Transcervical endoscopic procedures in the bitch
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Abstract
The use of transcervical endoscopic procedures has revolutionized artificial insemination and diagnosis of infertility in the bitch. Transcervical insemination can be utilized for intrauterine insemination; and is of great benefit during the insemination of chilled or frozen semen and poor quality semen (fresh, chilled or frozen) by improving pregnancy rates compared to vaginal artificial insemination, without the risks of anesthesia and surgery. The endoscope can be used to obtain endometrial culture, cytology and biopsy specimens in the awake, standing bitch, obviating the need for anesthesia and surgery. Endoscopy can also be used for hysteroscopy in the non-pregnant and postpartum bitch to evaluate possible anatomic or pathologic abnormalities of the endometrium. The unique vaginal and cervical anatomy of the bitch requires specialized equipment and training for successful transcervical cannulation, but with practice, clinicians can master this technique and provide useful diagnostics and a unique service to their clientele.

Keywords: Transcervical artificial insemination, culture, cytology, biopsy, hysteroscopy

Introduction
Advanced reproductive techniques in the bitch have increased tremendously over the last 15 – 20 years. The ability to successfully inseminate via intrauterine techniques has increased pregnancy rates and litter size and reduced the need for surgical insemination for intrauterine deposition of sperm. Diagnostic procedures such as endometrial culture, cytology and biopsy as well as hysteroscopy can now all be performed endoscopically rather than surgically. Furthermore, therapeutic procedures such as uterine lavage can now be performed to aid in the treatment of pathologic uterine conditions such as pyometra, mucometra and hydrometra. This paper details the development of these transcervical (TC) procedures, provides detailed descriptions of the techniques, and includes discussion on the various uses of each procedure in a reproductive practice.

Anatomy
A thorough understanding of canine reproductive anatomy is critical to success with TC procedures. The vestibule is short, approximately 2–6 cm long.1-4 The urethral papilla is located on the ventral floor of the vestibule, immediately caudal to the vestibule-vaginal (v/v) junction.1-4 The papilla can be both palpated and visualized. Recognizing the location of the urethral orifice is critical, as it is easily entered during the TC procedure and may cause discomfort for the bitch. The v/v junction is a muscular sphincter that normally provides the second barrier of defense for the cranial reproductive tract against advancement of bacteria or foreign bodies (the first barrier is the vulvar lips and the final barrier is the cervix).1-4 In some bitches strictures or narrowing of the vulvar lips or v/v junction may occur. Additionally, hymenal remnants may be present at the v/v junction (sometimes referred to as ‘tags’) or vaginal septums may begin at the v/v junction. Digital examination is recommended prior to any TC procedure to determine if any anatomic abnormality is present in the caudal reproductive tract, as they are easily missed when passing the endoscope into the vaginal canal due to the difficulty of insufflating the vestibule. The vagina is very long, extending from 10–30+ cm depending on the size of the bitch.1-4

The cranial vagina (also called the paracervical region) begins with the dorsal median fold (DMF), a well-defined fold arising from the vaginal ceiling, that runs from about 3–8 cm posterior to the cervix, leading up to the cervical tubercle.1-4 The most posterior point of the DMF is called the caudal tubercle.1-4 There is clear demarcation between the end of the DMF and the cervical tubercle itself and this is an important landmark during TC procedures.1 There is usually a narrowing in the vaginal canal adjacent to the DMF (particularly in maiden and small breed bitches) requiring gentle pressure to push through.1-4 There may be discomfort displayed by the bitch in this area, particularly as the endoscope is
moved through it. Once through this narrowing there is usually more room around the cervix itself. The most cranial point of the vagina is called the fornix, and it is a blind ended cul-de-sac. The size of the paracervix is important as it may prevent the use of some endoscopes while allowing the use of others; and is the limiting factor in many unsuccessful TC procedures (see below).

The canine cervix sits in a diagonal position at the uterovaginal junction, between 45 and 90 degrees at an upward angle from the vaginal floor. The internal os faces almost directly dorsal and the external os faces the floor of the vagina. The appearance of the cervical lumen and caudal tubercle varies greatly from bitch to bitch. In some bitches there are many small furrows giving the appearance of a head of cauliflower, while in others the furrows are fewer in number giving the appearance of the surface of the brain. The author has found that the morphology of the external os of the canine cervix is highly variable. The cervical lumen may be located directly in the center of the tubercle with a clear opening visible or it may be located deeper in the tubercle with a ventral slit and ‘ramp’ leading to the lumen, which requires manipulation of the cervix with the catheter or endoscope to enter. Because of the steep angle of the cervical lumen and the sharp angle of the uterine body after the cervical canal is traversed, passing a catheter may be difficult even if the cervical lumen can be catheterized. The lumen of the cervix is often narrower in maiden bitches and wider in those that have previously delivered at least one puppy vaginally.1

The character of the vaginal epithelium changes with the stage of the cycle. During proestrus the epithelium is pink and edematous. As the bitch progresses through proestrus the mucosa becomes lighter pink and the edema recedes due to dehydration of the vaginal tissues. As the tissues dehydrate, crenulation or wrinkling of the mucosa becomes evident. When the bitch enters estrus, the mucosa appears blanch white due to thickening of the vaginal wall from keratinization and the diminished bloody supply at the lumenal surface. Throughout estrus and the fertile period, the mucosa appears white and crenulated. An abrupt change in the epithelium occurs with onset of diestrus in that the epithelium becomes blanch red and the wrinkles flatten out. If the mucosa is touched it will blanch and then redden, a phenomenon called rosette formation. As the bitch progresses through diestrus and into anestrus, the mucosa continues to flatten and remains blanchy pink-red. The mucosa in late anestrus is deep pink-red and flat.

