TRANSCERVICAL INSEMINATION IN THE BITCH

Marion Wilson BVMS, MVSc, MRCVS
Glenbred Artificial Breeding Services Ltd.
The Glen, RD 9, Feilding, New Zealand

Introduction

Intrauterine deposition of semen is an essential component in the successful use of frozen semen; this can be achieved surgically or by the transcervical route. Over the last decade awareness that transcervical insemination (TCI) is possible has led to increasing use of this technique, which removes any risks associated with anaesthesia & surgery. In the past catheterisation of the canine cervix was considered impossible or only possible in anaesthetised patients but current techniques allow it to be a routine procedure, however it requires specialised equipment and expertise. The technique can be used for the insemination of fresh, chilled & frozen semen, for diagnostic procedures, as a research tool and possibly for therapy. The problems faced in any attempt to catheterise the canine cervix relate to its relative inaccessibility.

The purpose of this paper is to look at transcervical insemination in the bitch, with emphasis on the 'New Zealand' endoscopic technique. The limiting factors, problems and how to overcome them from a practical point of view will be considered. Uses for TCI technology will be discussed as well as insemination protocols.

Anatomy of canine reproductive tract

The vagina of the bitch is comparatively longer than that of most species; the author has found that in large breeds such as the St Bernard & Newfoundland the length from vulva to cervix can be up to 29cm.

A well-defined fold, the dorsal median fold (DMF) dominates the cranial portion of the vagina; it extends caudally from the vaginal portion of the cervix (cervical tubercle) ending in another less distinct caudal tubercle.[1] When standard insemination catheters are introduced into this area there is often some resistance and then a distinct 'give,' which may explain why some clinicians believe they do intrauterine inseminations routinely when in fact they are only inseminating into the paracervical area; the true cervix is to be found approximately 2.5 cm cranial to this pseudo cervix. The vaginal lumen in this paracervical area is significantly reduced by the DMF. Cranially the vagina is limited by the fornix and cervical tubercle; the fornix being a slit like space cranioventral to the cervical tubercle.

The cervix lies diagonally across the uterovaginal junction with the cervical canal directed caudo-ventrally from the uterus to the vagina. Consequently the internal orifice of the cervical canal faces almost directly dorsally whereas the external orifice is directed to the vaginal floor. Recent work by Watts et al[2] has revealed that the cervix is patent throughout the reproductive cycle of the bitch, but that cannulation can be performed more readily at some stages of the cycle than others.

TCI Equipment & techniques

The length of the vagina, narrow paracervical area, position of the cervical os, angle and diameter of the cervical canal present significant obstacles to the routine catheterisation of the cervix with standard equipment.
Two methods have been described which allow TCI to be performed routinely in the bitch; they employ different equipment & technique to overcome the obstacles presented.

'Norwegian' method

This technique was first described by Fougner et al[3] as a technique developed for the intrauterine insemination of foxes and was later utilised by Anderson[4] for the intrauterine insemination of frozen semen in the bitch. The equipment consists of a nylon sheath and metal catheter; these are produced in 3 sizes to suit different size bitches.
The Norwegian method has been described in several previous reports.[4,5] The technique requires palpation and fixation of the cervix through the abdominal wall; manipulation of the cervix enables catheterisation with the metal catheter.

'New Zealand' endoscopic method

This technique was developed as part of this authors degree programme.[6] The equipment used is a rigid cysto-urethroscope[a] which comprises a telescope with a 30° oblique viewing angle, a sheath, bridge and cold light source; the working length of the assembled endoscope is 29cm. A video camera can be attached to the endoscope but this is not essential. When this technique is being used for insemination, an 8 French-gauge urinary catheter is appropriate in the majority of bitches, although a 6 French-gauge is sometimes required in small or maiden bitches.

The bitch is restrained in a standing position on a specially designed platform on a table; the platform provides a tie point to the dog's collar and a canvas band around the abdomen which restricts sideways movement and discourages any attempt to sit. The author uses a hydraulic table and chair to ensure optimum position of the bitch relative to the operator during the procedure, these are particularly helpful when the endoscope is being used without the camera.

