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Collection of tissue and culture samples from the canine reproductive tract

Margaret V. Root Kustritz *

University of Minnesota, College of Veterinary Medicine, 1352 Boyd Avenue, St. Paul, MN 55108, USA

Abstract

Definitive diagnosis of reproductive tract infection or other disease often requires sampling of tissue, either for culture or histopathology. Indications, sample collection technique, possible side-effects and interpretation of results are reviewed. Pertinent facts include: (1) collection of uterine biopsy specimens via laparotomy was associated with higher yield of diagnostic samples and fewer side-effects than other less invasive techniques; (2) vaginal culture samples should be collected from the anterior vagina to minimize number of contaminants in the sample; (3) collection of culture samples from the anterior vagina during proestrus or estrus, in the presence of discharge originating in the uterus, was a non-invasive technique for assessment for uterine infection; (4) samples for bacterial culture from mucosal surfaces, including the vagina and penis, must be quantitated to allow interpretation, with moderate to heavy growth of any single aerobic bacterial organism relevant; (5) mycoplasma and ureaplasma are part of the normal flora of the genitourinary tract in dogs and bitches and, because most laboratories cannot give reliable quantitative results, interpretation of positive results often is difficult; (6) collection of prostatic tissue samples for cytology or culture was more likely to yield a correct diagnosis than submission of ejaculated prostatic fluid.
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1. Ovarian biopsy

Indications for ovarian biopsy include ovarian neoplasia and ovarian cystic disease. Place the bitch under general anesthesia, with preparation for sterile abdominal surgery. Make a ventral midline incision and use retractors to expose the ovaries. Each canine ovary lies within an ovarian bursa, an extension of the mesosalpinx, which is a fold of the peritoneum investing the ovary on each side. To visualize the ovary, a bursotomy must be performed. A wedge of ovarian tissue in the area of interest is removed and placed in formalin. The ovary is closed with absorbable suture in a cruciate pattern. The author is unaware of published studies evaluating the best method of closure of the bursa, if any, and possible effects on future fertility. The abdomen is closed routinely [1]. Removal of the ovary from its protective bursa may traumatize the uterine tube or cause adhesions to develop after sampling; the author is unaware of published studies describing incidence of complications after ovarian biopsy in bitches. The sample should be submitted to a pathologist experienced in interpreting reproductive tract lesions.

2. Uterine cytology

There are no traditional indications for endometrial cytology assessment in canine theriogenology. Cells may be collected at the time of hysterotomy, as described below for uterine biopsy, or may be retrieved

* Tel.: +1 612 624 7290; fax: +1 612 624 0751.
E-mail address: rootk001@umn.edu.

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transcervically. Sedate the bitch if necessary. Pass a narrow diameter, rigid endoscope to the level of the cervix. Pass a 4–7 Fr catheter through the biopsy port of the endoscope. Using the end of the endoscope to stabilize the cervix, rotate the catheter as you gently pass it through the cervix. Infuse and aspirate several milliliters of sterile saline [2]. Another technique that has been described for assessment of the endometrium is hysteroscopy, performed in anesthetized bitches with a 3–4 mm laparascope and air insufflation of the uterus [3]. The transcervical technique using a rigid endoscope as described above was reported successful for sample collection in 215 of 259 bitches (83%), ranging in size from a 3.5 kg Chihuahua to a 54.6 kg Mastiff [2]. Reported complications included vaginal inflammation, vaginal tearing and endometritis; these side-effects were most common when the instruments are forced and when samples are taken during anestrus [2]. Hysteroscopy was associated with inability to visualize the endometrium in bitches with excessive intrauterine discharge or escape of air through the cervix, and with development of petechia or ecchymoses on the endometrium in 50% of the bitches evaluated with this technique [3]. Retrieval of cytology samples via hysterotomy has all the potential negative side-effects of any procedure involving anesthesia and surgery.

