ISSN: 2583-8288
Popular Article
Published on: 28.03.2024

Artificial insemination - Useful tools in Canine breeding

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Introduction

Artificial insemination (AI) has been much more popular in bovine animals compared to canines since its inception. Artificial Insemination is one of the earliest techniques of assisted reproduction in animals, but took longer time to be implemented in dogs due to species-specific particularities. The first AI in a dog was reported by Italian physiologist Lazzaro Spallanzani in 1784. This insemination resulted in the birth of three puppies 62 days later. In past decades, progress in the knowledge of canine physiology and new advances in canine semen technology allowed these services to become available worldwide.

Artificial insemination (AI) in the dog is commonly practiced when the female does not accept breeding by a specific male when a male cannot mount due to physical problems, or when the male and female live far apart and cannot travel. Semen collection in the dog is a simple technique that requires very little training and can provide veterinarians with extremely important clinical information concerning the present and future fertility of their canine patients.

In Artificial Insemination (AI) the semen is collected manually from a stud male and thereafter deposited (inseminated) in the female so that fertilization can occur in the absence of natural mating.

Therefore, with the spread of canine AI dogs, breeders now may select stud dogs from all over the world to improve their kennel genetics, without transport-associated stress to the animals. Also, it is possible to save semen from valuable dogs into a sperm bank to be used in the next generations, after their death or the peak of reproductive age. In addition, breeders also are aware of the sanitary benefits associated with AI. Avoiding direct contact between the male and female, AI also prevents the spread of sexually transmitted diseases, such as those originating from *Brucella canis* or *Herpes virus*.

Indications for artificial insemination

The main indications for AI in dogs include both medical and breeding-management reasons. As a major potential advantage, AI may allow to a reduction of physical distances, the use of genetically valuable stud dog semen all over the world, and fighting the stress of transportation of animals and inbreeding. It is also an important technique whenever physical and behavioral abnormalities in the male or female prevent natural mating.

Semen collection

Collection technique

Semen collection in the dog is a relatively easy procedure, although requiring some training for optimization of the technique. Semen collection and evaluation are necessary to obtain good results in canine AI. Although practitioners are often asked to collect semen and perform AI without detailed semen analysis, every sample of semen collected should be evaluated (at least progressive forward motility, total sperm count, and morphology) before it is used for artificial insemination or cryopreservation. Semen evaluation before insemination warrants the male potential fertility and consequently may predict the fertility potential for the AI. In addition, when preparing semen preservation, a fertility certificate may be needed. In such cases, an andrological evaluation of the stud dog (breeding soundness evaluation or BSE) has to be performed. Semen collection should be performed before the physical exam or any stressful procedures on the stud or can be booked to another day

Semen can be collected from most dogs in the absence of a teaser, in a quiet and isolated room, where interruptions should be prevented, although

the presence of a bitch would allow better ejaculates. In reluctant males, stimulating estrus scent can be provided by the presence of a female in estrus or by using frozen-thawed swabs or gauze sponges taken from the vaginal secretions of estrus bitches.

Collection of semen should be prepared in advance, and intervals between collections or between the natural mating and collection should be registered, if the male is regularly used. Ideal intervals between collections are 2 to 5 days, whilst intervals longer than 10 days may result in an increased number of morphological abnormalities and decreased motility.

The most common method for semen collection in the dog is by digital manipulation, in the presence of a female. However, bitch presence, although desirable as it facilitates procedures. It should be noticed that when the collection is achieved in the presence of the bitch ejaculates present a higher concentration.

The use of manual massage is the most commonly used technique, although in the past semen was collected from dogs using an artificial vagina. The process is started with a massage of the dog prepuce at the level of the *bulbus glandis* until developing partial erection, followed by the quick retraction of the prepuce and penile exposure. If the collector is right-handed, semen must be collected from the dog's left side, with the operator holding the dog's penis with the right hand and the collection container in the left hand. During pelvic thrusting, rigid vials should be kept at a distance from the penis, to avoid trauma. When pelvic movements are finished and the dog lifts its rear leg, a 180º backward rotation of the penis should be obtained and the erectile penis should then be directed into the collection cone or the funnel. Some pressure may be applied with the thumb on the apex of the *glans penis*, at the level of the urethral process, to stimulate ejaculation. When a crystal-clear fluid (prostatic fluid) begins to flow into the collection tube, you can gently slide the collection cone off the penis. Watch for semen to flow in the collection tube.

Canine ejaculate consists of three fractions, with the first and third fractions consisting of prostatic fluid and the second being rich in spermatozoa **(Table 1)**. The first fraction, the presperm portion, is emitted in 0.5 to 1 minute and is colorless, with a volume range of 1-5 ml. It is expelled during the first stage of erection, at the moment of the presence of evident copulatory movement of the male. The second fraction, the sperm-rich portion, is also rapidly completed (1-2 minutes), and is grayish-white in color, with a volume of 1-3 ml. It is expelled when the thrusting movement of the male ceases and a full erection is observed. The third fraction comes from the prostate and may be up to 30-40 ml; it may take up to 5 to 30 minutes to be completed.

Semen Evaluation

Immediately after semen collection, it is evaluated for Volume, Color, Motility, Concentration, total sperm count, Sperm morphology, Live-dead spermatozoa, etc.

Table: 1 Main characteristics of the different fractions of the dog ejaculate.