The endoscope is introduced into the vagina and advanced through the vaginal folds by observing the direction of the vaginal lumen. In proestrus and early oestrus the rounded vaginal folds can make advancing the endoscope more difficult as they tend to fill the lumen; as oestrus progresses dehydration of the folds results in a more obvious route forward. The paracervical area with the DMF & crescentic vaginal lumen is a prominent landmark; the lumen at this point can be quite narrow in some bitches, requiring manipulation of the endoscope to the widest space. This may result in the endoscope being pushed to one side of the DMF in many bitches rather than continuing ventrally under the DMF. The vaginal portion of the cervix appears as a distinct tubercle with the cervical os facing caudo-ventrally or ventrally. The cervical os is located in the centre of a rosette of furrows in most bitches though in some it is less obvious. The catheter is advanced into the cervical os by manipulation of the endoscope and catheter. The ridgidity of the endoscope is used to move the cervical tubercle, line up the os and change the angle of the canal. Once the tip of the catheter is introduced into the os it is steadily advanced using a twisting movement to aid its passage through the cervical canal. For semen deposition it is passed in as far as it will go

[a] Storz Extended Length Cysto-urethoscope: Telescope 30° 10325 B, 3.5mm. Sheath 27027KL Bridge 27027NL

without force. Bitches in oestrus, exhibiting standing behaviour show excellent tolerance to the technique; the author has never found sedation necessary.
Air insufflation is often used in association with endoscopic techniques but when the above procedure is used in the oestrous bitch for insemination and general vaginoscopic assessment it is unnecessary. In the early development of this technique the author used a deflecting mechanism to aid in the manipulation of the catheter but with experience and the acquisition of the current endoscope this is no longer used.

Endoscopic TCI – the practical issues

The cost of the equipment means that it is a major decision to take on board endoscopic TCI in a practice. Veterinarians need to know what they can expect from the technology, how easy it is to learn and what problems & results they can expect.

1. The learning process

The technique is theoretically very simple but takes time, patience and practise to perfect. The attachment of a video camera to the endoscope allows direct training by an experienced operator and is extremely helpful in talking the trainee through the procedure but many people have taught themselves. Most find the prospect of getting the catheter into the os a challenge which they can confidently achieve in a relatively short time; mastering the peculiarities of all breeds and sizes however, takes longer. A thorough knowledge of the anatomy of the reproductive tract is essential and time spent studying post mortem material is invaluable.

2. The limiting factors / problems

For the technique to be widely adopted it is essential that it can be successfully applied to all, or at least, most bitches and it important that the majority of bitches can be inseminated using the same endoscope. Looking at the vast array of breeds presented with regard to size and shape this would appear unlikely but is in fact for the most part possible

a) length of the vagina

The maximum length of the vagina is a critical dimension; however no bitch has so far been examined where the cervix is beyond the reach of the equipment described, so vaginal length is not a limiting factor. Large breeds examined include Great Dane, St Bernard, Mastiff, Irish Wolfhound, Newfoundland, Borzoi, Afghan and Pyrenean Mountain Dog. The external size and shape of a bitch does not accurately predict the internal situation – some medium sized bitches e.g. German Shepherds will have vaginas as long as much larger bitches and Borzoi and Afghan bitches have surprisingly short vaginas.

b) diameter of the paracervix

The one limiting factor identified, is the amount of space in the paracervix– in a small percentage of bitches it is impossible to advance the endoscope through this area. This occurs in some maiden bitches of small or medium sized breeds and in some toy breeds e.g. Chihuahua. There are ways of overcoming this problem in some bitches but in others (probably less than 1%) access to the cervix is impossible; endoscopes of smaller diameter are available but they are also considerably shorter so only solve the problem for toy breeds where the vaginal length is short. The standard endoscope described here has been used successfully on many small/toy breeds e.g. Pugs, Pekingese, Griffon Bruxellois, Cavalier King Charles Spaniel & Miniature Dachshund so is suitable for the majority of bitches. However, where toy breeds are a significant part of the reproduction workload investment in a smaller scope may be necessary.
With the very small toy breeds restraint of the bitch whilst trying to manipulate a relatively long scope is a large part of the problem and sedation may be the answer.

c) identification of the cervical os

Theoretically once the endoscope has passed through the paracervix then it should be possible to catheterise all bitches. The ability to identify the os comes down to experience of what it looks like, where it is usually located and how to search for it. The cervical tubercle varies significantly in appearance in different bitches and at different stage of the oestrus cycle; the os at times is not found in the expected ventral position. There are folds in some bitches which can be mistaken for the os, but obviously there is no opening for the catheter –fluid issuing from the os identifies the correct location.

d) cannulation of the cervix

The angle and diameter of the cervical canal can also cause problems in completing the TCI procedure. Ensuring the catheter advances once the tip is in the os depends on apply pressure at the correct angle. Sometimes progress is limited by the diameter of the canal and a smaller gauge catheter is necessary.

e) visibility

Vaginal discharges resulting in poor visibility can cause significant problems, particularly in the learning phase, but there are several ways of overcoming this.

