Scientific Works. Series C. Veterinary Medicine. Vol. LXX (2), 2024
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

# ENDOSCOPIC TRANSCERVICAL INSEMINATION: A METHOD FOR SUCCESSFUL CANINE ARTIFICIAL INSEMINATION

**Cătălin MICŞA[1], Maria Roxana TURCU[1], Nicolae Tiberiu CONSTANTIN[1, 2], Dorin ŢOGOE[1], Alexandru DIACONESCU[1]**

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Splaiul Independentei Street, 050097, District 5, Bucharest, Romania
[2]Research and Development Institute for Bovine Balotesti, Bucharest-Ploiesti Road, km 21, 077015, Balotesti, Ilfov County, Romania

Corresponding author email: catalin_micsa@yahoo.com

***Abstract***

*Artificial insemination (AI) is one of the most frequently implemented assisted reproductive technologies for animals. The dog-breeding industry is extremely dependent on artificial insemination (AI), which enables the successful transfer of genetic material over large distances and its indefinite storage for future use in breeding programs. When natural reproduction is not possible due to male incapacity, receptivity, or physical impairment, AI may also be utilised. The manner in which AI is put into practice in canines differs and is dependent on the variety of sperm utilised. In particular, with preserved or fresh sperm, intrauterine insemination is performed through transcervical catheterization using an endoscope. Endoscopic TCI for frozen sperm offers the advantage of obtaining comparable or superior results while avoiding the requirements and potential risks associated with general anaesthesia and surgery. Undoubtedly, the capacity to perform all inseminations with fresh or refrigerated semen increases the conception rates. This article will centre on the endoscopic transcervical insemination (TCI) method of canine artificial insemination (AI).*

***Key words****: artificial insemination, endoscopy, reproduction, TCI.*

## INTRODUCTION

Transcervical catheterisation (insemination), or TCI, is a minimally invasive technique that involves inserting a tool through the vagina and cervix into the uterus. In large animals, this procedure is possible due to their size and the ability to control the cervix through the rectum (Thomassen et al., 2006; Fontbonne, 2006; Blendinger, 2007b). However, this was not feasible in small animals until about 25 years ago. Scandinavian veterinarians were successful in modifying a fox transcervical catheter for use in female dogs (Linde-Forsberg et al., 2001), which led to the subsequent documentation of a procedure involving the insertion of a catheter into the cervix of a dog using a cystourethroscope designed for humans (Johnston et al., 2001; Santos et al., 1997). The use of a human ureteroscope modified for dogs has enhanced canine transcervical insemination (TCI), enabling more effective cervical manipulation and faster procedures (Silva et al., 1995; Silva et al., 1996).

The TCI procedure, which utilizes advanced equipment, is becoming a standard technique in canine artificial insemination due to its ability to enhance fertility, particularly when using frozen or low-quality semen, as well as overcome the obstacle of a closed cervix that occurs near the end of estrus, when oocytes are still capable of being fertilized (Sum et al., 2009; Rodrigues et al., 2002).

The limited use of transcervical insemination (TCI) in cats has resulted in ineffective attempts to perform the procedure due to the small size of the feline vaginal entrance, which complicates endoscopic exams. Moreover, the procedure of gathering feline seminal material is more difficult compared to dogs, leading to a reduced need for intrauterine deposition of seminal material in cats. However, despite the challenges, TCI manoeuvres have been documented in cats (Feldman et al., 2004; Levy et al., 2007).

When it comes to canine reproduction, using frozen-thawed semen for intrauterine semen deposition has been considered (Nizanski, 2006; Wilson, 1993). However, the outcomes

may be affected by the low quality of the semen after thawing (Fukushima et al., 2010; Meyers-Wallen, 2007). Additionally, the offspring produced using this method are generally smaller compared to those acquired by inseminations using fresh semen (Linde-Forsberg et al., 1999). Intrauterine artificial insemination is a viable option when both the male and female are unable to reproduce naturally due to conditions such as vaginal strictures, orthopaedic issues including fractures, a history of refusing to mate or allow mating, or behavioural factors (Silva et al., 2003).

This study aimed to examine the specific characteristics and challenges associated with performing the endoscopic transcervical intrauterine technique in canines. Additionally, another object of this study was to determine whether challenges in performing the endoscopic transcervical intrauterine artificial insemination (AI) procedure can be resolved through equipment manipulation and practical experience.