Because the colon and bladder both sit dorsal to the reproductive tract, having a full rectum or descending colon or a full bladder may impact the ability to catheterize the cervix. Ensuring the bitch has urinated or defecated prior to attempting catheterization will make the procedure easier. If the bitch will not defecate at the clinic, placing a match (sulfur end in) or a small piece of a cotton applicator swab in the anus and then taking the bitch out to defecate may cause enough rectal irritation to stimulate a bowel movement. The simple act of defecation or urination is sometimes enough to allow passage through the paracervix or to allow presentation of the cervix in such a manner that catheterization is possible.

Types of endoscopes and catheters

Before discussing the types of techniques that can be performed, a brief discussion of the types of endoscopes available and the necessary equipment will be provided. Historically, the first descriptions of transcervical catheterization were with the Norwegian catheter system described Fougner in 1973 and Anderson in 1975. The first descriptions involved use of these catheters for intrauterine insemination (IUI). This system involves a hard nylon outer cannula with a metal stylet running through its center. The stylet has a rounded tip to minimize trauma to the reproductive tract during the catheterization procedure. The cannula is initially passed through the vaginal canal to the area of the dorsal median fold and then past the fold as far as possible into the paracervix. Then the stylet is advanced into the fornix. The cervix is grasped through the ventral abdominal wall and manipulated posteriorly to present the external cervical os toward the tip of the stylet. The tip of the stylet is moved systematically back and forth over the surface of the cervix until the lumen is located. There is a typical ‘cobblestone feel’ of the surface of the cervix and a distinct ‘give’ once the stylet enters the cervical lumen. Once the lumen is entered, the stylet is advanced proximally a few centimeters and then the bitch is inseminated while the cervix is occluded with digital pressure via the abdominal wall. If the stylet is not in the uterine lumen,
there is significant leakage between the stylet and outer cannula outside the bitch, making it obvious that uterine cannulation has not been successful. This technique requires significant practice to master. It can be performed in most small, medium and large breed dogs. Bitches that are very fat or have deep or tense abdomens are difficult to palpate the cervix, making this technique difficult to impossible. The Norwegian catheter may be used for IUI of fresh, chilled, or frozen semen. It has been used for uterine culture as well, but is not used routinely for this purpose (personal communication, Catharina Linde-Forsberg, 2012). Potential complications include trauma to the vaginal wall, cervix, or uterus and vaginal or uterine perforation.  

The first description of endoscopic transcervical catheterization (TCC) was made by Marion Wilson in 1993. She used a cysto-urethroscope for the procedure (Extended Length Cysto-Urethroscope, 27027KL, Karl Storz Imaging, Goleta, CA). The endoscope has a telescope with a 30° oblique viewing angle, an outer sheath, a bridge, and a cold light source. The working length of the endoscope is 29 cm. The system will accept as large as an 8 Fr polypropylene (PP) urinary catheter, although smaller catheters may be used. The diameter of the sheath is 22 Fr (7.3 mm). There is a smaller version of the same endoscope which will accept up to a 6 Fr PP catheter, with an outer dimension of the sheath being 17 Fr (5.7 mm). In smaller or maiden bitches, if the larger endoscope will not fit, the smaller one may allow entrance to the paracervical area. The main disadvantage of the smaller endoscope is that it is difficult to catheterize a tighter cervix because the catheter bends rather than threading up the cervical lumen. Minitube® (Minitube of America, Verona, WI) makes TCI catheters that are made of a sturdier plastic and they have a mandrel which makes the catheter more rigid (see below for discussion on catheters).  

The endoscope sheath can be fitted with an obturator which can be used to initially feed the sheath through the vaginal canal. This is particularly helpful with smaller bitches as the rounded end of the obturator is less likely to get caught in the vaginal folds particularly in the area of the dorsal median fold where the vaginal canal narrows considerably. A video camera can be attached to the telescope, which greatly facilitates the TC procedure. The 30 ° viewing angle provides excellent visualization of the entire vaginal canal, making this an excellent endoscope for vaginoscopy and vaginal exploration (i.e. for foreign bodies or masses). The bridge has a dorsal port that allows passage of a PP catheter or a small grasping or biopsy forceps beside the camera. There are two side ports on the sheath that allow for insufflation of air (or CO₂) or fluid administration.  

Because of the angle of view, and the open sheath, manipulation of the cervix is difficult with this endoscope. If the cervix is not at an appropriate angle, catheterization may not be possible. The inability to get under the cervix with the endoscope to access the lumen may preclude catheterization despite being able to see the location of the external os. Maiden bitches are more difficult to catheterize and may prove impossible to catheterize with this endoscope both due the small size of the paracervix and fornix as well as the lack of stretching of the cervical lumen that occurs following fetal delivery. Small and toy breed bitches may not be able to be catheterized because it is not possible to get the endoscope through the paracervix because there simply is not enough room. Giant breed bitches may not be able to be catheterized because the length of the endoscope is not sufficient to reach the cervix.  