Most of the problems encountered, result from not appreciating the anatomy of the tract or not having developed the knack of manipulation of the endoscope and catheter together. However, not all bitches are easy to catheterise because of difficult angles, ongoing poor visibility or fidgeting patients and occasionally will defy all attempts from even the most skilled operator.

3.Confidence in the technique

a)intrauterine deposition

With visualisation of the cervix there can be no doubt that the catheter is intrauterine; continued viewing of the insemination process ensures that the semen is deposited in the uterus without back flow occurring. A video camera allows the client to observe the intrauterine deposition of the semen as well as the operator.

b) safety

The risk of trauma or infection resulting from the procedure are important considerations. It is difficult to imagine that the plastic urinary catheter could ever perforate the vaginal or uterine wall during oestrus unless a pathological condition already existed. However the paracervical area can be traumatised by the endoscope by the use of inappropriate force; if advancing the endoscope causes obvious discomfort to the bitch then the procedure should be stopped. When examinations are performed during anoestrus and dioestrus the vaginal walls are thinner and more susceptible to trauma and the bitch is likely to be sedated so does not react to inappropriate handling; extreme care should be taken in these situations.

It has been suggested that TCI could introduce infection to the uterine environment. During proestrus and oestrus, bacteria are routinely isolated from uterus and vagina without apparently causing any problems perhaps due to a greater resistance to infection at this time.[7] Therefore, it is reasonable to assume that advancing a catheter from vagina to uterus at this time will not cause any problems. However, care must be taken to ensure no new infections are introduced as a result of inadequately cleaned equipment or from the environment through poor technique. During dioestrus the situation changes and the uterus, under the influence of high progesterone levels, may be particularly susceptible to trauma and infection requiring special care and aseptic technique at this stage.[2]

4. Cleaning

The equipment can be readily cleaned and disinfected; early reports of long drawn out procedures for endoscope cleaning are not a reality.

5.Results / Uses

a) TCI & frozen semen

This technique provides intrauterine deposition of semen, which is a vital part of frozen semen technology; equally important to the successful use of frozen semen are the timing of insemination, semen quality and bitch fertility. Endoscopic TCI, like any other intrauterine insemination method will only be successful if all these factors are taken into account. It does mean the bitch will be less stressed by the insemination procedure and repeat inseminations are possible using TCI.

Results from a trial comparing Norwegian & endoscopic transcervical insemination demonstrated that conception rates of 83.3% and average litter size of 7.5 were possible on bitches of unknown breeding history.[6] These results compare favourably with results from other trials using frozen semen indicating there are no undesirable effects from transcervical insemination. There are no trials making a direct comparison between surgical & endoscopic transcervical insemination so it is impossible to know if the results are better using TCI. The ability to do repeat inseminations has been reported to increase conception rates or litter size.[8,9] Where the semen is of lower quality repeat inseminations allow more semen to be inseminated over an extended period; repeat inseminations are also useful where the bitch is difficult to time.

b) TCI & chilled semen

Although chilled semen is not compromised to anything like the extent of frozen semen, it is reasonable to assume it is not equivalent to fresh semen. Therefore any technique which enables more sperm to achieve an intrauterine situation must be beneficial to the success rate. TCI allows this to be done without resorting to surgical insemination which may not be an acceptable option to the client for a non-frozen semen insemination.

c) TCI & fresh semen

In any insemination if the semen can be deposited intrauterine then it is reasonable to expect the results may be better than vaginal deposition. In the situation where the semen quality is compromised in any way then TCI will improve the chance of success.

The author uses TCI for all fresh & chilled semen inseminations with exceptional results for pregnancy rates and litter sizes.

d) Other uses for TCI

The endoscope can also be used for routine vaginoscopy to determine the progression through the reproductive cycle as well as for diagnostic vaginoscopy & cystoscopy.

The technique has been used for the hysterographic examination of the bitch and for diagnostic microbiology.

Recently Watts et al have used the method to study the intrauterine environment with respect to microbiology and cytology throughout the reproductive cycle of the bitch giving us valuable research information.[7,10] Use of this method during anoestrus and diestrus requires some modification to the technique. The vaginal mucosa is thinner and more susceptible to damage and bitches may not tolerate the endoscope so well when not in standing heat. The requirement for sedation has been reported, as well as the need for air insufflation.

With the ability to catheterise the cervix and following up on Watts' research comes the possibility of developing new diagnostic procedures & perhaps therapy.