It is crucial to determine the most suitable timing for AI performance in female dogs, as the proestrus, oestrus, and ovulatory phases can be lengthy and unpredictable (Arbeiter et al., 1991; Concannon et al., 1989; England et al., 2002). The LH surge is considered the most significant phase of the oestrous cycle as it is closely associated with all reproductive processes, ranging from ovulation to parturition. Research indicates that the LH surge takes place when progesterone levels reach approximately 2 ng/ml (de Gier et al., 2006). Clinical and reproductive parameters, vaginal cytology, hormonal tests, and ovarian ultrasonography are all methods that can be employed to ascertain ovulation (Jackson et al., 1979; Wright, 1990).

## MATERIALS AND METHODS

This study included five healthy female Golden Retrievers, three of which had given birth multiple times (pluriparous) and two of which had never given birth (nulliparous). The age of the dogs ranged from 24 months to 6 years, and their weight ranged from 26.8 to 33.2 kg. The female dogs had a detailed medical history of their reproductive system.

Three mature (1.5-3 years old) male Golden Retrievers with confirmed fertility were chosen based on an examination of their reproductive health, in accordance with the kennel's breeding programme and the quality of their semen. All animals, both females and males, included in this study are from a commercial kennel and are subjected to identical environmental, nutritional, and management circumstances.

The timing of insemination was determined using vaginal cytology, serum progesterone concentrations, and vaginoscopy (Macedo et al., 2012; Pretzer et al., 2006).

All female dogs were brought to the clinic for their initial evaluation 5 to 7 days after the owner noticed swelling or discharge in their vaginal area. Vaginal smears were obtained by inserting a dampened cotton swab into the posterior part of the vagina. Subsequently, swabs were delicately rotated onto glass microscope slides and dyed using Diff Quick (Blendinger, 2007a). Vaginal smears were examined during the initial appointment to determine the stage of the menstrual cycle. Vaginal smears were only evaluated for concerns about the cycle not proceeding as expected, as indicated by progesterone fluctuations (Hase et al., 2000; Hori et al., 2005).

The blood samples were obtained by drawing blood from cephalic veins. The blood was collected and sent to the laboratory for detection of serum progesterone levels. The serum progesterone concentration was measured during the initial visit and then every 3 to 4 days until the LH surge was identified, which is indicated by a progesterone value exceeding 2 ng/mL (Volkman, 2006). Following the LH surge, the concentration of progesterone in the blood was measured every 24 to 48 hours until ovulation was considered to have finished. After determining ovulation based on a progesterone concentration of 2-6 ng/mL, vaginoscopic investigations were started (Jeffcoat et al., 1989). Insemination was only carried out if ovulation was considered complete, indicated by a progesterone concentration over 6 ng/mL. Measurement of serum progesterone was discontinued after reaching a concentration higher than 10 ng/mL (Kim et al., 2007; Kutzler et al., 2003).

Reproductive monitoring began with the completion of anoestrus and the onset of proestrus. The proestrus and oestrous phases were identified by observing behaviour and reproductive factors, as well as analysing vaginal cytology and doing serum progesterone assays. During the proestrus phase, female dogs exhibited signs of male attraction, swelling of the vulva, a discharge of blood-tinged fluid from the vagina, and a reluctance to be mounted by males (Concannon, 2005). In contrast, during the oestrus phase, female dogs displayed male attraction, a discharge of blood-tinged fluid from the vagina, willingness to be mounted by males, and a deviation of the tail. The female dogs were assessed at intervals of 24 to 48 hours until the first artificial insemination was carried out in each dog.

## RESULTS AND DISCUSSIONS

The initial artificial insemination (AI) procedure was conducted during the period when the female dogs were sexually receptive to males, as shown by a vaginal cytology with more than 80% superficial cells and a progesterone concentration exceeding 6 ng/mL. The semen was collected just prior to artificial insemination using digital manipulation. This involved acquiring both the first and second ejaculate fractions (Nizanski, 2006).

Following collection, the sperm's total and progressive motility, speed, and morphology were assessed using bright-field microscopy at a magnification of 100x. The concentration of sperm was measured using a hemocytometer, and the morphology of the sperm was analysed using phase contrast microscopy at a magnification of 1000x (Kustritz, 2007). The semen collected from the male canines in this investigation exhibited a total motility of over 80% and a sperm speed exceeding 4 with CASA System (Computer Assisted Sperm Analysis).

