

Advanced reproductive techniques :

Artificial insemination in cats

MALANDAIN E.M., DVM, MSc, RAULT D.N., DVM, Dipl ECVDI
Alfort Veterinary College, Paris, France

History

Contrary to other mammal species, the practice of artificial insemination (AI) is unusual in cats. The first publication dates from 1970. AI is still not routine due to a few of animals potentially concerned and by the unresolved difficulties.

Over the past years, cat breeding had become more professional and the international exchange of animals has increased. Numerous infectious diseases potentially contracted during the mating have become a major concern for the breeders. As a result, breeders are searching alternative solutions for breeding.

Semen collection

The first problem in the tom cat is semen collection. Usually, this is performed in other mammals by digital manipulation or by vaginal collection. Digital collection is impossible for the cat due to the size of the penis. Vaginal collection is a useful method, but it requires training. If this practice is feasible with experimental animals (not all of them will accept the recolt), it's impossible in clinical practice. Therefore, the most frequently employed method for artificial insemination in breeder's cat is the electroejaculation, which use a specific probe (approximately 1 cm diameter, with three longitudinal electrodes placed ventrally) and an electroejaculator with a voltmeter ranging from 0 to 6 volts.

It's now well known that alpha 2 agonists can increase retrograde flow of spermatozoa into the bladder. That's why we use routinely an association of tiletamine and zolazepam or propofol for the anaesthesia of the tom cat (4) (5).

After the evacuation of faeces, the probe is lubricated and inserted 6-8 cm into the rectum. A tom cat urethral catheter (1mm diameter) is inserted in the urethra up to 5 cm in depth and connected with an insulin syringe without pusher. The semen is collected in the syringe by capillarity. (7)

Many different stimuli protocols have been described in the cat. (2) (3) (7) (9) (11). (8). We use a protocol which consists of 60 electrical stimuli, ranging from 2 to 5 V. Each stimulation consist of an increase from 0 to volts needed to provoke a rigid extension of the hind legs, 2-3 seconds at the desired voltage followed by an abrupt return to 0V. After 20 stimulations, the semen is collected in a small test tube, and a rest period of 2 min is observed.

Compared to vaginal stimulation, electroejaculation produces ejaculates in larger volume and lower spermatozoa than vaginal collection.

Examination of the Semen

The volume of the ejaculates of domestic cats is low (10 to 100µL by artificial vagina, 50 to 300µL by electroejaculation) which creates a problem for semen examination.

The color of semen can be easily evaluated. The intensity of the white of the semen reflects the concentration of spermatozoa. A small hematospermia is often seen when the urinary catheter harms the urethra but has no influence on semen parameter and fertility. Pyospermia can also be detected. A yellow coloration is a sign of urinary contamination.

The sperm motility is assessed under phase contrast microscope with 38°C warming plate. 10µL of semen are evaluated on a pre-warmed slide. Normal sperm shows motility ranging from 60 to 90%

The sperm morphology is evaluated by counting abnormalities with 10µL of semen, stained with eosin nigrosin. Acrosomal defects can be seen with Spermac stain or with a stain containing rose Bengal and fast green FCF. (14) (13). Usually, the normal semen shows at least 90% of intact spermatozoa.

The total number of spermatozoa is evaluated by diluting 10µL aliquot of semen, in saline at 1-10 to 1-100 dilution rates, depending on the sperm concentration, in a Toma chamber. It is difficult to evaluate the possibility of oligospermia in cats due to variability in the recolt technique among individuals and recolts. Only total azoospermia, with no retrograde ejaculation and high alkaline phosphatase concentration in the sperm can lead to a diagnosis of infertility. Often, sperm recolt by electroejaculation ranges from 3 to 120 million spermatozoa.

Artificial insemination

Anatomy of the genital tract of the queen

The cervix of the queen is located approximately 45 mm cranial to the vulva. The urogenital sinus narrows approximately 20 mm from the vulva with an average diameter of 3 mm. The constricted, cranial vagina, 25 mm long, permitted only the passage of a probe less than 1.5 mm in diameter (24)

Timing of induction of ovulation

No study has reported the influence of the day of ovulation upon fertility. It is recognized that during the first two days of estrus ovulation may fail, but it is not known if the third, fourth or fifth day would be better. Recent study, concerning follicular maturation and cervix patent may lead to advances. (1) (10)

Induction of ovulation

Ovulation can be induced by two methods

- 100/250 UI of hCG
- Vaginal stimulation (5 stimulations with a sterile cotton swab at 30 min of interval are effectiveness)

It has been supposed that stimulation by hCG could modify the endocrine environment which disrupts the oviductal embryo transport (6).

