305 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

324

325 Semen collection by Urethral Catheterization after Pharmacological Induction (UrCaPI)

326

327 The sperm collection technique of UrCaPI, described in the past couple of decades,⁵¹ overcomes
328 some of the most important disadvantages encountered with the AV and EE techniques. UrCaPI
329 does not require any specific equipment (artificial vagina or electroejaculator) or a trained cat
330 (necessary for AV collection) and can be performed in any country without restrictions. Although
331 semen collected with UrCaPI show macroscopic and microscopic differences from other semen
332 collection technique (see below), it can be cryopreserved⁵⁷ and used for in vitro fertilisation⁵⁷ or

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

341

342 The reported dose of medetomidine for this technique is 130 µg/kg IM. This stimulates contraction
343 of the vas deferens, with consequent release of sperm cells into the urethra, without inducing
344 ejaculation.⁵¹ Despite many anaesthetists being reluctant to use this dose, it has been proven to be
345 safe and well tolerated.⁵² As always before anaesthesia, a cardiological examination is
346 recommended to exclude individuals with cardiovascular disease. Results of sperm collection after
347 administration of high doses of medetomidine (120 µg/kg) or dexmedetomidine (60 µg/kg) and low
348 doses of the two drugs (50 µg/kg and 25 µg/kg, respectively) have been compared;⁵⁶ it was observed
349 that high doses of both drugs allow the collection of good quality sperm, while low doses were in
350 general unsatisfactory in terms of both sperm collection and sedation level. In addition to these
351 protocols, other doses of medetomidine (such as 80 or 100 µg/kg) and dexmedetomidine (such as
352 5 or 25 µg/kg associated to other anesthetic agent to reach an adequate level of sedation) are
353 reported in the literature for successful semen collection by UrCaPI.^{55,59,60}

354

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

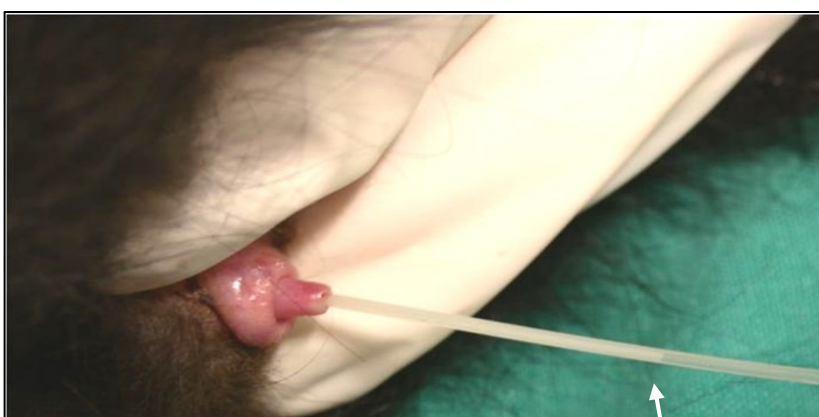
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372 *Figure 4. Semen collection by urethral catheterisation after pharmacological induction. Semen enters the*
373 *catheter (arrow) by capillary action. In this image the catheter has been pulled caudally to show semen*
374 *collected.*

375
376

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

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

399 Sperm evaluation and insemination dose

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

407

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

419

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

427

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

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BOX 3

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

Semen	Site of insemination	of Number of spermatozoa (10⁶)	of Conception rate (%)
Fresh	Intravaginal	80	77.8 ⁷¹
Fresh	Intrauterine	8	80 ⁷³
Frozen/thawed	Intrauterine	50	57.1 ⁷⁸

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437

Artificial insemination techniques

438 Fertilisation in the cat is possible using both artificial intravaginal insemination (AIVI) and artificial
439 intrauterine insemination (AIUI) techniques. As reported above, and based on investigations
440 performed under similar experimental conditions, approximately 10 times more fresh sperm are
441 necessary with AIVI than with AIUI to obtain an 80% conception rate in the cat.^{71,73} Additionally,
442 Tsutsui et al⁷⁸ demonstrated that approximately five times more frozen sperm were needed
443 compared with fresh sperm to achieve a 57.1% conception rate with AIUI. Early reports detailed
444 surgical procedures for intrauterine insemination, but techniques for transcervical insemination
445 have subsequently been described, avoiding surgical risks and postsurgical complications.
446 Laparoscopic oviductal AI has also been reported using low sperm numbers (2 million sperm per
447 insemination dose), and resulting in high fertilisation and pregnancy rates;⁷⁹ to date, however, this
448 remains an experimental procedure.