A rigid endoscope with a cover and catheter port or working channel is preferable for vaginal examination in bitches compared to a flexible endoscope. Rigid endoscopes enhance vaginal navigation and facilitate the insertion of flexible catheters and brushes. Regardless of their size, rigid endoscopes can be used for the majority of dogs. Typically, an endoscope should have a length of at least 30 cm to reach the cervix in medium to large dogs. It should also have a tiny diameter to pass through the cranial section of the vagina in small and medium dogs. Authors typically suggest an optical angle ranging from 6° to 30° in order to enhance the visualisation of the cervical aperture. A commonly used instrument for the assessment of vaginal and transcervical insemination (TCI) is a cystoscope with an enlarged urethra and a diameter of 3.5 mm (Concannon, 2004; Linde-Forsberg, 1991).

During the AI procedure, the female dog was placed in a standing position on a table with an antiskid surface and manually restrained by the owner. The front part of the table was inclined at an angle of around 30 degrees towards the ground, causing the hindquarters of the female dog to rest on the higher section of the table.

Typically, the procedures of vaginal examination and intrauterine insemination are generally well-tolerated by individuals without the need for anaesthesia. On average, these procedures may be conducted on female canines while they are positioned upright on a veterinary examination table. Under some circumstances, such as anoestrus and/or limited lumen, an anxious female dog, or vestibulitis/vaginitis, it may be appropriate to provide sedation (e.g., medetomidine, 10-20 μg/kg IV) or general anaesthesia in order to reduce the risk of vaginal trauma and/or damage to equipment (Macedo et al., 2012).

After external cleaning of the vulvar area, the cervix was visualised using a TCI cystoscope (Karl Storz, Tuttlingen, Germany) that had a xenon cold light source and camera. The images were shown on a monitor. Vaginal insufflation was accomplished using a rectal insufflation bulb. A CH-5 transcervical catheter was inserted through the cervix into the uterine body, and semen was gradually introduced for insemination. Following the introduction of semen, the catheter was filled with 1 ml of air to facilitate the deposition of the remaining semen. After the AI procedure, the hindquarters were kept raised for 10 minutes to reduce the possibility of backflow, and manual stimulation was applied to the perineal region (Linde-Forsberg, 2001).

Each female dog was artificially impregnated two times during their reproductive cycle, with

a 24-hour gap between each insemination. Only fresh semen that had been collected beforehand was utilised, as frozen-thawed semen was not suitable due to its low quality after thawing (data not provided). A volume of 5.4 ml was used during insemination in order to reduce backflow.

The entire duration of the procedure, from the introduction of the endoscope to its withdrawal after AI, and the time taken for catheter introduction from the endoscope introduction through the vulva to the introduction in the cervical os, did not exceed 10 to 15 minutes.

The diagnosis of pregnancy was conducted through abdominal palpation and ultrasonographic examination between 25 and 30 days after the estimated LH surge (Fontbonne et al., 2006; Taverne et al., 1985).

There were no observable clinical indications of infection in any of the female dogs during the pregnancy diagnosis. Four out of five female dogs became pregnant, resulting in a conception rate of 80%. The litter size varied from 7 to 11 puppies, and all of them were delivered via caesarean section. Due to the absence of pregnancy in one female dog (the oldest one being 6 years old), a statistical analysis was not conducted. Nevertheless, there were no discernible variations in age, parity, serum P4 concentrations during LH surge and TCI, or sperm quality between the female dogs that successfully conceived and the one that did not conceive.

## CONCLUSIONS

Precise identification of the ovulatory phase is crucial for determining the specific days for insemination. Hence, it is essential to conduct a methodical monitoring of the oestrous cycle utilising various techniques.

Furthermore, in the current study, progesterone levels were measured every two days, as suggested by other researchers, in addition to observing any changes in behaviour. Therefore, it was feasible to determine the exact day of the LH spike, enabling the identification of ovulation. The female dogs were artificially impregnated when the concentration of progesterone in their bodies exceeded 6 ng/mL. This was done to ensure that they were impregnated during their fertile phase, which includes the time of ovulation and maturation of the eggs.

In this investigation, the intrauterine insemination technique was conducted following the method described by Wilson (2003). However, the bitches were restrained on an antiskid surface table with an inclination of approximately 30 degrees to enhance the movement of semen to the upper uterine horns.

The difficulty in catheterizing the cervical canal, which was seen in certain animals, was due to factors such as the placement of the deep cervical tubercle and increased movement, but we achieved success in cervical transposition. This involved the simultaneous manipulation of both the endoscope and catheter, with the catheter positioned precisely in front of the cervical os.

