



## Semen Collection, Cryopreservation and Artificial Insemination in Dogs

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### Review

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### ABSTRACT

Today, the methods of artificial insemination (AI), that have recently become a biotechnological phenomenon, have being used in dogs especially for those partners that are largely unable to mate because of differences in their body sizes. AI is likely only by diluting the semen collected in order to protect from the environmental conditions, by using some short- and long-term preservation methods and by transferring into the females with proper techniques. Therefore, in this brief review, some data about the approach to the male at the time of semen collection, as well as the collection itself (digital manipulation, conical plastic rubber along with hand massage, and electrical stimulations (the electro-ejector)), dilution (Tes, Bes, Hepes, Pipes, Tris, Tes/Tris and commercial extenders, at 1:1-1:5 dilution rate), short (at +4°C) and long-term (in ampoule, pellet and straw) frozen storage (within liquid nitrogen, at -196°C) of semen and AI methods (intravaginal catheter, Norwegian intrauterine catheter, endoscopic intrauterine method and surgical intrauterine technique) are given in dogs.

**Keywords:** Dog, Semen collection, Freezing-thawing, Artificial insemination

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## Introduction

AI is the transfer of semen collected from the male with appropriate techniques and methods into females of the same species with appropriate techniques and methods. The realization of this application is possible by obtaining semen from male in large quantities, in good quality and in accordance with the technique. In this respect, it is necessary to know and apply different semen collection and evaluation methods specific to animal species. AI method, which has shown great development in recent years and has become a biotechnological phenomenon today, has led to the emergence of a major industry in the subjects covered. For this purpose, it has become an industry and trade branch with international relations in terms of the production of the tools and materials used and the frozen semen trade. With the AI, there is a chance to evaluate the male genotype most appropriately and widely, and this method is the most elegant and easy approach in terms of preventing the spread of infectious genital organ (venereal) diseases and increasing the rate of offspring. AI activities in recent years have come to the presence especially for the purpose of protecting the health of animals rather than breeding studies.

The first (empirical) AI in the world was applied to mares by Arab tribes in the 13th century. After the widespread use of AI in Soviet Russia at the end of the First World War, it later found an important application area in European countries and the USA. Scientifically, the first AI studies were initiated in 1780's by the Italian Physiologist Abbe L. Spallanzani (Ucar, 2000). In Türkiye, these studies first started in sheep in 1936, in mares in 1939, and in cows in 1949.

In this context, research has focused on storage possibilities by increasing the volume and viability of semen without losing their characteristics. As a result of these intense scientific studies, information has been revealed over time that semen can be stored both in the short- and in the long-term without losing their fertilisation capacity. There are many situations in which AI can be used when caring for dogs. The main reason for AI in dogs is that the male and female cannot mate for various reasons (Baran, 2015) and it is the request of the animal clients (Ucar, 2000). In bitches, the most common reasons for this are the problems such as vaginal stenosis, conformational defect, weakness in the hind legs, psychological problems and pain. Again, the AI can be tried in cases of unknown infertility problems. In males, weakness, skeletal system problems such as arthritis, early ejaculation and conformational defects are some of

the reasons for the lack of natural mating. Psychological problems such as the lack of experience and adverse upbringing are among the indications for AI.

Some animal clients want AI to control and prevent infectious diseases. Infectious agents that can be transmitted from male dogs to bitches by natural mating have the ability to be transmitted by AI.

However, with AI, infections that can be transmitted from bitches to males are prevented. All bitches and male dogs to be used in AI should be free from sexually transmitted (venereal) infections, especially Brucella.

In some breeds of male dogs, prostate bleeding and haemospermia can be seen after mating. This bleeding may be associated with Von Willebrand's disease, but it has also been seen in those without the disease. The AI can be used in dogs at risk of such a problem. Since there is no contact, the possibility of bleeding will be greatly reduced. The AI in dogs can be performed upon the request of the breeder or dog owner without any problems. The AI can also be performed in order to use pure breeds and valuable breeding male dogs more efficiently and to allow semen transport (Baran, 2015).

