



Can we use reproductive biotechniques in cats like it is used in dogs?

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Abstract

In recent years, there has been a great interest of purebred cat breeders in the possibilities and benefits of implementing of consistent veterinary care on reproduction. This includes tools available in the field of Assisted Reproductive Technologies (ART). As in dogs before, there is a tendency to reduce the reproductive potential of purebred cats, which is partly due to the nature of the selection of animals for reproduction on the basis of phenotypic features, omitting the control of their fertility. It is worthy to note hazardous specificity of this selection where inbreeding is commonly used. If all this is imposed on the frequent occurrence of infectious diseases in a domestic cat, then the population of purebred cats appears as a group of animals, which is a kind of challenge for a specialist of reproduction. This is a paradoxical phenomenon, because regarding non-purebred cats, the main challenge for the veterinary community is to limit their population. Some of the most important barriers to the implementation of advanced control of reproduction of purebred cats over the years have been: 1) difficulties with sperm collection, 2) technically troublesome artificial insemination, 3) numerous and difficult to recognize and treat forms of fertility disorders. In recent years, progress has been observed within each of these areas. It is possible to implement into practice extremely valuable tools that are helpful in monitoring the reproductive status of these animals. The breakthrough was the development of effective methods of semen collection, its evaluation and preservation and the development of increasingly effective methods of artificial insemination. Therefore, there is a real prospect of organizing cat reproduction centers operating within veterinary facilities dealing with small animals. The time is coming to carry out veterinary care for cat reproduction at such an advanced level as it has been developed in recent decades for dogs. Development in this area is facilitated by progress in research on the field of the use of ART in programs of rescue of endangered feline species where domestic cat is treated as model animal.

Keywords: cat, ART, semen, insemination

ART that can be used in cat clinical practice. Collection of sperm cells.

Almost every day, owners of reproductive males come to university outpatient clinics with a request to assess the fertility of the male. Sometimes it is an assessment of the fertility of a young individual before introducing it to the breeding program. However, more often, it is a request to perform fertility control of an individual with history of unsuccessful mating. A recommendable method of sperm collection is the technique developed in recent years by Prof. D. Zambelli. It involves pharmacological stimulation of sperm passage from the epididymis to the urethra using alpha-2-mimetic agents (3), such as medetomidine. The drug is usually administered in relatively high doses (100-140 µg / kg of body weight). After about 10-15 min. it is necessary to gently insert a tom cat catheter into the urethra to a depth of about 8 cm. Urethral sperm will spontaneously move to the lumen of the catheter, which is a capillary vessel. After a while, the catheter should be carefully withdraw from the urethra and the semen should be suspended in 100-200 µl of physiological saline or Tris buffer or other sperm extender. Medetomidine doses may be reduced. Dexmedetomidine may also be used. The use of the above drugs in combination with ketamine should not interfere with the movement of spermatozoa. Usually, it is possible to collect 10-20 µl of semen from urethra. These spermatozoa are highly concentrated. The number of sperm collected in this way is most often within range 5-50 x10⁶ of sperm cells (usually 10-20 µl) in fertile cats. In our center, we have collected sperm using this method from over 300 individuals and its effectiveness in terms of successful collection is about 90% (2, 4, 5, 6, 13).

Semen quality assessment

Is carried out macro- and microscopically. As mentioned, the volume of urethral semen is small. Normal urethral semen is white or gray-white in color. Usually, its quality in microscopic evaluation is not

so good if compared with the semen of a dog. Sperm motility in fertile males usually ranges from 60-70% (7). It is assumed in practice that the minimum percentage of sperm with normal motility should be higher than 60%. An important element of sperm evaluation is the determination of sperm morphology. One of routine method of sperm staining can be used for morphology assessment as Giemza stain, DiffQuick® or Nigrosine-Eosine, which allows to assess sperm morphology and the percentage of live and dead sperm. The specific feature of a domestic cat sperm cells is high percentage of morphological defects. Sometimes in fertile males, the percentage of sperm with normal morphology is not much higher than 50%. It is usually assumed in cats that >50% of sperm with normal morphology and >70% of living sperm suggest that the individual is fertile.

Due to the fact that in many fertile male cats (domestic and wild felids) spermatogenic function is characterized by relatively poor microscopic characteristics of sperm cells, there is a need to verify the commonly assumed in other species opinions and criteria for normal/abnormal fertility and to determine specific seminological reference values. It should be noted that the quality of sperm in the group of purebred male domestic cats is usually lower than in mixbred.

Sperm preservation

If the semen is not used for artificial insemination immediately after collection, the ejaculate should be preserved in appropriate medium e.g. in Tris-citric acid-fructose/glucose buffer with the addition of 20% yolk – in this medium it can be stored for a longer time at a temperature of 5°C. In cats, sperm survive in these conditions usually 1-2 days. So in practice it is possible to collect the semen, add a medium, slowly (1 hour) cool it to a temperature of 5°C and keep in a liquid state until artificial insemination (AI) and reinsemination the next day or ship it to another place for AI. Sperm preservation at low temperatures (liquid nitrogen -196°C) consists of dilution of urethral sperm in the medium Tris-citric acid-fructose/20% yolk with the addition of 4-8% glycerol. The sperm suspension should be slowly cooled (1.5-3 hours) to a temperature of 5°C. Then the equilibration is carried out for 1-1.5 hours. Semen is drawn into 0.25 mL straws containing 3-10 x10⁶ sperm in each. Straws are frozen for about 10 minutes in nitrogen vapour at -120°C or in a programmable freezer (2,12). The freezability of the male's sperm, as in other species, varies from individual to individual. There are males, where the results of freezing are good. There are also individuals from which it is not possible to freeze sperm successfully independently on initial semen quality. It is advisable that semen intended for artificial insemination and previously stored in a liquid state or subjected to cryopreservation, shows a motility of at least 30-40%. It is obligatory that detailed records have to be kept at the ART centre for each insemination dose - stored/shipped/received.