In this study, it was found that all five artificial insemination (AI) procedures were able to successfully perform cervical catheterization in all female dogs. However, the level of difficulty varied across the procedures. Nevertheless, Wilson (1993) provided evidence of changes in the position of the cervical os during the progression of oestrus, likely due to dehydration and contraction of the vaginal folds. It was not feasible to insert a catheter in all the female dogs in that study, especially those with a lengthy vagina and a cervix that was out of reach of the equipment. Some of the dogs had a cervix that was positioned towards the front, while others had a cervix that was positioned towards the front and to the side (Wilson, 2003).

One additional aspect that hindered the process of inserting a catheter into the cervical os in this study was the presence of serosanguinous vaginal discharge. This discharge made it difficult to see the cervical os in certain female dogs. While serosanguinous vaginal discharge was present in the majority of female dogs during vaginoscopy prior to intrauterine artificial insemination (AI), it was only necessary to aspirate the discharge in 15% of the AI procedures. In this study, the recommended method of vaginal discharge aspiration, was successfully performed using a urinary catheter.

The technique is fundamentally simple but requires a significant amount of time, patience, and experience to achieve expertise. It

necessitates a comprehensive understanding of the reproductive tract's anatomy, and examining anatomical specimens is quite beneficial.

Attaching a video camera to the endoscope enables direct instruction under the guidance of a skilled operator, as it provides a visual representation of the ongoing procedure. The obstetrician is able to mentally perceive and comprehend the desired outcome.

Many people consider inserting the catheter into the os to be a difficult task, but it can be accomplished with confidence in a relatively short amount of time. However, it takes longer to become proficient in handling the unique characteristics of different breeds and sizes.

In order for the procedure to be widely embraced, it is crucial that it can be effectively administered to all, or at least the majority, of female dogs. Additionally, given the costly nature of the equipment, it is imperative that a significant number of female dogs can be inseminated by applying the exact same endoscope. Considering the wide variety of breeds available in terms of size and shape, it may appear improbable, but it is actually mostly feasible.

Understanding the constraining variables for each step of the procedure is crucial.

By visualising the cervix, it is absolutely certain that the catheter is inside the uterus. By continuously observing the insemination process, we can ensure that the semen is deposited in the uterus without any backflow. The use of a video camera allows both the client and the operator to witness the intrauterine deposition of the semen.

Overall, by assessing the behaviour and reproductive indicators, conducting serial serum progesterone assays, and examining vaginal cytology, it was possible to determine the LH surge day and ovulation. These procedures proved to be efficient in identifying the optimal time for performing artificial insemination (AIs).

There are precautions taken to keep the procedure as clean as feasible, but it is rarely aseptic. No infections associated with vaginal endoscopy have been identified (Stasi et al., 2001).

The challenges associated with performing the endoscopic transcervical intrauterine artificial insemination procedure in female Golden Retrievers were successfully and resolved by skilful equipment management and practical expertise. The specific characteristics of the technique, such as the challenge of cervical os catheterization, the resistance encountered during semen intrauterine deposition, and the occurrence of backflow, were consistent among the five artificial insemination methods examined in the female dogs that were tested.

One significant constraint of this investigation was identified. The findings of this study were validated through the use of ultrasound to examine the foetuses and gestational sac. However, the exact number of puppies was not established through visual examination. Hence, it remains ambiguous if this approach yields a typical duration of pregnancy and regular deliveries.

Furthermore, the accuracy of the confirmed number of foetuses using ultrasonography may not be as precise as those observed upon delivery. Hence, it is imperative to investigate the duration of pregnancy, the process of giving birth, and the quantity of offspring per birth in forthcoming studies.

The endoscope should not be seen as an exclusive procedure, such as artificial insemination, but rather should be utilised in all relevant circumstances in order to improve experience and proficiency.

Endoscopic vaginal endoscopy is a rapid, non-invasive procedure that can provide additional diagnostic information regarding the phase of the oestrous cycle and to conduct transcervical artificial insemination

## REFERENCES


Arbeiter, K., Dobretsberger, M., Muller, E. and Holzmann, A., 1991. *Indirect detection of ovulation and fertilization in the dog by progesterone level testing.* Zentralbl. Veterinarmed. A 38: 696–701.

Blendinger, K., 2007a. *Physiology and pathology of the estrous cycle of the bitch*. In 56th Congresso Internazinale Multisala SCIVAC, Rimini. Proceedings of the 56th SCIVAC Congress, pp. 73–77.