### Approaching A Male Dog During Semen Collection

If semen is to be collected from a male dog for the first time, it is imperative that an oestrous bitch be present (sometimes this may not be necessary if the male is very eager). The presence of an oestrous bitch in the environment can increase the quality of the semen (especially sperm concentration) to be obtained. For this purpose, if a bitch in oestrus cannot be found, a spayed female can be brought into oestrus by administering a low dose of oestrogen. Also, dog pheromones can be used. These are vaginal swabs for oestrous bitches stored in hydroxy benzoic acid or frozen form (Baran, 2015).

In recent studies conducted, marked increases in the number of spermatozoa collected have been noted with the administration of 0.1 mg/kg PGF2 $\alpha$  to the male dog 15 minutes before the semen is collected. Male dogs respond better to vaginal swabs than synthetic chemicals. The experience of the person collecting the semen can also affect semen collection during this application (Figure 1).



Figure 1. Cotton swabs used in taking vaginal swabs (Anonymous).

For male dog, due to the fact that the environment located is different from the living place, he is affected by environmental factors, the stress experienced and especially the smell taken in the clinical environment and the sounds of different animals (from cats and other dogs in the clinic) adversely affect the concentration during semen collection. It will be useful to have the owner of the male dog next to the animal during semen collection. All kinds of disorders (orchitis, prostatitis, pain originating from the feet, waist and hips) that may cause the male dog to feel pain during the semen collection process are other conditions that should be considered while collecting semen (Baran, 2015).

### Semen Collection Techniques from Male Dogs

Today, semen can be collected from dogs in three different ways; a) digital (finger) manipulations, b) artificial vagina and c) semen collection with an electro-ejaculator (Baran, 2015).

#### Collection of Semen with Digital Manipulation

Researchers used two different techniques for semen collection from dogs with penile massage. The first of these; hand massage technique, which is applied by passing the rubber conical sheath adapted from the artificial vagina of the bull over the penis, and the other is the digital (finger) manipulation technique.

In the first method, the conical rubber plastic (contact funnel) that provides the connection between the artificial vagina and the graduated collection glass can be used. The presence of an oestrous bitch in the environment (especially for inexperienced first-time ejaculating dogs) facilitates semen collection. The semen should be collected on a non-slippery surface and in a quiet area. The practitioner stands to the right of the male dog with the conical semen collection tool in his left hand. He tries to stimulate the erection by sliding his right hand back and forth on the preputium. The bulbus glandis begins to swell as the first sign of erection. An erection occurs when the cavernous body of the penis fills with blood. The erection is stimulated nervously by an increase in blood pressure in the penis, during which the return of venous blood is partially inhibited and the penile arteries expand. Before the erection is fully realized, the preputium is pulled back to expose the bulbus glandis and the conical semen collection tool is passed over the penis.

If a complete erection occurs before the bulbus glandis is protruded from the preputium, the animal will feel pain and the erection will then quickly disappear. The thumb and index fingers of the hand holding the collecting funnel begin to apply light and rhythmic pressures to the back of the bulb. After the pelvic thrusts reach their peak (approximately in 15-30 seconds), the penis is turned caudally with the rubber funnel. The first and second fractions of the ejaculate is attained in the first 1-2 minutes. The second sperm-rich fraction of semen, which has three fractions in total, has a milky appearance and an

average volume of 0.5-5 ml (Ucar, 2000; Baran, 2015) (Figure 2).

Approximately 1-3 minutes after the semen is ejaculated, a clear liquid (3rd fraction) originating from the prostate begins to come. This part is the last fraction and can be 5-40 ml in volume in 5-45 minutes.

In this method, it is very difficult to catch the onset of the sperm-rich fraction, and often parts of the ejaculate unintentionally mix. Again with this method, smegma preputi contained within the preputium while semen is being collected may contaminate the semen sample (Figure 3).

With the help of a glass funnel and graduated collection goblet, semen can be collected in the most comfortable way by finger manipulations. Most dogs are predisposed to semen collection with digital manipulations. Semen can be collected while the animal is on the ground and standing.