Endometrial epithelial cells were degenerated in appearance late in diestrus and throughout anestrus [4]. Bacteria commonly were present during proestrus and estrus but were not retrieved from the uterus at other stages of the cycle in normal bitches [2,4]. Polymorphonuclear leukocytes (PMNs) were the most prevalent white blood cell type during proestrus, estrus and diestrus, and lymphocytes and macrophages were most common during anestrus [4]. The author is unaware of published studies evaluating when during the bitch's estrus cycle practitioners are most likely to collect diagnostic cytologic specimens from the uterus or the probability of identifying uterine disease by examination of cytologic specimens with the true incidence of uterine disease in dogs.

3. Uterine biopsy

Indications for uterine biopsy include prognostic assessment for fertility, and evaluation of degree of cystic endometrial hyperplasia and inflammation. Uterine biopsy is contraindicated in bitches with intrauterine fluid; this can easily be diagnosed by ultrasonography. Place the bitch under general anesthesia and prepare for sterile abdominal surgery. Make a ventral midline incision and expose the uterus. Palpate the uterus and collect biopsy specimens from abnormal and normal areas (for comparison). Compress the uterine horn to ensure retrieval of a full-thickness specimen, using a 2–4 mm skin biopsy punch. Use a 25 gauge needle, carefully remove the sample collected and place it in fixative. The resulting hole may be used for collection of cytology or culture specimens. Close the biopsy site with absorbable suture in a simple interrupted or cruciate pattern [1]. Another technique that has been described involves use of an endoscope as described for uterine cytology. A biopsy forceps is passed transcervically and endometrial biopsy samples retrieved [5]. Complications of the surgical approach are those of anesthesia and surgery. Some speculate that manipulation of a diseased uterus may lead to release of endometrial prostaglandin with luteolysis and possible alteration of the estrus cycle; however, these concerns are not substantiated in the veterinary literature. The endoscopic technique is associated with low probability of collecting a diagnostic sample; in one report, only 31.3% of 45 samples collected were in agreement with pathologic samples retrieved at post-mortem [5]. Another negative side-effect of this technique is development of hematomucometra after sample collection (that was reported in 8 of 12 bitches sampled [5]). The sample should be submitted to a pathologist experienced in interpreting reproductive tract lesions.

4. Uterine culture

Indications for uterine culture include infertility and a history of reproductive tract infection, such as pyometra. The author feels that anterior vaginal culture during proestrus or in the presence of pathologic discharge originating from the uterus is sufficient for documentation of uterine infection and performs direct uterine culture only if the uterus is exposed for some other reasons, such as uterine biopsy or Cesarean section (see Section 7, below).

Uterine culture samples can be collected using the techniques described for collection of uterine cytology specimens, or by introduction of a culture instrument through the hole created during uterine biopsy. Complications are as for those procedures. Presence of bacteria during proestrus and estrus was not uncommon; these bacteria are members of the vaginal flora, which will be described below [2,4,6]. Bacteria were rarely present in normal bitches during other stages of the cycle [6,7]. Of 22 uterine cultures collected at the time of elective ovariohysterectomy, 21 (95.5%) were negative [8]. Anaerobic bacteria and mycoplasma were not considered normal flora in the canine uterus [6].

5. Vaginal cytology

Vaginal cytology is a valuable tool in assessment of stage of the estrus cycle and character of vulvar discharge associated with disease of the genitourinary tract. Moisten a cotton-tipped applicator swab; neither the swab nor the fluid used to moisten it need to be sterile. A speculum may be inserted or the swab itself inserted at the dorsal commissure of the vulva so as to avoid the ventral clitoral fossa. Advance the swab at a 45° angle to about half the length of the swab, rotate the swab and pull it straight out. Roll the swab onto a glass slide, allow it to air-dry and stain for interpretation. No negative side-effects are reported for this procedure. The author has, on occasion, known the cotton to become detached from the applicator stick during the procedure; the cotton always easily was retrieved by digital vaginal examination.

