

Techniques of artificial insemination by fresh, chilled and frozen semen

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Artificial insemination (AI) is becoming more common in canine reproduction, permitting the use of shipped semen, assistance for geriatric or subfertile males, coverage of dominant females, and advanced reproductive technology such as intrauterine deposition of semen. AI may be performed with fresh, chilled, or frozen semen. All instruments should be clean and free of any chemical contamination. After semen has been collected and evaluated, it can be deposited in the cranial vagina of the bitch using a rigid insemination pipette of appropriate length, or into the uterus via transcervical catheterization. Access to the uterus via laparoscopy or laparotomy is less desirable due to invasiveness. Semen (the second fraction) may be diluted with extenders and chilled for later or distant use (within 48 hr), or extended and frozen in liquid nitrogen (in straws or pellets) for longterm storage. Phosphate-buffered egg yolk diluent or Tris-buffered diluent is used most often; several commercial extenders are available. Chilled semen should be warmed for evaluation before use. Frozen semen should be thawed as directed by the cryopreservation center, evaluated, and immediately inseminated.

METHODS TO PERFORM INTRA-UTERINE AI IN THE DOG

Intra-uterine AI in the dog can be done transcervically either by way of the Scandinavian (or Norwegian) catheter, or by using a rigid fiberoptic vaginal endoscope to visualize the cervix and a dog urinary catheter or a human urethra catheter to transverse it. Intra-uterine AI can also be accomplished by invasive methods such as laparoscopy, or full abdominal surgery. In some countries the latter methods may be illegal or not considered ethically acceptable.

PALPATION OF THE CERVIX

It is absolutely essential for the person who wishes to perform canine AI to learn how to locate the cervix by abdominal palpation in order to be able to deposit the semen in the

correct place and to avoid injuring the bitch. The bitch should have an empty stomach and bladder to facilitate the procedure. For training purposes it is recommended to use the single-use plastic canine vaginal AI-catheters (Minitüb GmbH, Tiefenbach, Germany).

Because the urethral opening of the bitch is located at the pelvic brim, it is surprisingly easy for the AI catheter, or a thin rigid endoscope, to be unintentionally introduced into the urinary bladder. Apart from the hazards of perforating the bladder with the catheter, it is obvious that no pregnancy would follow after an AI. Thus, the correct position of the catheter should always be checked by palpation before depositing a semen dose. If the catheter is in the urinary bladder, the cranial part of the vagina and the cervix can be palpated above the catheter. The walls of the urinary bladder usually are thinner than those of the vagina, and the tip of the catheter stands out more distinctly than if it were in the vagina.

To palpate the cervix, an AI catheter is introduced into the vagina of the bitch. The introduction of the catheter is facilitated if the vulva is elevated until it is just below the anus (like when the bitch stands for the male dog). When the tip of the catheter is introduced as far as to immediately cranial to the pelvic brim, it should be palpated. Cranially the vagina in most bitches slopes slightly downward. In some breeds, however, especially the sight hounds with a very arched loin, the vagina has a more dorsal direction. The cranial end of the catheter should now be lowered closer to the abdominal wall to become more accessible to palpation. When the catheter tip can be palpated and its correct position in the vagina thus checked, it is carefully introduced further, under continued palpatory control, until it reaches the paracervical area. This is the narrow, cranial portion of the vagina created by the dorsal, median post-cervical fold and can be palpated as a 1 to 2 cm long, firm structure. It ends at the cervix, which in a bitch in estrus is a 0.5 to 1.5 cm hard, rounded-to-ovoid freely movable structure. It is usually not possible to pass the outer protecting sheath of the Scandinavian catheter, which has a diameter of 10 mm, into the paracervical area. Also the thinner plastic AI catheter, which has a diameter of 5 mm, may be too wide to introduce into the paracervical area in some bitches, especially those of the smaller breeds, or those that have not given birth to a litter of pups. Once the cervix has been identified the corpus uteri and the uterine horns can be palpated in front of this structure. Lower the tip of the catheter and then close the tip of the thumb against that of the index finger above the catheter, then lift the cranial end of the catheter in such a way that the

cervix and the uterine horns are pulled upward between the fingers. Their size and consistency then become evident. (This method of palpating the uterus is also very useful for early pregnancy detection and to examine bitches with suspected pyometra).