Recently, a human ureteroscope has been adapted for TC endoscopic procedures. The ureteroscope (Karl Storz Imaging, 27002L) has a telescope with a 5° end on viewing angle, an instrument port, sealing system and a cold light source. The working length of the ureteroscope is 43 cm, the outer diameter is 9.5 Fr (3.15 mm) and the system will accept up to a 6 Fr catheter. Standard urinary PP catheters will not work with this ureteroscope as they are not long enough. Minitube® makes a TCI catheter (56 cm) that allows 6-10 cm of the catheter to enter the uterus during an insemination procedure. The ureteroscope has a seal for the working channel through which the catheter passes directly through the center of the instrument. There are two side ports which allow either fluid administration or air (or CO₂) insufflation. The seal can be used repeatedly but tends to tear after multiple uses making insufflation less effective as the seal becomes less tight. These adaptors can be replaced but they are expensive, so making them last as many procedures as possible is ideal.
The ureteroscope has some clear benefits over the cysto-urethroscope. First, its small diameter allows it to be passed easily through the paracervical region of the smallest bitches, regardless of their parity. Second, its length allows it to be used in the largest of bitches since the cervix is easily reached. The 5° angle of view allows the ureteroscope to be placed directly in front of the cervical lumen allowing better access to the cervical lumen. In cases where the cervix is angled to the side, downward or upward, the end of the ureteroscope and the catheter can be used to manipulate the cervix into position to allow catheterization of the cervix even in the most difficult of positions. The ureteroscope can be ‘walked’ up the external os of cervixes with a ‘ramp’, allowing catheterization of these more difficult cases. The ureteroscope is small enough to be passed through the cervix and into the uterus for hysteroscopy.

Once the handling techniques are mastered, the length of time to perform TC procedures is markedly shorter with this instrument than with the cysto-urethroscope. Uterine culture, cytology and biopsy can be performed using the ureteroscope. Cystoscopy can also be performed with this instrument but visualization is not as good as with the cysto-urethroscope. The disadvantages of this instrument are that the amount of light put out by the telescope is much less than the cysto-urethroscope and the angle of view is also much narrower, so this is not a good instrument with which to perform vaginoscopic examination; and the channel in the ureteroscope is quite small, minimizing the number and size of instruments available for use with it. It is very easy to pass this ureteroscope into the urethra with no signs of distress from the bitch. Due to the small size of the end of the ureteroscope, inadvertent or sudden movement by the bitch may hold a greater risk of vaginal perforation; that said, the small size of any perforation will likely heal quite uneventfully without any surgical intervention.

Minitube® has a TCI shunt which can be used to help keep the vaginal canal insufflated continuously during a TC procedure with the ureteroscope. This shunt is basically a large diameter (36 Fr) Foley catheter. It comes in two lengths (21 cm and 16 cm). The catheter is passed beyond the vestibule and the Foley balloon inflated with up to 100 ml of air. The correct amount of air is usually obvious when the bitch’s tail flags as if a copulatory lock has been obtained. The Foley is seated at the v/v junction by pulling caudally against the muscular sphincter there. Then an insufflation hand pump attached to the ureteroscope is used to distend the vagina with air. This air also distends the paracervical area, often lifting the cervix up slightly and making the cervical lumen more accessible. The main advantages of the shunt are that it requires less continuous insufflation by the operator and that it increases the amount of room in the vaginal cavity by keeping it distended with air. When one is learning how to use this instrument, not having to continuously insufflate is helpful. The main disadvantage is that the ureteroscope is less able to be manipulated cranially and caudally because it is ‘locked’ in the O ring in the center of the shunt. If the bitch moves suddenly, there is less control over the end of the ureteroscope and a greater risk of perforating the vaginal canal. Other disadvantages are that if the bitch has a stricture, hymenal remnant or septum, the shunt may be too large to pass through it so a seal cannot be established; and that the ureteroscope tends to ‘stick’ to the O ring if the procedure takes more than a few minutes because any lubrication applied to the ureteroscope will dry – this may result in the O ring popping out of the metal holder, an abrupt loss of air from the vagina and possibly frightening the bitch.

Catheter choices include a standard length urinary PP catheter or the Minitube® TCI catheters. When using the cysto-urethroscope either catheter can be used. With the ureteroscope, only the Minitube® catheters will work. Benefits of the PP catheters include the ability to watch the semen (or fluid) enter the uterus and to know the catheter is empty when semen can no longer be seen; the rigidity of the 8 Fr PP catheter when trying to catheterize a highly mobile cervix; the ability to heat mold the catheter at a slight angle facilitating entry into the cervix; and the ability to cut off the end of the catheter and aspirate fluid that is pooling in the fornix when it obscures the view of the cervical lumen. The disadvantages of the PP catheters include the flexibility of the smaller catheters resulting in them bending rather than threading into the cervical lumen, the need to ‘burp’ the catheter to bleed it of air before threading it all the way into the uterus (this can result in the catheter slipping out of the cervix if the bitch moves suddenly and the need to re-catheterize), the relatively large amount of residual fluid left in the catheter after insemination (up to 0.5 ml in an 8 Fr), and their shorter length such that they can not be

216
used with ureteroscope. The PP catheters can be re-sterilized up to three times as long as they do not become bent during the catheterization process.