Interpretation of vaginal cytology will not be covered in detail here. Most common interpretations are: (1) degree of cornification, indicative of stage of the estrus cycle; and (2) character of the discharge. Degree of cornification varies in a predictable manner throughout the estrus cycle. Four cell types are described. These are parabasal and intermediate cells (non-cornified cell types) and superficial and anuclear squame cells (cornified cell types). Proestrus is defined cytologically by increasing numbers of cornified epithelial cells and decreasing numbers of PMNs; estrus by 100% cornification with greater than 50% anuclear squames; diestrus by abrupt return to complete non-cornification and influx of PMNs in the first couple of days; and anestrus by scant number of epithelial cells, all of which are non-cornified [9]. Determination of character of discharge directs one to further diagnostics (Table 1) [10,11]. Finally, some vaginal or vulvar neoplasms exfoliate easily and can be diagnosed by collection of cells by impression smear. The best example of this is transmissible venereal tumor, a round cell tumor with pale blue or colorless cytoplasm, small distinct clear intracytoplasmic vacuoles, and numerous mitotic figures [12].

6. Vaginal biopsy

There are few indications for vaginal biopsy. Vaginal prolapse is differentiated from vaginal neoplasia by signalment of the bitch, site from which the mass arises, and variation in size of the mass with stage of the estrus cycle. Most vaginal tumors are not malignant and bleed excessively when biopsied. Biopsy of the vaginal wall in bitches with vaginitis most often results in a diagnosis of chronic ulcerative vaginitis, a diagnosis which does not lead to specific further diagnostics or treatment [10].

Table 1
Rule-outs for vulvar discharge in female dogs

Character of discharge	Rule-outs
Sanguinous	Normal estrus cycle—Proestrus, estrus Coagulopathy—VWD, hemophilia, anticoagulant rodenticide poisoning Vaginal neoplasia Blood cell parasitism—Babesia, Ehrlichia Subinvolution of placental sites
Mucoid	Late pregnancy Urinary tract disease Vaginitis Parturition—post-partum [lochia]
Purulent	Pyometra Vaginitis

Some bitches with vaginitis have numerous follicular lesions in the vaginal wall; these are lymphoid follicles and are a non-specific sign of inflammation. Finally, the occasional bitch with vaginitis may have infiltration of eosinophils, plasma cells or lymphocytes in the vaginal wall [13]. The author has not found vaginal biopsy to be a useful diagnostic tool.

7. Vaginal culture

Indications for vaginal culture include any disorder of the genitourinary tract associated with vulvar discharge and anterior vaginal culture in proestrus for diagnosis of uterine infection. Use a long, guarded swab whenever possible, so as to capture the sample for culture from the anterior vagina with as little contamination as possible. Long culture swabs are available commercially for use in bitches. Equine culture swabs can also be used. No negative side-effects were reported for this procedure. The vagina is not sterile and a larger number of contaminants or normal flora are routinely cultured from the caudal vagina (2.2 isolates per bitch in anestrus and 2.3 isolates per bitch in proestrus) than from the cranial vagina (0.7 isolates per bitch in anestrus and 1.0 isolates per bitch in proestrus) [14]. A larger number of organisms are cultured during estrus than during diestrus or anestrus [15] and a larger number of organisms are retrieved from bitches with reproductive tract disease than from normal bitches [16].