INTRA-UTERINE INSEMINATION USING THE SCANDINAVIAN CATHETER

The Scandinavian catheter consists of a 1 - 2 mm wide steel catheter with a 0.75 mm to 1 mm diameter tip, and comes in three different lengths: 20, 30 or 40 cm. It is used together with a 10 mm diameter outer protecting nylon sheath. The medium sized catheter fits most small and medium sized bitches. The equipment can be obtained from the Norwegian Fur Breeders' Association, P.O. Box 136, Økern, N-0509 Oslo 5, Norway.

Intra-uterine AI with the Scandinavian catheter is performed with the bitch standing on the floor or on a table. Sedation is very rarely needed; on the contrary, most bitches in estrus freely accept this type of handling. In case a light sedation should be required for instance in a very large, obese or nervous bitch, 1 - 3 mg/kg xylazine IM or IV can be used. The inner steel catheter, with the tip within and protected by the nylon sheath, is introduced into the vagina. The cranial end of the nylon sheath is palpated in front of the pelvic brim as previously described. If the tip of the catheter sheath has been lowered closer to the abdominal wall the cervix usually is found a few cm in front of and above it. The steel catheter then is introduced through the sheath until its tip reaches the ventral fornix. The cervix is fixed between the thumb and the index finger and, by applying a slightly downward traction at the corpus uteri, it is tilted so that the angle of the cervical canal becomes more horizontal.

The tip of the catheter is then carefully withdrawn while pushing it repeatedly against the surface of the cervix in search of the opening of the cervical canal. The sensation when this opening is found can in most cases be described as the sensation of touching cartilage, i.e. "crispy". Once the opening has been found, fix the catheter and start working the cervix against the catheter. The cervical canal is 5 to 10 mm long, and not always completely straight. Thus, a slight pressure may have to be applied, while rotating the catheter to ease it through. In most bitches, the tip of the catheter easily can be felt in front of the cervix in the corpus uteri. In some bitches, however, the sensation is not as distinct. The syringe containing semen is connected to the catheter and the semen slowly infused into the uterus. Sometimes there is a resistance to infusion depending on whether the opening of the catheter is pressing against the endometrial mucosa. A light downward traction of the corpus uteri or the cervix usually alleviates the situation and allows semen infusion.

To check that the catheter really is in the uterus of the bitch 1 - 2 ml of physiological saline can be infused. If the catheter is in the right position in the uterine body the fluid can easily be infused. If, on the other hand, the catheter is in the paracervical region, there will be an almost immediate backflow of saline between the catheter and the nylon sheath.

The catheter is removed and the hindquarters of the bitch are elevated and the bitch kept in this position for 5 - 10 minutes after the AI to minimize backflow of semen and to facilitate uterine transport of spermatozoa toward the oviducts. The bitch should also be feathered around the perineal region as this is believed to stimulate uterine contractions.

To learn this technique requires some practice, but once learned it is a quick method, usually being accomplished within minutes. It is recommended that, initially, organ specimens be obtained for training purposes and anatomical study. It is also an advantage if especially the first attempts are made in bitches that have given birth to one or more litters, as they are usually much easier to catheterize. Perforations may occur if the catheter is introduced blindly or with force. Provided that the catheterization is performed under careful palpatory control, however, the technique is completely safe for the bitch. Some bitches are more difficult to catheterize, particularly those belonging to some of the giant breeds, as well as obese or nervous animals. Using the Scandinavian catheter only between 2 and 3.5% of attempts at intra-uterine catheterization were unsuccessful.