Characteristics	**1st fraction**	**2nd Fraction**	**3rd Fraction**
Volume	0.1-2 mL (average 0.33 mL)	0.1-3 mL (average 1.17 ml)	1-2 to >20 ml Quite variable depending on the animal.
Colour	clear or opaque	greyish-white white, milky-white	clear, transparent
Consistency,	watery	watery-milky	milky watery
Character	Prostate secretion with admixture of epithelial cells, urine, bacteria and sperm cells	sperm cells suspended in seminal plasma	prostate gland secretion
pH (average)	6.37	6.10	7.20
Duration	5-90 sec. (average 13.5 sec)	5-300 sec. (average 52.4 sec.)	60 sec-20 min. (average 6 min. 55 sec.)

Timing of ovulation in the bitch

Canine proestrus and estrus last on average 9 days each with ovulation taking place 3 days after onset of estrus (or day 12 after onset of proestrus). However, ovulation can occur as early as 5 days or as late as 27 days after onset of proestrus. Therefore, it is very important to check the female's behavior, perform vaginal smears every 2-3 days starting on the first day of proestrus to catch early ovulation and

draw blood samples to measure progesterone once behavior and/or vaginal smear indicate estrus. Estrus is indicated by acceptance of the male or by a degree of vaginal cornification of >70. Ovulation occurs 3 days after the onset of estrus. Serum progesterone has a concentration of (values are approximate) 2.0-3.0 ng/ml on the day of the peak of luteinizing hormone (LH), 4.0-8.0 ng/ml on the day of ovulation, 10-25 ng/ml during the 2 days following ovulation, which is when oocytes are reaching maturity in the ampullae of the oviducts and fertilizations are taking place. Ovarian structures can be visualized with ultrasound using 5.0 to 7.5 sectorial MHz probes; follicular growth can be followed and ovulation can be estimated based on disappearance of the hypoechogenic areas representing follicles (which become luteinized) and on the appearance of a hypoechogenic area at the periphery of the ovary representing follicular fluid accumulation within the ovarian bursa.

Fresh and Refrigerated Semen

In most countries of the world, canine AI is performed using fresh semen. When properly performed, the success of AI with fresh semen is equal to the success of natural breeding, i.e. >80%. Although shipment of fresh undiluted semen can be done provided that travel time does not exceed a few hours (and provided also that prostatic fluid is normal), it is always better to dilute semen as spermatozoa lose very rapidly their fertilizing ability when maintained in seminal plasma. Semen extenders protect the sperm membrane from temperature variations as well as from mechanical trauma during transport, providing also stable pH and temperature conditions. Antibiotics such as streptomycin and penicilline should be used especially when storage is prolonged for more than a few hours especially when using egg yolk-based extenders where bacterial growth is enhanced.

Insemination Technique

Fresh semen can be deposited in the cranial portion of the vagina through a plastic catheter. Rigid catheters used for large animal uterine flushing work well although they need to be shortened for use in bitches. Intravaginal insemination is easy and widely practiced and conception rates following the use of fresh or refrigerated semen are good. Ideally, the bitch should have an empty stomach and be contained in a standing position. The catheter is inserted from the dorsal vulvar commissure (just like the cotton swab for vaginal smear) and its positioning at the end of the vagina is verified through abdominal palpation: the cervix (easily palpable during estrus) is identified and the tip of the catheter must be palpated just caudally. Once all the semen has been flushed from the catheter the hind legs of the bitch are elevated and kept in this position for 5-10 minutes (a procedure which is widely believed to help spermatozoa cross the cervix, although no scientific data have ever been produced).

Frozen semen must be inseminated into the uterus, as thawed spermatozoa are short-lived and cannot move vigorously enough to cross the cervix in numbers adequate to achieve a good conception rate. A catheter has been used for AI in bitch. One hand identifies and holds the cervix while the other one pushes the sheath until it reaches the par cervix, after which the steel tip of the catheter is carefully worked through the cervix into the uterus. The disadvantages of this catheter are a difficult learning process and the fact that it cannot be used in large-size bitches (because the cervix cannot be palpated with one hand).

The cervix can be passed also with a rigid endoscope. A human cystoscope or cysto ureteroscope is best used. A complete set of endoscopies (CO2 insufflator, light source, etc.) is necessary, which makes the technique expensive. Alternatively, intrauterine insemination can be performed surgically or laparoscopically. The surgical approach is faster, both are without complications and the conception rate is in the 60%-70% range.

Table: 2 Artificial insemination schedules for dogs, according to the type of semen used.

Semen	Doses	Expected spz Semen survival	Insemination schedule	Expected fertility
Fresh	150-200x106 spz/ml (extended)	4-6 days	-Every other day, when P4 rise above 4ng/mL, up to 3 times. -Day 1 to 4 post-ovulation -P4 levels between 8 & 15ng/mL	-80-90% (either with transcervical or vaginal deposition)
Chilled	150 - 200x106 spz/ml (extended)	24-72hrs	-Breeding once or twice 2-4 days post ovulation (P4 = 4 -10ng/mL).	- 80-90% (either with Transcervical or vaginal deposition

			-Day 2 to 4 post-ovulation -P4 levels between 8 & 15ng/mL	
Frozen	50-300x106 spz/ml (extended)	12-24 hrs.	-Twice, at P4 levels above 8 ng/mL and estrus vaginal cytology -Day 5 to 7 post-ovulation -P4 levels between 18& 28 ng/mL	-45% if vaginal deposition -67-84% if transcervical or intrauterine

Conclusion

Success rates for artificial insemination and the key-issues to obtain good results by using canine artificial insemination are proper timing of the insemination, the use of adequate number of viable sperm cells *per* dose**,** good semen preparation and handling**,** Adequate deposition of semen in the female reproductive tract