The presence of a bitch in oestrus facilitates semen collection, but is not always necessary. In this method, the presence of bitches in the environment can increase the number of sperm in the semen.

The semen collector, wearing a glove on one hand, gently massages the caudal part of the bulbus glandis from the left hind part of the male dog and over the preputium. During semen collection, contact of the rubber glove with the collected ejaculate may adversely affect motility, so contact should be avoided.

When semi-erection occurs, the preputial sheath is retracted. An erection is achieved after the retraction of the preputium and pressure applications to the posterior part of the bulbus glandis. When pelvic thrusting starts, the penis may hit the semen collection goblet. To avoid haemorrhage and trauma, the semen collection goblet is kept away. If the male dog is reluctant to semen collection, ejaculation can be stimulated by holding the collection cup with the thumb of the hand and touching it to the urethral formation (urethral orificium) of the glans penis. After the pelvic thrusts are finished, the penis is turned backwards between the dog's hind legs. At this time, rhythmic pressures should be applied to the posterior part of the bulbus glandis between the thumb and index fingers. When the semen collection process is completed, the penis should be washed with an antibiotic solution and it should be allowed to enter the preputium (Baran, 2015).

### ***Semen Collection with Conical Rubber Plastic and Hand Massage***

A collection goblet is placed on the conical rubber plastic end and vaseline or a similar lubricant is applied into the plastic. The presence of an oestrous bitch in the environment is beneficial (Figure 4).

While semen is being collected, it should be ensured that the male sniffs the vulva region of the bitch. In this way, the male dog feels more comfortable and can give more semen. One person stands to the right of the male dog, allowing him to sniff the bitch. The person collecting the

semen stands on the right side of the male dog and holds the conical rubber plastic in his right hand (Figure 5).



Figure 2. Collection of semen from the dog by hand massage (Baran, 2015).



Figure 3. Sperm-rich 2nd fraction (Baran, 2015)



Figure 4. Conical rubber plastic, lubricant pomade and collection tube (Anonymous).

By gently massaging the penis from the outside of the preputium, the collector inserts the penis into the conical rubber plastic after the erection is formed. After pelvic thrusting movements, the rubber is held in the palm and the penis is turned back by applying pressure to the

posterior part of the bulbus glandis. This method has a disadvantage since the three fractions of the semen cannot be collected separately from each other (Baran, 2015).



Figure 5. Semen collection with conical rubber plastic and hand massage (Anonymous).



Figure 6. Electro-ejaculatory device (Baran, 2015).

### Collection of Semen with Electrical Stimulations (Electro-Ejaculator)

It is possible to collect semen from dogs with an electro-ejaculator. However, this method, when applied under general anaesthesia, should not be used unless it is necessary (Figure 6).

This method requires a rectal probe and electro-stimulator. After the bipolar rectal probe is inserted into the rectum in accordance with its technique, the ejaculation centre is stimulated at regular intervals with electrical 140-180 mA current and 10-20 Volt voltages and semen is collected. In the electro-ejaculation method, the

semen volume obtained is greater than in natural mating due to over-stimulation of the prostate gland. However, it is not a preferred method due to the possibility of urine mixing with the ejaculate obtained. It can be used in cases where semen cannot be obtained from very valuable stud dogs by other methods (Baran, 2015).

### Storage of Dog Semen

#### Short-Term Storage of Dog Semen

When semen is cooled or transported, it should always be diluted (extension). Extender helps to stabilise the pH, conserve energy, and protect the sperm membrane against the injuries caused by shaking and temperature differences during transportation.

Compared to frozen semen, higher pregnancy rates are obtained as a result of insemination with extended and cooled semen. The potential fertility of extended and cooled canine semen is preserved due to three factors, as follows;

- Reducing sperm metabolism at low temperature,
- Protection from cold shock by adding extender,
- The resistance of canine sperm cells to cold shock is high.