Vaginal stimulation can be ineffective if the queen is stressed due to transportation or Manipulation, and some breeders are reluctant to perform it themselves.

Timing of insemination

As a single mating can result in pregnancy, cat spermatozoa can be fertile at least 26-29 hours. AI practice 49 hours after the induction of ovulation suggests that ovocyte resist at

least 15 hours. If only one insemination is performed (as in the case of non experimental cats, because only one electroejaculation is done) it can be preferable to perform IA immediately or in the 24 hours following the induction of ovulation. (20; 22; 21) (17; 18; 16) (15) (11)

Number of spermatozoa per AI

Fresh AI

Sojka stated that 5 million spermatozoa could prove sufficient for a pregnancy to occur. But, Tanaka showed that 80 million spermatozoa for vaginal deposition increase the fertility rate to 80% (16,6% of conception with 20 millions of spz). But for these experienced, the authors used a mix of the semen of several cats to obtain the appropriate number of spermatozoa.

The problem for the private cat breeder is the availability of only one electroejaculate with the aim of achieving a pregnancy. This electroejaculate contains more often 10-20 millions of spermatozoa. In practical use, it is then extremely difficult to predict a percentage of chance for pregnancy. (19) (18) In our experienced, with a proper follow-up of follicular maturation, and an induction of ovulation with vaginal stimulation, we obtained a conception rate of 50%, with a mean of 9.5 millions of spermatozoa.

For uterine insemination, fewer spermatozoa are needed (0.1 to 2 millions). Tsustui showed a good fertility rate (80%) with only 10 millions of spermatozoa inseminated into the uterine horn.

Frozen AI

At the present time, few studies have been conducted on frozen AI. The number of spermatozoa required, as well as the frozen method, needs to be improved before its proposal in practice: the problem of semen collection is the same, and the mean sperm mobility after thawing is around 30% (versus 70% before thawing). (20) (12) (23). Several electroejaculation should be performed on the same Tom Cat, for the collection of enough motile spermatozoa for the AI.

Vaginal insemination

Vaginal insemination is the easiest way to inseminate a cat. Sedation is generally not necessary. Semen is deposited in the cranial vagina using a sterile urethra catheter, and the queen is left and held with fore limbs upright for 10 minutes. The volume of the semen must be carefully notice, because with more than 0,25mL, retrograde flow of semen is frequently noticed. But, in our experience we did not correlate this flow back with a decrease of conception rate.

Intrauterine insemination

Intrauterine insemination increases undoubtedly the rate of pregnancy with a single electroejaculate, and would be a prerequisite for the development of IA with frozen semen. Several ways to perform it have been described.

Laparotomy/Laparoscopy

The laparotomy or laparoscopy is an efficient way to inseminate a queen, but owners are often reluctant to perform it. (20; 22; 21, 23)

Transvaginal catheterization

Recently, transcervical catheterization was shown to be feasible, but it must be further developed before used routinely : the catheterisation during estrus has been reported to be difficult, and vaginal perforation has occurs. For the moment no AI has been decribed with this method. (25) (1)

Ultrasound Guided Transabdominal Puncture (UGTP)

Recently, we test a new method for the intrauterine insemination of the queen (Malandain et Rault, unpublished) UGTP was performed under general anaesthesia (propofol IV). After identification of one of the horn, transabdominal puncture was performed, using a needle. The success of catheterization of uterus was confirmed by the instillation of a tiny amount of air, easily visualised with ultrasonography inside the horn. The semen is then deposited directly in the uterus. The preliminary trial showed that conceptions can be obtained with this technique, but more experienced is needed to objective the conception rate, and the potential adverse effects.

With more studies on this subject and new possibilities such as transcervical catheterization as well as the determination of the optimal day of the induction of ovulation, AI will be in a near future as valuable in the queen as in the bitch.

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