6. Insemination protocols

As already indicated, success with fresh, chilled or frozen semen depends on several factors; insemination at the optimum time is essential. Surgical insemination ethically only allows for a single insemination but with TCI repeat inseminations are possible and may be preferable. When moving from surgical to TCI it is appropriate to re evaluate insemination protocols along with the 'new' technology.

The following thoughts on insemination timing form the basis for the insemination protocols developed by the author in conjunction with endoscopic TCI

- As thawed semen only survives for a short time the aim when using frozen semen is to inseminate when the ova are immediately ready for fertilisation,
- Assuming that ovulation occurs approximately 48hrs after the LH peak, maturation of the ova takes a further 2-3 days and ova remain viable for 2-3days then the optimum insemination timing is LH + 4 -7
- If we have a spread of ovulation, maturation & life span then logically 2 inseminations may prove more successful than a single insemination when using frozen semen
- Our options for identifying this optimum time for insemination are vaginal smears, vaginoscopy, progesterone and LH assay; of which progesterone assay is the most popular.
- We apply a general protocol to interpret progesterone levels for insemination timing but what occurs in each individual is what is important. Experience shows that the initial rise in progesterone level is not always clear-cut and there is significant variation in progesterone profiles between bitches; thus identification of the optimum timing is more difficult in some bitches. Again, repeat insemination may be more successful than a single insemination
- If 2 inseminations are performed using the insemination dose normally used for a single surgical insemination this becomes 'expensive' in terms of semen used and there is definite resistance to doing this from clients. In a small trial there was no significant difference in pregnancy rate or litter size between 2 groups of bitches where the insemination dose was reduced to 25% in the second group – in both groups the dose was divided into 2 inseminations.
- The protocol adopted by the author for endoscopic TCI using frozen semen is therefore to divide the 'normal' dose between 2 inseminations performed at LH +5 & 6 or 7
- Chilled or fresh semen lives longer making insemination timing less critical; a single insemination only is required between LH + 4 & 7 as long as the bitch has been timed on progesterone assay. Repeat inseminations are only performed where bitches are presented without progesterone workup and they look 'early' on smear & vaginoscopy. With compromised semen 2 inseminations are performed at LH + 4 & 6

Summary

The most frequent comment expressed is 'why would I use TCI when I can readily inseminate surgically?'

The benefits of using endoscopic TCI for frozen semen come from being able to achieve the same or better results without the need and risks of general anaesthesia & surgery. The ability to do all fresh and chilled inseminations this way will certainly improve conception rates without the owner having to make a decision about exposing their bitch to the risks of anaesthesia and surgery. The other potential uses open up a whole new field for canine theriogenology. Above all the client response to the technique is overwhelmingly positive. At times the learning process will be discouraging but the end result is worth the effort. The endoscope should not be treated as something special for frozen semen insemination but be used at every opportunity in order to develop experience and expertise in all situations.

References

1. Pineda M H, Kainer R A, Faulkner L D: Dorsal median postcervical fold in the canine vagina. Am J Vet Res 34: 1487-1491, 1973

2. Watts J R, Wright P J: Investigating uterine disease in the bitch: Uterine cannulation for cytology, microbiology and hysteroscopy. J Small Anim Pract 36: 201-206, 1995
3. Fougner J A, Aamdal J, Andersen K: Intrauterine insemination with frozen semen in the Blue Fox. Nord Vet Med 25: 144-149, 1973
4. Andersen K: Insemination with frozen dog semen based on a new insemination technique. Zuchthyg 10:1-4, 1975
5. Linde-Forsberg C: Achieving canine pregnancy by using frozen or chilled extended semen. Vet Cin North Am Small Anim Prac 21:467-486, 1991
6. Wilson M: Some aspects of artificial insemination in the bitch, using frozen semen. MVSc Thesis, Massey University, New Zealand, 1992
7. Watts J R, Wright P J, Whithear K C: Uterine, cervical and vaginal microflora of the normal bitch throughout the reproductive cycle. J Small Anim Pract 37: 54-60, 1996
8. Farstad W, Anderson-Berg K: Factors influencing the success rate of artificial insemination with frozen semen in the dog. J Reprod Fertil Suppl 39:289-292, 1989
9. Linde-Forsberg C, Forsberg M: Fertility in dogs in relation to semen quality and the time and site of insemination with fresh and frozen semen. J Reprod Fertil Suppl 39:299-310, 1989
10. Watts J R, Wright P J, Lee C S: Endometrial cytology of the normal bitch throughout the reproductive cycle. J Small Anim Pract 39: 2-9, 1998