Semen extenders should have general properties such as proper osmotic pressure, nutrient medium, absorbing metabolic residues and protecting spermatozoa from the harmful effects of cold. Cooling the semen, whose spermatological properties have been determined and extended, to +5°C requires special care. Because, in sudden temperature changes below 17°C, irreversible changes may occur, especially in the acrosome (Ucar 2000; Ucar 2004).

For an overall viability (71% at 24 h or 67% at 48 h) of sperm with physiologically-intact acrosomes (55-60%) of extended/cooled semen (in Tris-based extender), the steps of centrifugation (at 3,000 g for 5 min), dilution (between 1:4 to 1:8 as semen-extender), glycerol concentration (2%, v/v) and glycerolisation temperature (at 4°C) are all critically advisable in dogs (Ucar, 2000).

As a result of cooling the semen collected without the addition of a cryoprotectant substance, the viability of the sperm cells is very short. During cold shock, the phospholipids in the extender interact with the lipid structure of the plasma membrane of sperm cells and provide protection. Lipoproteins that bind to the sperm cell membrane help maintaining cellular integrity (Yanagimachi, 1988) in semen storage (Ucar, 2000; Baran, 2015).

#### Long-Term Storage of Dog Semen

##### Extenders used in freezing dog semen

In dog breeding, as in other animal species, it is of great importance to prevent breeding-related (venereal) diseases, improve genetic structures, benefit from superior individuals to the highest extent, obtain elite breeders and protect gene resources.

In this context, in addition to revealing the spermatological characteristics in many dog breeds, researches have been carried out for many years to freeze the semen by extending with proper solutions, to store for

short- and long-term, to use for AI when necessary, and subsequently to achieve high fertility.

Today, various solutions are used as semen extenders for the short- or long-term (freezing) storage of dog semen.

Among them, although zwitterionic (bipolar) solutions such as Tes (N-Tris [hydroxymethyl] methyl-2 amino-methane sulfonic acid), Hepes (N2 [hydroxymethyl] piperazine-N-2-ethane sulfonic acid) and Pipes (piperazine-N, N-bis-2-ethane sulfonic acid) with a better buffering capacity compared to Tris are used, more ready-made commercial preparations (Laiciphos, Biociphos, Biladyl, Triladyl, Andromed, etc.) have been preferred for freezing dog semen in recent years (Silva and Versteegen, 1995) (Figure 7).



Figure 7. Cooling semen in a water container (Anonymous).

Indeed, it is reported that in dog semen frozen with three different extenders consisting of Laiciphos, Biociphos and Tes/Tris, post-thaw sperm motilities were 65%, 70% and 50%, while the corresponding viable sperm ratios were 78%, 80% and 65%, respectively. When the effects of adding three different potassium buffers (KHCO<sub>3</sub>, K<sub>3</sub>PO<sub>4</sub>, KOH) to Pipes, Bes, Tes, Tris extenders on semen freezing were examined, the best sperm motility was obtained after thawing with the extender 50% Pipes/KOH + 25% sodium citrate + 25% dextrose (along with 20% egg yolk and 9% glycerol) at a 1:2 dilution rate (Silva and Versteegen, 1995).

When semen is frozen in liquid nitrogen vapour, the values of spermatological characteristics may change because the sperm cells are adversely affected by cold shock (Ucar, 2000; Uysal et al., 2005). It has been reported that perforations in cell membranes lead to the loss of enzymes (Yanagimachi, 1988), especially in the acrosome, which are involved in fertilisation, and that sperm cells in

this condition lose their ability to fertilise (Muller and Kircher, 1978; Weitze, 1981; Ucar 2000; Ucar 2004).

It is known that semen extenders containing different and varying amounts of cryoprotectants protect the sperm cells from the cold effect during the aforementioned freezing stages, regulate the exchange of substances in their environment, and minimise the adverse effects of ice crystallisation around the membranes (Moce et al., 2003).

Since seminal plasma contains protective factors for spermatozoa as well as factors that increase cell sensitivity, sperm cells in ejaculated semen are more sensitive to cold shock than epididymal semen and are more unfavourably affected (Weitze and Petzoldt, 1992). In the storage of dog semen, egg yolk has been used at a rate of 1-20% to protect sperm cells from the deleterious effects of freezing processes.