The TCI catheters made by Minitube® come in 4, 5, 6 and 8 Fr sizes and are either single port or dual port catheters. Fluid flows proximally from the side port openings (up the uterine horn) regardless of single or dual port status. This helps prevent backflow as long as the liquid is injected very slowly and excessive volumes are not used. They are marked every centimeter on the outside of the catheter, so it is easy to determine exactly how far into the uterus the catheter is seated. They are further marked every 5 - 10 cm with a double or triple hash mark to indicate a set length along the catheter. These catheters come with a removable mandrel (a thin metal stylet) that makes the catheter more rigid during the catheterization process. Once the catheter is seated fully in the uterus, the mandrel is removed. Careful attention should be paid to ensure the end of the stylet does not protrude from the side ports; as can happen if the mandrel becomes bent at all during attempts to catheterize the cervix. Once the mandrel is removed a syringe adaptor must be attached. Care should be taken to fully seat the adaptor on the end of the catheter so there is no leakage and the syringe does not fall off. During the learning process, there are many steps needed with these catheters so having an assistant available is helpful. As one becomes more dexterous with the endoscope and catheters, insemination procedures can be performed by a single person. They are dark gray in color so you cannot see the contents of the catheter as they enter the uterus. The 5 Fr catheter has an inner volume of 0.2 ml, so once all the semen is inseminated, a column of air of 0.2 – 0.3 ml should be injected to completely empty the catheter. The smaller catheters have lower inner volumes, so injecting at least 0.2 ml should be adequate for either 3 or 4 Fr catheters. Because of the small luminal size, these catheters empty almost completely, leaving no residual ejaculate in the catheter, assuming air has been injected following the ejaculate. The manufacturer recommends single use for these catheters, but the author routinely cleans and gas sterilizes them (see next section) up to three times as long as they do not become to bent or distorted during a given procedure.

Sterilization of equipment

Following use of the endoscopes, shunts and catheters, they should be rinsed thoroughly in tap water to rinse away any blood, sperm, cells, or bacteria. Both the inside and the outside of the endoscope, catheters and shunts should be rinsed. After rinsing, sterilization of the metal portions of both endoscopes is recommended with glutaraldehyde (Cidex®, New Brunswick, NJ) for 20 minutes. Then the disinfectant is rinsed clean with tap water, followed by distilled water. The metal is dried with canned air - in particular the ureteroscope should not be allowed to air dry because the channel is so narrow that it can take days for it to dry completely and molds may grow on the interior of the channel in the interim. The cysto-urethroscope can be air dried if it will not be used for at least 24 hours – it should be tapped on end several times during this period to encourage any water drops from clinging to the sides. The seal for the ureteroscope can also be placed in glutaraldehyde and rinsed thoroughly. After initial rinsing as described above, the catheters can be rinsed with Alconox® (Alconox Inc, White Plains, NY), followed by ample tap water to remove any soap residue, and then finally with distilled water, prior to drying completely with canned air. The catheters are then gas sterilized with ethylene oxide (ETO) prior to their next use.

Cytobrushes (see endometrial cytology section) are rinsed initially as above, soaked in Alconox® for 5 – 10 minutes, rinsed thoroughly with tap water followed by distilled water and towel dried prior to ETO sterilization. The biopsy forceps (see endometrial biopsy section) are rinsed with water and cleaned with a soft toothbrush to remove all cells and tissue from the jaws. They are sterilized for 20 minutes in glutaraldehyde rinsed in tap water, followed by distilled water and towel dried. Application of instrument lubricant to the jaws will prolong the life of the instrument prior to ETO or steam sterilization. The TCI shunt catheter is rinsed in tap water, soaked in Alconox®, rinsed with tap water and distilled water, air dried for 24 hours or dried with canned air and then either ETO sterilized or steam autoclaved (using pouch mode on the autoclave). The metal portions of the TCI shunt are rinsed and soaked in glutaraldehyde, rinsed with tap and distilled water and then hand or air dried. They do not need to be autoclaved or gas sterilized but they can be if desired.
The author currently uses the cysto-urethroscope for routine vaginoscopy (masses, foreign bodies, searching for the source of vulvar bleeding, etc) and for uterine lavage during the treatment of pyometra. The 30° angle of view and increased amount of light emitted from the larger telescope is highly beneficial for these procedures as well as for cystoscopy. The author currently uses the ureteroscope for all TC inseminations, uterine cultures, cytologies and biopsies as well as for hysteroscopy.