Blendinger, K., 2007b. *Techniques of artificial insemination by fresh, chilled and frozen semen*. In: 56th Congresso Internazinale Multisala SCIVAC, Rimini. Proceedings of the 56th SCIVAC Congress, pp. 87–89.

Chapwanya, A., Clegg, T., Stanley, P., et al., 2008. *Comparison on the Immulite and RIA assay methods*

*for measuring peripheral blood progesterone levels in Greyhound bitches*. Theriogenology 70, 795–799.

Concannon, P.W., 2004. *Canine breeding management and artificial insemination: techniques and caveats*. In: 29th World Congress of the World Small Animal Veterinary Association, Rhodes. Proceedings (online). Available from: Access 14/12/08.

Concannon, P.W., McCann, J.P., Temple, M., 1989. *Biology and endocrinology of ovulation, pregnancy and parturition in the dog*. Journal of Reproduction and Fertility 39s, 3–25.

Concannon, P.W., 2005. *Understanding and monitoring canine pregnancy*. In: 30th World Congress of the World Small Animal Veterinary Association, Mexico City. Proceedings... (online). Available from: Access 15/01/2009.

de Gier, J., Kooistra, H. S., Djajadiningrat-Laanen, S. C., Dieleman, S. J. and Okkens, A. C., 2006. *Temporal relations between plasma concentrations of luteinizing hormone, follicle-stimulating hormone, estradiol-17beta, progesterone, prolactin, and alpha-melanocyte-stimulating hormone during the follicular, ovulatory, and early luteal phase in the bitch*. Theriogenology 65: 1346–1359.

England, G., Concannon, P.W., 2002. *Determination of the optimal breeding time in the bitch: Basic considerations*. In: Concannon, P.W., England, G., Verstegen, J., et al. (Eds), Recent Advances in Small Animal Reproduction. International Veterinary Information Service, Ithaca, New York.

Feldman, E.C., Nelson, R.W., 2004. *Canine and Feline Endocrinology and Reproduction*, third ed. Saunders Company, Philadelphia.

Fontbonne, A., 2006. *How to perform transcervical catheterization in the bitch*. In: 31st World Congress of the World Small Animal Veterinary Association, Sidney. Proceedings, pp. 721–722. Available from: Access 01/15/2009.

Fontbonne, A., Malandain, E., 2006. *Ovarian ultrasonography and follow-up of estrus in the bitch and queen*. Waltham Focus 16, 22–29.

Fukushima, F.B., Malm, C., Henry, M., et al., 2010. *Site of intrauterine artificial insemination in the bitch does not affect sperm distribution within the uterus.* Reproduction in Domestic Animals 45, 1059–1064.

Hase, M., Hori, T., Kawakami, E. and Tsutsui, T., 2000. *Plasma LH and progesterone levels before and after ovulation and observation of ovarian follicles by ultrasonographic diagnosis system in dogs*. J. Vet. Med. Sci. 62: 243–248.

Hori, T., Hagiuda, K., Kawakami, E. and Tsutsui, T., 2005. *Unilateral intrauterine insemination with prostatic fluid-sensitized frozen caudal epididymal sperm in beagle dogs.* Theriogenology 63: 1573–1583.

Jackson, P. G. and Nicholson, J. M., 1979. *The use of ultrasound to monitor fetal life in a pregnant bitch*. Vet. Rec. 104: 36

Jeffcoate, I.A., Lindsay, F.E.F., 1989. *Ovulation detection and timing of insemination based on hormone concentrations vaginal cytology and the endoscopic appearance of the vagina in domestic bitches*. Journal of Reproduction and Fertility 39s, 277–287.

Johnston, S.D., Kustritz, M.V.R., Olson, P.N.S., 2001. *Breeding management and artificial insemination of the bitch*. In: Johnston, S.D., Kustritz, M.V.R., Olson, P.N.S. (Eds.), Canine and Feline Theriogenology. Saunders Company, Philadelphia.

Kim, Y., Travis, A.J., Meyers-Wallen, V.N., 2007. *Parturition prediction and timing of canine pregnancy.* Theriogenology 68, 1177–1182.

Kustritz, R., 2007. *The value of canine semen evaluation for practioners.* Theriogenology 68, 329–337.

Kutzler, M.A., Mohammed, H.O., Lamb, S.V., et al., 2003. *Accuracy of canine parturition date prediction from the initial rise in preovulatory progesterone concentration*. Theriogenology 60, 1187–1196.