In recent years, in sperm freezing studies (Smith, 1985; Uysal et al., 2007), many researchers have benefited from BSA, one of the seminal plasma components, and reported that BSA, a membrane stabiliser, improves the protective effect of egg yolk on sperm membranes (De Leeuw et al., 1993), and even in combination with BSA, egg yolk concentration can be reduced.

While some researchers (Trimeche et al., 1997) used BSA as high as 6% (w/v) to benefit from the cryoprotectant effect in semen extender in dogs, others used 5% (w/v) as protein supplement. Uysal et al. (2005) examining the effects of BSA on freezing dog semen in their study found that; the highest post-thawing spermatozoa motility (50.5%) and HOS-test value (58.5%) were obtained with the M-Tris extender containing 10% egg yolk + 10 mg/ml BSA as compared to the results obtained using BSA and egg yolk alone in the extender. Therefore, they stated that the combination of egg yolk and BSA better protected the spermatozoa from cold shock due to the synergistic effect. In addition to evaluating the effectiveness of different extenders in the freezing of dog semen, it is also aimed to reveal the effect of BSA (Muller and Kircher, 1978), used as a membrane stabiliser to reduce the injury to sperm cells caused by cold shock during freezing/thawing processes, on some post-thawing spermatological parameters.

#### Freezing and Thawing Dog Semen

In canine semen freezing studies, the pellet method was initially adopted rather than the ampoule method and successful pregnancy rates were achieved. In today's studies, semen can be frozen in 0.25 ml and 0.5 ml straws. It has been reported that after freezing, dog semen can be stored for more than 12 years and this period leads to a minimal (adverse) effect on fertility rate of spermatozoa. Moreover, frozen dog semen has an average post-thawing viability of 12 hours *in vivo* (Ucar, 2000; Baran, 2015).

In freezing dog semen, although the optimal concentration of glycerol, which is the most commonly used cryoprotective substance, varies between 3-4%, this ratio varies according to the type of diluent used (Ucar 2000; Ucar 2004). In general, most livestock ready-to-freeze straws are kept 4 cm above the liquid nitrogen

(LN2) level for 5 minutes and immediately plunged into the LN2 (Trimeche et al., 1997; Ucar 2004).

In a thesis study, dog semen was successfully extended in Tris-fructose-citric acid extender containing 2% glycerol (v/v) and 20% egg yolk and frozen, with the help of a semi-programmable biological freezer. This was achieved by pre-cooling from +4°C to -9°C at -0.5°C /min, -9°C to -20°C at -40°C /min, and -20°C to -120°C at -100°C /min, followed by plunging the semen directly into LN2. In this way, a post-thaw motility of 40-70% (mostly 40-45%) was obtained, even with a small number of individuals used (Ucar 2000).

In some studies, very low glycerol ratios fail to provide sufficient cryoprotective effect post-thaw, while very high glycerol rates cause cryoinjury on the acrosome. However, low glycerol rates (up to 2%, v/v) in extended semen stored at +4°C do not cause non-physiological injury in terms of *in vitro* AR (Yanagimachi, 1988), while higher ratios (up to 6%) adversely affects physiological AR (Ucar 2000; Ucar 2004). In order to freeze dog semen in pellet form, generally “4% glycerol, 11% lactose and 20% egg yolk” or “4% glycerol with skimmed milk-glucose” extender is used (Ucar, 2000; Baran, 2015). For freezing dog semen in straws, “3% glycerol buffered with egg yolk- Tris - fructose - citric acid” extenders are generally preferred (Baran, 2015). Generally speaking, sperm motility after thawing should be  $\geq 40\%$  and the rate of abnormal spermatozoa to be  $\leq 30\%$  (De Leeuw et al., 1993; Ucar 2004).