Resulting whelping rates using frozen-thawed semen has been reported to be 84.5%, and 71% when performed by skilled inseminators, an average of 65% with fresh and chilled semen and 52% with frozen-thawed semen, in a larger field study involving also less-experienced inseminators. This technique can also be used for intra-uterine infusion of contrast medium for hystero-graphic examination of the bitch.

INTRA-UTERINE INSEMINATION USING ENDOSCOPIC VISUALIZATION OF THE CERVIX

Transcervical intra-uterine insemination can also be accomplished with the aid of a rigid fiberoptic endoscope and a urinary or angiographic catheter, on the standing bitch, and without sedation.

Wilson used a rigid cysto-urethroscope, 30 cm in length and 4 mm in diameter with an oblique viewing angle of 25°, together with a 23 Fr gauge stainless steel sheath. A dog urinary catheter, 6 - 8 Fr gauge, is passed through the operating channel of the sheath. The endoscope is introduced into the vagina and advanced until the external os of the cervix can be visualized and the urinary catheter is manipulated into the cervical opening and further into the uterus. To guide the endoscope through the sometimes tortuous vaginal vault it can be quite helpful to let the urinary catheter lead the way by a few cm, thus indicating the right direction. Similarly to when using the Scandinavian catheter it can also be established by abdominal palpation whether the tip of the endoscope is correctly positioned in relation to the cervix. When the semen has been inseminated into the uterus, the endoscope is removed. After the intra-uterine semen deposition, the bitch's hindquarters are elevated and the AI catheter is removed, and the bitch is left in this position for 5-10 minutes, to minimize backflow of semen.

Using endoscopic AI with frozen-thawed semen, whelping rates of 25% (3 of 12 bitches) and 80% (32 of 40 bitches)

es) have been obtained. A significant advantage of this technique is that it allows direct visualization of the cervical opening. Still, it involves manipulation of the scope and catheter and requires some practice. In addition, to be able to catheterize bitches of all sizes with this method, several endoscopes of varying width and length would be required. Although the equipment is expensive, for practitioners specializing in canine reproduction and AI it should be a good investment to obtain at least one endoscope of medium size which fits most average sized breeds. The endoscope is also of great help when training to perform transcervical catheterization with the Scandinavian catheter.

INTRA-UTERINE INSEMINATION USING LAPAROSCOPY

Abdominal laparoscopy should offer a somewhat more acceptable alternative to full surgery for AI in the dog. The technique has been described by Wildt and Silva *et al.*. A 60 to 73% pregnancy rate has been reported by AI using laparoscopy, but the number of bitches in those studies were few.

INTRA-UTERINE INSEMINATION USING SURGERY

Surgery to perform intra-uterine insemination has been reported. Various surgical procedures have been used, with

the bitch under general anesthesia and in dorsal recumbency. The ventral abdomen is clipped, and after routine surgical preparation a 4 - 6 cm incision is made midway between the pubis and the umbilicus, through the linea alba. The uterus is elevated through the incision, and the needle of the syringe containing the semen is inserted into the lumen of the uterine body at a 45° angle with the bevel of the needle up. (In another technique, a intravenous catheter of small size (Braunüle) is introduced into the lumen of the cranial uterine horn on each side). The semen is slowly injected into the uterus. It should flow easily with obvious distention of the uterine horns, or else the needle should be repositioned. A saline moistened gauze is held over the injection site after the needle is withdrawn. After 1 min the gauze is removed, the uterus replaced into the abdomen and the wound closed using routine methodology. To avoid backflow of semen the bitch should be positioned with its rear elevated as she recovers from anesthesia. Around 60% pregnancy rate has been reported after surgical AI in the dog, but like with laparoscopic AI results are based on limited experimental studies and no field data are available for evaluation using either method. Whether it is ethically acceptable to resort to surgery to achieve pregnancies is debatable. The method, although advocated by some, is considered by many to be unethical and unacceptably stressful for the bitch. The risks for infection, etc. associated with surgery in general and the limited number of surgical AI's that can be performed in a given bitch are two obvious disadvantages. The method is also costly and time-consuming.