**Basic catheterization technique**

The bitch should be restrained in a standing position on a table. The use of a hydraulic table is quite helpful as the bitch can be raised or lowered to a comfortable position and adjusted as needed during the procedure. Either a wide belt around the abdomen or an assistant can help hold the bitch in position. No pressure should be applied to the abdomen during the procedure as this will obscure the view obtained by the endoscope. Sedation is rarely necessary, particularly when using the ureteroscope because there is virtually no pressure when passing through the paracervix. If sedation is needed, an extremely low dose of either acepromazine, butorphenol or dexmedetomidine may be administered. Sedation is most likely to be needed when procedures are performed during diestrus or anestrus, when uterine lavage will be performed due to discomfort the bitch may have as the uterus is distended, or during hysteroscopy. Care should be taken not to sedate the bitch so much that she will want to sit down or rest on the abdominal belt or assistant’s arm. In the author’s experience, almost all bitches tolerate this procedure very well during any stage of the cycle, particularly when using the ureteroscope. Warming the end of the scope with a hot water bottle or rice bag so that the bitch is not startled when the cold metal is introduced into the vaginal canal is also very helpful.

The endoscope is introduced at a steep angle to the vulvar lips (60 - 80° from horizontal) and the tip of the endoscope is introduced at the dorsal commissure of the vulva, and directed dorsally, so that the urethral papilla is avoided. The urethra has a streaky red – white appearance and is easily identified when the endoscope is introduced. If the urethra is entered, the endoscope is removed and reintroduced more dorsally. Once the tip of the endoscope clears the pelvic rim the operator’s end of the endoscope is raised to horizontal and insufflation commences. With the cystoscope, minimal insufflation is needed during estrus, while with the ureteroscope, repeated or continuous insufflation is helpful. The lumen of the vaginal canal is located (using the tip of the catheter as a guide) and the endoscope is maneuvered along the vaginal folds to the paracervix. When the DMF is identified, the endoscope is passed along one side of it. If there is not enough room on one side, sometimes backing up and changing sides will allow for easier passage. The more edema that is present, the more difficult the passage, while when there is marked crenulation, passage is easier. If the endoscope is being passed during diestrus or anestrus it is very important to remember that the vaginal wall is much thinner during these stages and so perforation is much more likely to occur with sudden movement or excessive pressure.

Once the endoscope is maneuvered past the DMF, the cervical tubercle is visible suspended from the dorsal wall of the vagina. If the fornix is reached, the endoscope should be pulled caudally 1-2 cm to locate the cervix. Typically, the cervical os is pointing ventrally so the endoscope must be moved underneath the cervix, moving it from one side to the other until the lumen is located. Watching for bloody discharge or for bubbles of air coming from the lumen can sometimes assist in its location. The catheter is lined up with the opening and advanced forward, using the catheter and the endoscope as needed to manipulate the cervical opening into position. If the cervix is pointing sideways, moving the endoscope to the opposite side and pushing anteriorly with the catheter can help reposition the cervix. Do not forget that sending the bitch out to urinate or defecate can radically change the position of the cervix, so if it is difficult to catheterize immediately, sending the bitch out to eliminate may be very helpful. Once the catheter is started in the lumen, rapidly twirling the catheter in one direction or the other can help thread the catheter through the cervix and into the uterus.

If using a PP catheter, as soon as the second port is at the cervical os, ‘burping’ of the catheter of all air should be performed if a TCI is being performed. If other diagnostic or therapeutic procedures are being performed, burping is not necessary so the catheter can be immediately threaded several centimeters into the uterine lumen. Sometimes the catheter will become ‘stuck’ at the junction of the
internal os and uterine body or at the body-bifurcation (b/b) junction. If this occurs the catheter should be backed out slightly and re-advanced using a rapid twirling motion. The catheter should be passed as far in the uterus as possible for AI, culture, cytology and lavage, while for biopsy it is stopped once the biopsy instrument is just inside the uterus.

The learning curve is quite steep with TC procedures, but once enough bitches have been inseminated it becomes easier to learn how to manipulate the cervix and catheter for success. Initially there will be many bitches that are not possible to catheterize but with patience and practice, most bitches can be successfully catheterized. The author recommends practicing only on bitches in estrus until competency is high and then adding on additional procedures during early proestrus, diestrus and anestrus when the procedure will be more difficult or associated with higher risks.

The cervix can be catheterized during any stage of the cycle but the procedure is easiest during proestrus and estrus. This is because the bitch is receptive to the procedure, and the paracervix region is more relaxed making it easier to pass the endoscope through the vagina. Reported complications include endometritis, mucometra, hematometra, vaginitis and vaginal perforation. These complications are more common when procedures are performed during diestrus or anestrus compared to proestrus or estrus. During diestrus and anestrus the vaginal wall is thin and easier to perforate if too much pressure is applied or the bitch moves suddenly. Repeated catheterizations may cause infection.

Transcervical insemination

Transcervical insemination can be used for fresh; chilled; and frozen semen. Insemination can be performed either once or twice during the fertile period. With high quality semen, a single insemination may result in normal pregnancy rates and litter size. Pregnancy rates over 70% can be expected if frozen semen motility is over 40 to 50%. Use of multiple breedings will increase the likelihood of having adequate numbers of viable spermatozoa available at the site of fertilization when oocytes become mature and this will result in increased pregnancy rate and litter size. Intrauterine breeding doses of 200 x 10⁶ sperm are associated with pregnancy rates >80% when quality semen is used. Successful pregnancy may be possible with doses as low as 30 – 50 x 10⁶ sperm per insemination.
**Endometrial culture**

Cultures are best performed in proestrus and estrus. This allows for culturing very close to the time of breeding for an accurate representation of uterine flora. If they are performed in the first two to three days of proestrus, results will be obtained before it is time to breed, allowing for appropriate use of antibiotics. It is important to differentiate normal flora from pathologic bacteria, and this can be a difficult distinction.