To dilute dog semen, 1:1 to 1:8 rates (1:4 rate mostly) are frequently used (Ucar 2000). These rates may vary depending on the concentration of spermatozoa in the semen collected. In order to thaw pellet-frozen semen, it is also necessary to thaw in the diluent used in the freezing protocol or in a tube containing physiological saline at 37°C for 30 seconds (Ucar, 2000; Baran, 2015). According to the straw method, a water bath at three different degrees and temperatures is often used to thaw frozen dog semen. Among them, the first is thawing at 37°C for 30 seconds (or at 35°C for 60 seconds) for 0.25 straws, while the second is at 75°C for 6.5 seconds and at 70°C for 8 seconds for 0.5 ml straws (Ucar, 2000; Uysal et al., 2007).

While dog semen is diluted to be 100 million doses per ml, the sperm concentration per straw is between 25-50 million (De Leeuw et al., 1993).

### Artificial Insemination Techniques in Dogs

Four main insemination techniques are used in dogs (Ucar, 2000; Baran, 2015):

- Intravaginal with Catheter,
- Intrauterine with Norwegian Catheter,
- Intrauterine by Endoscopic Method,
- Intrauterine by Surgical Technique.

#### Intravaginal Insemination with A Catheter

It is a preferred method because it is easy to apply and does not require any special tools. Vaginal insemination for bitches is a method in which the catheter is advanced with a plastic catheter from the vaginal cavity until

resistance is encountered and the semen is released into the cranial of the vagina.

#### Application

The dog is taken to an appropriate place. The tail is pulled to the side and the vulva is exposed. Vulva is carefully washed with a liquid (physiological saline, etc.) and dried well. Due to the toxic effect of disinfectants on spermatozoa, disinfectant should not be used for washing the vulva. The hind legs of the animal are lifted slightly apart. It is provided to make an angle of approximately 45-60 degrees with the ground. The vagina is opened and the catheter is advanced into the dorso-cranial direction of the vagina. After a little progress made, the catheter is adjusted horizontally. The catheter is then advanced until it encounters a vaginal resistance, that is, in front of the cervix, and the semen is deposited therein. In order to prevent the backflow of sperm, the bitch is kept in the same position for 15-20 minutes with her hind legs up in the air and the vulva (clitoris) area is massaged. The inseminated dog should be kept calm for about 1 hour and care should be taken not to apply pressure to the abdomen.

In bitches, it is somewhat difficult to perform intrauterine insemination by trans-passing the cervix due to anatomical reasons such as the long vagina, narrowing in the paracervical region, and ventral angle of the cervix. Intravaginal insemination has been suggested by many researchers and studies have been carried out on this subject because it is easy to apply with both fresh and frozen semen (Ucar, 2000). In most studies, pregnancy rates in intravaginal insemination with fresh semen are quite high. By this method (especially with fresh semen), 80% success is achieved in insemination (Baran, 2015) (Figure 8).



Figure 8. Intravaginal insemination set (Baran, 2015).

#### Intrauterine Insemination with A Norwegian Catheter

The aforementioned method was developed by Dr. John Fougner in 1970 as a catheter used for intrauterine insemination of foxes. Later, it has started to be used successfully for the first time in dogs in the Scandinavian countries (Ucar, 2000). Therefore, it is referred to as a

Scandinavian catheter in some books (Scandinavian stainless steel catheter) (Figure 9, Figure 10).

### Application

First, the thin tube made of hard plastic is slowly inserted into the vagina and brought to the front of the cervix (Figure 11).

First of all, the place of the hard plastic rod is determined from the abdomen with the left hand and the position of the uterus is tried to be determined. Then, the steel catheter is slowly passed through this sheath again,

and the tip of the catheter is fixed, trying to bring in facing with the tip of the cervix, and slowly inserted in to provide trans-cervical access. During intrauterine insemination, the hindquarters of the bitch should be lifted up in the air (Figure 12).

The semen is slowly transferred through the inserted catheter into the cervix. In transcervical insemination with a Norwegian catheter, two 0.5 ml straws (75 million spermatozoa each) are used.

It requires technical expertise. This has been caused by the rather narrow-short cervix and posture-angle in bitches, as suggested (Baran, 2015) (Figure 13).



Figure 9. Tips of scandinavian steel catheters (Anonymous).