Artificial insemination (AI)

Artificial insemination of queens is technically difficult. It may be stated that this is one of the most important barrier hampering development and practical use of ART in pedigree cats. The diameter of the vagina is extremely small in female cats. Even the introduction of a catheter of only 2 mm of diameter is very difficult. An additional difficulty is that vagina is specifically long. Technically, catheterization of the uterine cervix is sometimes a challenging task. Not only this is a difficulty. It should be remembered that in a domestic cat, ovulation is stimulated by the act of copulation. Thus, before artificial insemination, the queen should be additionally stimulated to induce ovulation. AI in queens is best carried out in spontaneous estrus. Induction of estrus with gonadotrophins can sometimes cause metropathy or cystic ovarian disease. It should be emphasized, that in general female cats are sensitive to the administration of gonadotrophins. Ovulation is induced in the estrus, not in proestrus. In order to carry out stimulation at the optimal time, history regarding the cat's behavior should be collected and an ultrasound of the ovaries should be performed. The finding of a strong estrous behavior and confirmation of the presence of large follicles on the ovaries confirms the appropriate phase of the cycle for ovulation induction. If we examine a queen that is to be subjected to artificial insemination these days (having overt estrus), we can perform vaginal cytology. The collection of a swab, through mechanical irritation of the vagina, can contribute to ovulation. The presence of keratinized cells on a clear background in the cytological examination confirms the estrus.

Ovulation can be stimulated by repeated vaginal irritation mechanically with a cotton swab. hCG can also be administered in small doses of about 75-100 IU in estrus. If the queen does not enter spontaneous heat we may decide on a pharmacological stimulation of estrus and ovulation. The pathology of the genital organ should be excluded in such females before. The dose of 70-100 IU eCG should be administered first, and after about 80 hours we carry out hCG injection (75 IU).

Artificial insemination is usually performed twice 1-3 days after hCG administration. A prerequisite for the success of artificial insemination with preserved semen is the administration of semen into the uterus. Therefore queen should be in fully sedated during AI. To catheterize non-surgically the uterine cervix, we use cannulas used in dentistry or small-sized rigid endoscopes, e.g. a sialoendoscope. Semen is transcervically deposited into the lumen of the uterus. Female should be kept in a position with hindquarter elevated for several minutes after AI (9,10,11). After a few days after insemination, it should be checked whether ovulation has occurred. For this purpose, the concentration of progesterone in peripheral blood is measured. Concentrations above 5 ng/mL confirm ovulations. After the 20th day after insemination/mating, an ultrasound diagnosis of pregnancy should be made. According to this method, pregnancies have already been obtained in cats in both private and university clinics. In the Wrocław center, for the first time in our country, the described litter was obtained as a result of artificial intrauterine insemination of queen in 2018 (1).

Pregnancy monitoring

Confirmation of pregnancy and care for a pregnant queen have already become a routine veterinary activity. In healthy, fertile females, the first ultrasound examination is carried out about 20 days after mating, then you can assess the development and estimate the number of embryos. The next examination is recommended around the 40-50th day of pregnancy and if necessary – a few days before the expected birth. In problem females who have previously lost their pregnancies, we conduct sequential ultrasound examinations after the diagnosis of pregnancy, usually 1x a week. On the occasion of ultrasound examination, we also measure the concentration of progesterone in peripheral blood. Low concentrations or rapid decrease in progesterone usually support the decision to administer progesterone analogues, e.g. MPA (5 mg every 3 days) to maintain pregnancy.

To determine the day of delivery, ultrasound fetal biometry is used. In the first half of pregnancy, it is most convenient to make measurements of the diameter of the inner chorionic cavity (ICC). In the second half of pregnancy, we carry out measurements of the biparietal diameter (BPD).

Examples of biometric formulas:

ICC cats: Number of days to birth = $(\text{mm} - 62.03)/1.1$

BPD cats: Number of days to give birth = $(\text{mm} - 23.39)/0.47$

Specific formulas have been developed for particular breeds of cats (8) that allow for a relatively precise determination of the day of delivery, thus making it easier to make decisions about how to provide obstetrical assistance - conservative or by caesarean section. Before parturition, bradycardia occurs in fetuses and intensification of peristaltic movements of the fetal intestines. In queens, the level of progesterone reaches baseline values as late as after the last placenta has been expelled.

Obstetrical aid have been described in many textbooks, so they are not the subject of this paper.

Conclusion

In recent years there has been great progress in the use of ART in cats, as well as the implementation of strict veterinary care and monitoring of reproductive function in female and male felids. Semen collection and preservation have been elaborated. This trend meets the huge interest of the breeders' community collaborating with the veterinary services. The development of cat breeding and its popularity entail new challenges in the diagnosis of the reproductive system and in the biotechnology of reproduction. It should be hoped that the next years will bring more and more optimal solutions in the field of ART of females, because this procedure, especially AI, is still technically difficult. However the establishment of Cat ART Center similar to Dog ART Center/Semen Banks started to be realistic. Having knowledge, equipment and skills many private specialist and universities are ready to carry out such a service.

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