For this procedure, sterile saline will be infused into the uterine lumen and then aspirated out and saved for culture.\(^{10-14}\) The author uses a 12 ml syringe of saline, a 3-way stopcock, a 6 ml syringe to collect the sample and a dual port Minitube® TCI catheter. The 12 ml syringe is placed in line with the catheter via the stopcock and the 6 ml syringe attached at a 90 degree angle. The catheter is passed as far into the uterus as possible. A small volume of saline is infused at a time (1-3 ml depending on bitch size). The stopcock is turned ‘off’ to the saline syringe and ‘on’ to the collecting syringe. Then the catheter is moved slightly forward and backward while an assistant gently aspirates. Excessive negative pressure on the collecting syringe will result in the catheter port aspirating endometrial tissue into the port, preventing collection of fluid. This is usually obvious if the catheter will not move easily forward and back. If no efflux is obtained, an additional 1-3 ml of saline is infused. This is repeated until 2-3 ml of efflux is collected. In some cases, backflow of saline out of the cervix will be visible. Care is taken not to get the catheter ports too close to the internal os of the cervix as fluid from the vagina can be aspirated and this will contaminate the sample (if air is found to be filling the collection syringe, the ports are too close to the cervix).

The efflux fluid is divided into two aliquots. One to two milliliters are centrifuged, similar to a urine sediment, and the supernatant removed. The pellet is re-suspended and a culture swab used to soak up the pellet suspension. This is then placed in transport medium (Amie’s without charcoal is ideal) and submitted for aerobic, *Mycoplasma* and *Ureaplasma* cultures. The remaining milliliter is centrifuged similar to a urine sediment and the supernatant is removed and the pellet re-suspended and smeared on a slide and stained with Wright stain or a modified Schorr’s trichrome stain (Diff-Quick®) for cytologic assessment (see below).

Aerobic bacteria are commonly isolated from uterine cultures during proestrus and estrus in low numbers and are similar to the organisms found in the same bitch’s vaginal canal and cervix.\(^{12,14,21,22}\) Bacteria should not be present in the uterus in any significant numbers during diestrus or anestrus, while they are commonly found in the vaginal canal during these stages of the cycle.\(^{10,12-14,21,22}\) Quantification of bacterial numbers is essential for interpretation.\(^{12,14,21}\) Isolation of multiple organisms or a single organism in low numbers is likely not significant, while isolation of a single (or two) organism in high numbers should be considered potentially pathologic.\(^{12,14,21}\) Concurrent evaluation of endometrial cytology can aid in determining the significance of a positive culture (see below). Anaerobes, mycoplasma and ureaplasma are not considered normal uterine flora.\(^{12,14,21}\) Bacterial species found in the uterus are similar to those found in the vagina.\(^{10,12-14,21}\) It does not appear that the bacteria found in the uterus during the TC culture procedure are transported there on the TC equipment, since the same numbers and types of bacteria were found in the uterus on post mortem examinations, where care was taken to avoid vaginal contamination prior to sample acquisition.\(^{12}\) Common pathogens include *Escherichia coli*, *Pasteurella multocida*, *group G Streptococci*, *Staphylococcus intermedius*, and *Proteus mirabilis*.

**Endometrial cytology**

Endometrial cytology can be performed along with cultures in proestrus or estrus or along with biopsy in very early anestrous.\(^{13,14,23,24}\) If cytology is obtained with cultures, the centrifuged efflux pellet provides an excellent sample. If performed in conjunction with biopsy, a small cytobrush (Olympus, BC-201C-1006, 1050 cm working length, brush diameter 1 mm, Melville, NY) can be used to obtain the samples just prior to biopsy. The cytobrushes are very small and highly flexible, so that if the cervical lumen is not lined up so it can be easily catheterized, manipulation of the cervix with the cytobrush will not be possible making samples unobtainable. Cytobrush samples are immediately rolled onto a glass
slide and stained with Wright stain or Diff Quick® and provide excellent cellularity and cellular detail. There are reports of a higher incidence of endometritis associated with cytobrush sampling or when samples are taken during diestrus. In the author’s experience, however, this has not been the case using the 1 mm cytobrush. Repeated sampling reportedly increases the chances of endometritis.

Clumps of endometrial cells appear normal in size, shape and number during proestrus, early diestrus and late anestrus, while they occur in lower numbers during estrus and appear degenerate in late diestrus and early-mid anestrus. These findings are consistent with the regeneration of the epithelium that occurs during anestrus. Single endometrial cells are seen in early diestrus and late anestrus while clumps of endometrial cells are seen in proestrus, estrus, and diestrus and late anestrus. Naked nuclei are seen during proestrus and early diestrus. Bitches with cystic endometrial hyperplasia (CEH) had degenerate endometrial cells, single endometrial cells, foamy cytoplasm and rare neutrophils. Altered numbers of single endometrial cells and naked nuclei are a good differentiator of disease.