Figure 10. Three different sizes of norwegian catheters (Baran, 2015).



Figure 11. Advancement of rigid plastic tube from the vagina to the front of the cervix (Anonymous).

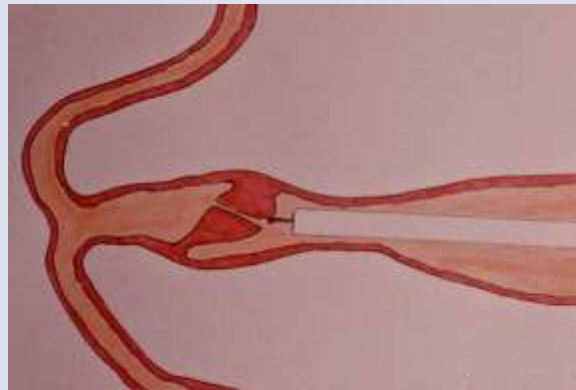


Figure 12. Trans-cervical AI with a Norwegian catheter (Anonymous).



Figure 13. Passing the steel catheter through the plastic tube and depositing the semen (Anonymous).



Figure 14. Endoscopy device used in intrauterine insemination (Anonymous).

### ***Intrauterine Insemination by Endoscopic Method***

Insemination with this method requires experience, time and patience. By connecting the camera to the endoscope device, it provides a lot of convenience for the catheter passed through the endoscope. For this purpose, an extended cystourethroscopy is used.

This instrument consists of a telescope with a 30 degrees of oblique angle of view and a cold light source. The working length of the endoscope is 29 cm. Insemination is performed with a 6 to 8 number French urinary catheter.

In the application of this method, it is extremely important to immobilise the animal. For this purpose, the bitch, which is on a special hydraulic platform, should be tied with a band from the abdomen to prevent her from sitting and moving left and right after being tied to her leash.

#### ***Application***

The endoscope is inserted into the vagina and looked inside through the vaginal wall. In early oestrus and pro-oestrus, the vaginal wall of the bitch is oedematous and fills the lumen of the vagina. With the onset of oestrus, this wall is dehydrated, allowing better observation. The caudal projection of the dorso-median wall is quite narrow. The vagina-facing side of the cervix may not be observed because it is caudo-ventral or ventral direction.

Without anaesthesia and sedation, many oestrous bitches do not experience discomfort during trans-cervical insemination. The catheter should be advanced into the cervical canal with careful manipulation of the endoscope. The operator, observing while depositing the semen, stops if the semen flows backwards and tries again by changing the position of the catheter. In some small breed dogs, smaller diameter endoscopes should be used because the cervix is too narrow. At the right time, the pregnancy rate is over 80% in cases where the fertility of both male and female dogs is good and the semen is introduced into the uterus (Baran, 2015).

### ***Intrauterine Insemination by Surgical Technique***

In this technique, the animal is kept hungry before the operation and general anaesthesia is then applied. The operation is performed by an experienced Veterinarian by opening the cornu to the uterus from a suitable site (median or lateral). As insemination dose, 5-10 million spermatozoa are sufficient for frozen-thawed semen and 2-5 million spermatozoa for fresh semen (Figure 14).

#### ***Application***

First, the ovaries are examined and recorded in terms of follicular and luteal developments. Fresh or frozen-thawed semen drawn into the injector is deposited inside from the midline and a non-vascular part of the cornu uteri, as revealed by the operation. After this procedure, a sufficient dose of GnRH should be administered to the animal to stimulate for stimulating ovulation (Baran, 2015).

### **Conclusion**

To obtain successful semen collection, as in all other animals, preliminary evaluation, processing, freezing-thawing and AI results in dogs; All protocol steps must be carried out with great care and attention, starting with the collection of semen, performing the AI process and following up the result. For the reliability of the results to be obtained, an 'integrity' of the collective contributions of both male (libido, semen, genetics, health, etc.) and female individuals (oestrus, body structure, health, nutrition, shelter, etc.) throughout the AI process must be addressed in (Ucar, 2004).

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