Lévy, X., Fontbonne, A., 2007. *Determining the optimal time of mating in bitches: particularities*. Revista Brasileira de Reprodução Animal 31, 128–134.

Linde-Forsberg, C., 1991. *Achieving canine pregnancy by using frozen or chilled extended semen*. Veterinary Clinics of North America – Small Animal Science 21, 467–485.

Linde-Forsberg, C., 2001. *Intra-uterine insemination in the dog using the scandinavian trans-cervical catheter and a comparison with other methods*. In: Concannon, P.W., England, G., Verstegen, J. (Eds), Recent Advances in Small Animal Reproduction. International Veterinary Information Service, Ithaca, New York.

Linde-Forsberg, C., Holst, B.S., Govette, G., 1999. *Comparison of fertility data from vaginal vs intrauterine insemination of frozen–thawed dog semen: A retrospective study*. Theriogenology 52, 11–23.

Macedo, S. P., Malm, C., Henry, M. R., Telles, L. F., Figueiredo, M. S., Fukushima, F. B., Neves, M. M., de Oliveira Cavalcanti, G. A., Chaves, M. S., Mascarenhas, R. M., de Albuquerque Lagares, M. and Gheller, V. A. 2012. *Endoscopic transcervical intrauterine artificial insemination in Labrador Retriever bitches*. Res. Vet. Sci. 92: 494–500.

Meyers-Wallen, V.N., 2007. *Unusual and abnormal canine estrous cycles*. Theriogenology 68, 1205–1210

Nizański, W., 2006. *Intravaginal insemination of bitches with fresh and frozen-thawed semen with addition of prostatic fluid: use of an infusion pipette and the Osiris catheter.* Theriogenology 66: 470–483.

Pretzer, S.D., Lillich, R.K., Althouse, G.C., 2006. *Single, transcervical insemination using frozen–thawed semen in the Greyhound: a case series study*. Theriogenology 65, 1029–1036.

Rodrigues, B.A., Rodrigues, J.L., 2002. *Endocrinologia reprodutiva na cadela*. Clínica Veterinária 40, 50–58.

Santos, S.E.C., Vannucchi, C.I., 1997. *Inseminação artificial em cães*. Clínica Veterinária 2, 22–24.

Silva, A.R., Cardoso, R.C.S., Silva, L.D.M., 2003. *Principais aspectos ligados à aplicação da inseminação artificial na espécie canina*. Revista Portuguesa de Ciências Veterinárias (RPCV) 98, 53–60.

Silva, L.D.M., Onclin, K., Lejeune, B., et al., 1996. *Comparisons of intravaginal and intra-uterine insemination of bitches with fresh or frozen semen*. Veterinary Record 17, 154–157.

Silva, L.D.M., Onclin, K., Snaps, F., et al., 1995. *Laparoscopic intrauterine insemination in the bitch*. Theriogenology 43, 615–623.

Stasi, K. and Melendez, L. 2001. *Care and cleaning of the endoscope*. Vet. Clin. North Am. Small Anim. Pract. 31: 589–603

Sum, S. and Ward, C. R. 2009. *Flexible endoscopy in small animals*. Vet. Clin. North Am. Small Anim. Pract. 39: 881–902.

Taverne, M. A., Okkens, A. C. and van Oord, R., 1985. *Pregnancy diagnosis in the dog: a comparison between abdominal palpation and linear-array real-time echography*. Vet. Q. 7: 249–255.

Thomassen, R., Sanson, G., Krogenaes, A., et al., 2006. *Artificial insemination with frozen semen in dogs: a retrospective study of 10 years using a non-surgical approach*. Theriogenology 66, 1645–1650.

Volkmann, D.H., 2006. *The effects of storage time and temperature and anticoagulant on laboratory measurements of canine blood progesterone concentrations*. Theriogenology 66, 1583–1586.

Wilson, M.S., 1993. *Non-surgical intrauterine artificial insemination in bitches using frozen semen*. Journal of Reproduction and Fertility 47s, 307–311.

Wilson, M.S., 2003. *Endoscopic transcervical insemination in the bitch*. In: Concannon, P.W., England, G., Verstegen III, J., Linde-Forsberg, C. (Eds). Recent Advances in Small Animal Reproduction. International Veterinary Information Service, Ithaca, New York.

Wright, P.J., 1990. *Application of vaginal cytology and plasma progesterone determinations to the management of reproduction in the bitch*. Journal of Small Animal Practice 31, 335–340.