449

Artificial intravaginal insemination

451 The first pregnancies after AIVI, using fresh or frozen semen, were reported in the 1970s by Sojka
452 et al¹ and Platz et al⁴⁴. The procedures described are simple and the equipment needed is generally
453 readily available. Sojka and colleagues¹ used a 20 gauge, 9 cm long needle with a bulb, connected
454 to a 0.25 ml syringe. Much more recently, a 1.5 mm diameter, 9 cm long nylon probe connected to
455 a 1.0 ml syringe was used for insemination by Tanaka et al.⁷¹ During vaginal insemination, sperm is
456 deposited in the anterior vagina or posterior cervix. It is usually recommended that the queen is
457 under general anaesthesia or heavy sedation for the procedure.^{1,44} She is then generally maintained

458 in dorsal recumbency with the hindquarters elevated for 15–20 mins post-insemination to minimise
459 sperm backflow.^{44,71}

460

461 Artificial intrauterine insemination

462 In 1992, conception was reported after surgical uterine deposition of sperm using laparoscopy.⁷⁶

463 This AIUI procedure was performed for research purposes and has not been applied in clinical
464 practice. AIUI after laparotomy has also been described, involving infusion of semen into the uterine
465 horn with the greatest number of ovarian follicles or ovulations.^{74,78}

466

467 Various techniques for uterine insemination by cervical catheterisation have been proposed, and
468 different catheters have been developed. Catheterisation has been performed blindly using a 2 mm
469 glass speculum,⁸⁰ and also with the aid of a modified polypropylene urinary catheter (2.7– 2.8 mm)
470 used as speculum,^{81,82} to facilitate the insertion of a 3 French gauge (Fr; 1 mm) tomcat catheter
471 through the cervix; these methods are unsuitable for animals with a narrower cranial vagina lumen
472 (<2 mm).^{83,84} In 2001, another procedure for uterine insemination by transcervical catheterisation
473 was proposed by one of the present authors (Zambelli).⁸³ This method allowed the uterine lumen
474 to be reached using a 3 Fr catheter, modified with the addition of a rounded tip needle (0.65 mm)
475 at the cut end, inserted into the vagina and through the cervix guided by transrectal palpation.^{83,84}
476 Success rates for cervical catheterisation using this method depend on training and experience of
477 the practitioner.⁸⁴

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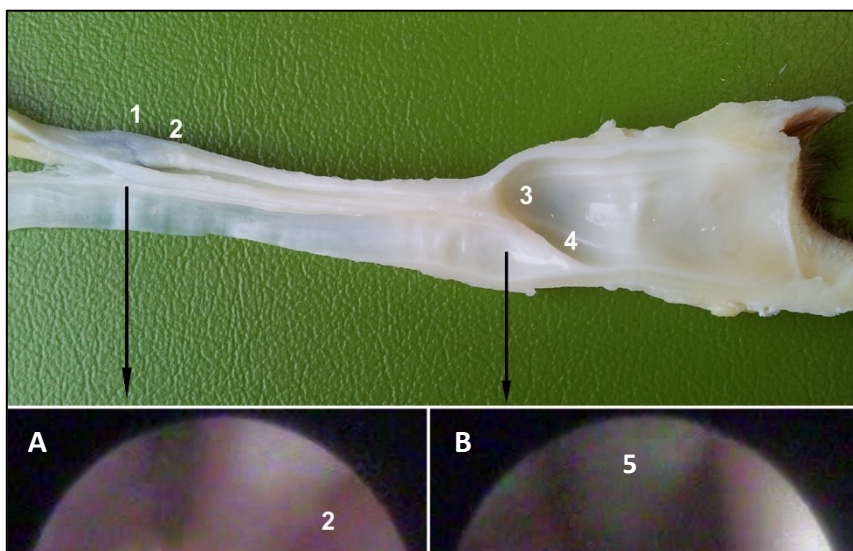
479 Endoscopic transcervical catheterisation has more recently been described for the first time in cats
480 (Zambelli and colleagues) for different purposes, including AI.³² For this procedure, the queens were
481 positioned in sternal recumbency with the pelvis slightly elevated using a cushion (Figure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

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



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

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



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

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

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548 Pull quotes:

549 AI should be considered only for those animals in which natural mating is not practicable.

550 Deslorelin is classified as a superagonist and has a receptor affinity that is 200 times superior to
551 that of endogenous GnRH.

552 For any AI programme, it is recommended that queens should preferably be in anoestrus or
553 postoestrus (interoestrus) and showing pre-treatment basal levels (≤ 2 ng/ml) of serum
554 progesterone.

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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