



Assisted reproductive technologies used in small animal veterinary practice - possibilities, obstacles and future

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Abstract

Assisted Reproductive Technologies (ART) are currently used in animals usually in three main situations: 1. As a form of treatment of subfertility and infertility in females and/or males, 2. As a method to obtain genetically valuable progeny in relatively short time in healthy fertile animals, 3. As a modern smart tool to obtain progeny in endangered animal species in programs of rescue of wild animals threatened extinction.

Generally the efficiency of reproductive biotechniques in dogs and cats is lower in comparison to obtained in farm animals and in human. Independently of the aim of their use, there are some techniques, which are better developed in dogs and some others seems to be better developed in cats. It may be assumed that simple, clinical techniques are well elaborated and more frequently used in dogs while more advanced techniques are better developed in cats. The level of effectiveness of ART is conditioned by anatomical and physiological factors specific for species, general demands for their use in veterinary practice and research, and general interest of breeders and scientific community in such activity.

Keywords: dog, cat, Assisted Reproductive Technologies, In Vitro Fertilization, infertility.

Introduction

On the field of small animal reproduction ART may be classified into group of:

1. **Simple methods** of fertility optimization and treatment: conventional treatment of sub-/infertility, estrus induction, monitoring of estrous cycle, detection of moment of ovulation, semen collection, its assessment and preservation, intravaginal and deep intrauterine insemination (2,4,5,7);
2. **Advanced methods** of ART as oocyte in vitro maturation (IVM), in vitro fertilization IVF-classical and Intracytoplasmatic Sperm Injection (ICSI) (3), embryo in vitro culture (IVC);
3. **Specialized methods** of ART as microsurgical epididymal sperm aspiration (MESA) or percutaneous epididymal sperm aspiration (PESA) (6), sperm collection from the testis by testicular fine needle aspiration (TEFNA), testicular extraction (TESE) or microsurgical testicular sperm extraction (MicroTESE).

For years there was great demand of breeder's community to use ART in dogs

As a form of control on reproduction and a method to optimize fertility potential. Therefore, the whole procedure of **simple techniques** as semen collection, assessment, preservation and Artificial Insemination (AI) are elaborated and commonly used in veterinary world. All these procedures are easy to perform by vet practitioner. There are many specialized veterinarians on this field, as well as many private and academic centers of biotechniques of reproduction providing semen banking and shipment. In other words, the commercial demands pressed veterinary community to develop and achieve progress on this field. However, in vitro maturation of oocytes and IVF are extremely difficult procedures, which have not been developed in dogs, due to specificity of ovulation in dogs, which takes place in very early stage of oogenesis (prophase of first meiotic division). Next stages of the oocyte maturation are finalized not in the follicle, but in specific oviductal environment, difficult to mimic in *in vitro* conditions. This unique phenomenon hampered the progress on this field in canidae for years. The fact of obtaining first healthy dog litter after IVF in Cornell (1) did not changed this situation. Unfortunately, this is one of the most important barrier and obstacle of progress regarding the use of advanced ART in dogs in veterinary practice and in programs of rescue of endangered canidae.

In cats the situation is different

For years the more advanced ART have been developing in cats partly due to the fact that scientific community was searching for the useful reproductive methods allowing to rescue of wild felidae species listed on IUCN Red Book. Fortunately, IVM and IVF may be performed with success, which is partly associated with more classical ovulation in felidae, which takes place 'classically' in metaphase of second meiotic division. Therefore, many procedures elaborated and developed before in human and farm animals were found to be useful also in cats. However, for several years it was not great demand from the side of cat breeders to develop simple ART techniques. Recently this situation has changed and we observe great interest of pedigree cat breeders in biotech reproductive service and the demand to perform procedures used routinely for years in dogs. Unfortunately, some simple ARTs are extremely difficult in cats and useful techniques such as repeatable, atraumatic semen collection were not available in the past but some of them have been elaborated recently. Nevertheless, up to now non-surgical AI is extremely difficult and seems to be the most important barrier retarding the practical use of reproductive biotechniques in veterinary daily practice.

Assisted Reproductive Technologies used in clinical practice

Semen collection in dogs is simple and easy to use method performed for years in veterinary practice. Manual stimulation of glans penis is performed for this purpose. Usually, fractionated semen is collected for sperm evaluation, prostatic fluid assessment, cryopreservation and/or AI. In cats collection of semen is more difficult. Recently, the commonly used method of semen sampling is collection of urethral sperm by administration of α -mimetic drugs (medetomidine, dexmedetomidine), which was developed by Zambelli. Concentrated sperm cells are suspended in medium and undergo evaluation and further procedures.

Gold standard for semen assessment in dogs and cats is Computer Assisted Semen Analysis (CASA) completed by morphology assessment and evaluation of morpho-functional cell features with the use of fluorochrome markers assessed on Flow Cytometer or fluorescent microscope.

It is relatively easy to preserve semen in liquid state and in low temperatures in dogs and cats. Many commercial extenders are available. Very popular in academic centers is extender based on Tris buffer. Usually, glycerol is used as cryoprotectant. Good results of semen post-thaw longevity are obtained if surface active substance is added to medium e/g/ sodium dodecyl sulphate (SDS). Semen is frozen in 0.25 (cat) or 0.5 (dog) French straws.

Artificial Insemination (AI) in dogs is technically easier when semen is deposited into vagina. However, the results of such insemination are poor, especially if cryopreserved semen is used. Better results are obtained with intrauterine insemination. The most popular method of semen deposition is currently Trans Cervical Insemination (TCI) with the use of rigid endoscope, elaborated on the basis of human cystourethroscopy or uretheroscopy. In cats, even intravaginal insemination is technically difficult and results of it are poor. Therefore currently, some centers are developing practically useful and efficient method of intrauterine insemination. In our center we use thin cannulas (human gynecology or stomatology) or sialoendoscope used for examination of lacrimal duct in human.

The gold standard of monitoring of estrous cycle in bitches (spontaneous ovulators) is clinical examination in combination with vaginal cytology, P4 and LH concentration measurements, as well as ultrasound monitoring of ovarian follicle development. This procedure is well elaborated. In cats (stimulated ovulator) the cycle is monitored by clinical examination and ultrasound observation of follicles. Vaginal cytology swabbing may provoke premature ovulation. Progesterone concentration is measured for confirmation of ovulation and to monitor development of corpora lutea.

IVF and ICSI are practically not used in dogs as was mentioned. These methods are optimized in cats treated as model animal in projects on the use of IVM and IVC-ET in programs of rescue of endangered animal species.

Conclusions

Simple ART are better elaborated in dogs and more advanced methods are more commonly used in cats, especially in research. Due to demands from the side of pedigree cat breeders in the next years probably we will observe rapid progress of methods of semen collection-preservation and especially AI in this species. Challenging for the future is elaboration in small animals advanced ART methods used recently in human medicine as MESA and PESA, which are so useful in symptomatic treatment of male infertility.



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