Polymorphonuclear leukocytes are the most common white blood cell type seen in all stages of the cycle except anestrus, when lymphocytes and macrophages predominate. Neutrophil numbers are highest during proestrus and estrus or associated with intrauterine infection. Lymphocytes and plasma cells are seen in large numbers during late anestrus and are also associated with uterine infection and subinvolution of the placental sites. Eosinophils were noted during proestrus, estrus and in bitches with SIPS. Watts created a leukocyte scoring system which allows one to compare number and types of WBC in a cytology sample to established norms and determine if inflammation is present (Table 1). Bacteria were normally seen during proestrus and estrus but not during other stages of the cycle. The presence of intracellular bacteria is a hallmark of pathologic inflammation.

### Table 1. Interpretation of endometrial cytology throughout the estrous cycle

<table>
<thead>
<tr>
<th>Stage of cycle</th>
<th>Endometrial cell morphology</th>
<th>Types of leukocyte normally present</th>
<th>WBC score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proestrus</td>
<td>Non-degenerate</td>
<td>Neutrophils</td>
<td>Low numbers</td>
</tr>
<tr>
<td>Estrus</td>
<td>Non-degenerate</td>
<td>Neutrophils</td>
<td>Low numbers</td>
</tr>
<tr>
<td>Diestrus</td>
<td>Non-degenerate to degenerate</td>
<td>Neutrophils</td>
<td>Low numbers</td>
</tr>
<tr>
<td>Early – mid anestrus</td>
<td>Degenerate</td>
<td>Lymphocytes, Neutrophils, Macrophages</td>
<td>6, 3, 1</td>
</tr>
<tr>
<td>Late anestrus</td>
<td>Non-degenerate</td>
<td>Lymphocytes, Macrophages</td>
<td>7, 2</td>
</tr>
</tbody>
</table>

Adapted from Watts 1998. White blood cell score is the ratio of each cell type compared to each other.

Interpretation of endometrial cytology is in its infancy, so clear evidence of pathology needs to be documented with additional positive diagnostics (i.e. culture, ultrasonography) and evidence of clinical disease (i.e. discharge, infertility, absorption, abortion). Cervical discharge, cervicitis and vaginitis commonly accompany endometritis. Endometritis was found to be bacterial in origin 70% of the time and non-infectious 30% of the time. Use of computerized cellular morphometric studies allows differentiation of healthy uterine growth, differentiation and involution from uterine pathology.

### Endometrial biopsy

Endometrial biopsy can be performed at any stage of the cycle but it is not recommended during diestrus using TC techniques as the risk of taking vaginal flora into the uterus and seeding the biopsy sites with bacteria is very high. Biopsies will yield the most information when they are taken during stages of the cycle when there is endocrine activity resulting in increased glandular function and avoiding stages of endometrial repair (mid–late anestrus). So proestrus, estrus or very early anestrus (while the glandular epithelium is still under the influence of progesterone but after progesterone has dropped to baseline) are
the best stages to take samples transcervically. Bitches sampled during proestrus or estrus should not be
bred on that cycle in case unnoticed uterine perforation occurs during the biopsy; which could result in
sperm peritonitis at the time of breeding. Biopsy at any stage of the cycle may result in local endometritis
which in turn may develop into pyometra, so bitches should be treated with antibiotics prophylactically
for seven days after the procedure.26 There are reports of hematomucometra and endometritis/pyometra
following biopsy during proestrus, diestrus and early anestrus.26

Biopsy can be performed with the ureteroscope or the cysto-urethroscope but it is more difficult
with the cysto-urethroscope.26 A 65 cm (working length or longer) long oval jaw biopsy forceps (5 Fr) is
passed into the cervical lumen via the endoscope. Once the forceps are in the uterus, the jaws are opened
and the forceps advanced slowly and gently until slight resistance is felt (author’s method). Alternatively,
the biopsy forceps may be palpated through the abdominal wall and gentle pressure applied manually
as the jaws are closed to ensure tissue is pushed into the jaws.26 The jaws are closed and the forceps quickly
pulled posteriorly to take the sample. The tug of tissue can be felt when it releases from the wall. The
forceps are removed and the sample gently teased out of the jaws using saline flush and the tip of a 25
gauge needle.26 The samples are very small (1-2 mm or less) so care should be taken to avoid too much
handling with the needle as this can cause tissue trauma rendering the samples impossible to interpret.
Three to four biopsies should be taken at different locations in the uterus. If the forceps are advanced,
meet resistance and then there is no resistance, the forceps should be removed—it is possible that the
forceps can penetrate the uterine wall and biopsies of mesentery or intestine may be obtained instead of
endometrium. Biopsies should be submitted for processing in formalin, for routine staining and
interpretation, by a reproductive pathologist familiar with canine uterine histology and pathology. Studies
are currently underway to determine if biopsy samples obtained by endoscopic biopsy techniques are
comparable to those taken surgically or after ovariohysterectomy. One prior study showed that only
31.1% of samples were in agreement, but this was done early in the development of the biopsy technique,
so may not still be true today.26

**Hysteroscopy**

Hysteroscopy is easy with the ureteroscope but is not possible with the cysto-urethroscope in non-
postpartum bitches, while the cysto-urethroscope will pass through the post partum cervix up to day 17-
23.10,13 Hysteroscopy may be performed in late proestrus or estrus when the uterine wall is thicker,
however there is glandular activity and erythrocyte diapedesis present, so the development of endometritis
may be more likely when progesterone rises post-ovulation.27 During proestrus and estrus, the cervix is
edematous and relaxed so passage of the endoscope is often easier (author’s experience). Alternatively,
hysteroscopy can be performed during anestrus when there will less chance of the development of
endometritis after the procedure. Regardless of when the procedure is performed, a week’s course of
prophylactic antibiotics is indicated.27

Early in proestrus, when there is heavy bloody discharge present, visualization is more difficult.14
In anestrous, bitches are less willing to stand quietly and since the uterine wall is thinner, any sudden
movement may increase the risk of perforation of the uterine or vaginal walls. Bitches in late proestrus
and estrus, stand quite receptively to hysteroscopy. It is imperative that the bitch stands without moving
during the procedure, so bitches that are anxious or nervous or are panting heavily are best sedated or
anesthetized to minimize the risk of perforation.10,13,14 Any bitch being examined during anestrus should
be anesthetized to reduce the risk of perforation of the thinner uterine wall. The cervix needs to be
sufficiently relaxed to allow passage of the ureteroscope. It is much easier to perform hysteroscopy in
multiparous bitches that have whelped vaginally than nulliparous bitches. Depending on cervical
anatomy, some bitches may not be able to be examined while others are quite simple and may be
inadvertently examined during other TC procedures by inadvertent movement of the bitch or endoscope.

Hysteroscopy is performed by simply following the TCI catheter into the uterus while insufflating
slowly but continuously. Having an assistant available to insufflate is best so the operator can concentrate
on directing the endoscope up the uterine horns. Overinsufflation may cause discomfort from uterine
stretch receptors and so should be stopped momentarily until the bitch is standing quietly again.
Discharge in the uterine lumen may obscure visualization. Infusion of small amount of saline through the side ports can help clear bloody or purulent discharge from the uterine lumen and improve visualization. Alternatively, saline can be continuously infused, instead of air, to distend the uterus. It is critical to be able to visualize the endometrium at all times during the procedure. It is possible to examine both horns in their entirety in some bitches, while in others positioning of the intestinal tract or bladder may prevent traversing the entire length of horn. The size of the bitch may also dictate how much horn can be visualized; in some giant breed bitches the endoscope may not be long enough to reach the tips of both horns. Biopsy of specific lesions can be taken during the hysteroscopic procedure if indicated.

The normal endometrium is homogenously pink in normal bitches. During diestrus, helical folds of endometrium may be present. The endometrium may be irritated during the procedure resulting in development of petechia due to removal of the epithelium in areas where the endoscope touches the uterine wall.

**Uterine lavage**

Bitches being medically treated for pyometra will benefit from uterine emptying early in the course of treatment. Uterine lavage can be performed with either the cysto-urethroscope or ureteroscope, but is generally easier with the cysto-urethroscope because of the increased field of view, brighter telescope, and ability to use a larger catheter (8Fr rather than 5 Fr or smaller). The cervix is catheterized with the largest catheter possible in a routine manner, using caution when passing the endoscope through the paracervix because the wall is thin during diestrus and perforation is more likely (perforation with pyometra can result in significant peritonitis, so excessive pressure should never be applied). A liter bag of saline or lactated Ringer’s solution is attached to the end of the PP catheter and fluids are run in as quickly as possible. If they do not flow freely they can be hooked up to a fluid pump and forcibly infused. Typically, efflux is immediately seen from the cervix, but if it is not, an appropriate amount of fluids should be added so as not to over-distend the uterus. Aspiration through the catheter can be attempted to recover fluid and purulent discharge if it does not flow freely from the cervix. Having an ultrasound close by during the procedure is helpful. The catheter can easily be visualized with the ultrasound and the operator can easily assess how well the uterus is being evacuated or if the uterus is becoming over-distended without adequate emptying. Once the bulk of the purulent discharge is flushed out, the catheter can be removed. Local antibiotics can be instilled prior to removing the catheter if most of the purulent discharge has been emptied. If excessive discharge remains, the antibiotics will likely not be effective in the face of a large amount of organic material. Lavage provides an almost immediate improvement in demeanor in a bitch with a closed pyometra, so should be considered if the bitch is depressed or febrile or if uterine emptying does not begin early in the treatment course.

**Conclusion**

Transcervical insemination has been used for many years in the bitch with good success. With the advent of better equipment and with more experienced operators, more TCIs and fewer surgical inseminations are being performed, with very high pregnancy rates. Encouraging breeders to use this new technology makes breeding safer and less stressful for the bitch and allows for more than a single breeding thus increasing litter size and pregnancy rates. Furthermore, the use of TC endoscopic procedures has opened up a whole new world of diagnostics for the subfertile bitch. Previously, the only way to obtain such diagnostics was to do surgery and many breeders did not want to be so aggressive with diagnostics to perform them. Interpretation of samples is still in its infancy and work needs to be done comparing biopsy samples obtained via endoscopy to surgical samples to ensure accurate diagnoses are being made. Cultures of large numbers of single organisms are most significant. Cytologic assessment of endometrial pathology is possible and needs to be further compared to biopsy results. Continued research into the uses of and diagnostic results from TC endoscopic procedures are necessary and are occurring.
References