Organisms most commonly isolated from the vagina of normal bitches are listed in Table 2 [8,16–23]. Note that organisms most commonly isolated from bitches with vaginitis are virtually identical [16,24,25]. Because reproductive tract infection is caused by overgrowth of normal flora, the laboratory performing the culture always should be asked to provide a quantitative result,

Table 2
Commonly isolated aerobic bacteria from the vagina of normal bitches and female dogs with vaginitis

Aerobic bacteria isolated from normal bitches	Aerobic bacterial isolated from female dogs with vaginitis
E. coli	*E. coli*
Streptococcus canis	*Streptococcus canis*
Pasteurella multocida	*Staphylococcus intermedius*
Staphylococcus aureus	beta-hemolytic *Streptococcus*
Streptococcus sp.	*Staphylococcus aureus*
beta-Hemolytic *Streptococcus*	*Pasteurella multocida*
alpha-Hemolytic *Streptococcus*	*Proteus mirabilis*
Bacillus sp.	
Proteus mirabilis	
Staphylococcus intermedius	
Staphylococcus sp.	

Organisms listed in order of frequency with which they are cultured from normal or affected bitches.

with culture of any single organism with moderate to heavy growth, or 3+ to 4+ growth on a four-point scale, considered relevant. Anaerobic bacteria are not commonly cultured from the normal vagina but have been retrieved from reproductively normal bitches [19].

Mycoplasma and ureaplasma organisms are part of the normal flora of the canine genitourinary tract. Because they are difficult to culture, many laboratories are hesitant to provide quantitative results, making interpretation of a positive result difficult. In one study comparing mycoplasma and ureaplasma culture results between clinically normal bitches with no fertility history ($n = 16$), known fertile bitches ($n = 10$), known infertile bitches ($n = 27$) and female dogs with vaginitis ($n = 22$), there was no difference in rate of isolation between the groups for either organism [26]. Mycoplasma was cultured from 88% of dogs in that study and ureaplasma from 51% [26].

8. Prostatic cytology—manual ejaculation or prostatic massage

Indications for assessment of prostatic fluid include any clinical signs of prostate disease (discharge of bloody fluid from the prepuce or penis unassociated with urination, apparent hematuria, rectal tenesmus), poor semen quality, infertility, and recurrent urinary tract infections in an intact male dog. Prostatic fluid most easily is collected by manual ejaculation, as described recently in the veterinary literature [27]. Histologically, the prostate is the only secretory tissue in the male dog's reproductive tract [28]; therefore any portion of the ejaculate can be used to assess prostatic fluid cytologically. The first and third fractions of the ejaculate contain fluid from no other portion of the reproductive tract and collection of these samples may allow better localization of disease processes. There are no published negative side-effects of manual ejaculation in dogs. The author is aware of one dog that demonstrated priapism after manual ejaculation that was relieved after 60 min by cool hydrotherapy.

Prostatic massage is indicated in those dogs too painful or apprehensive for successful collection of prostatic fluid by manual ejaculation. Allow the dog to void urine completely. Sedate the dog if necessary. Catheterize the bladder with sterile technique, remove any remaining urine, flush the urinary bladder with 5 mL of sterile saline and aspirate it back. Retract the catheter to the level of the prostatic urethra, guided by rectal palpation. Perform transrectal massage of the rectum for about 1 min. Occlude the urethral orifice. With a clean syringe, slowly inject 5 mL of sterile saline and aspirate it back. The two samples can be evaluated concurrently to help localize disease to the prostate or urinary bladder [29]. There are no reports of negative side-effects of this technique.

Interpretation of prostatic fluid cytology specimens is complicated by dilution of all cell types. Centrifugation of any sample collected and examination of the resulting pellet is more likely to yield a diagnosis than is examination of a sample taken directly from the collection vessel. Interpretation also is complicated by the presence of urinary tract infection; one investigator recommends pre-treating for 24 h with intravenous ampicillin, which will concentrate in the urinary bladder but not penetrate the prostate, before collection of samples [30]. Inflammatory change in prostatic fluid is greater than 80% correlated with inflammation identified histologically [30].

9. Prostatic cytology—fine-needle aspirate

Indications for prostatic fine-needle aspirate (FNA) are as those for prostatic cytology as described above

and for identification of lesions seen on ultrasound. While the author always has been cautious with aspiration of potentially infected prostatic tissue and subsequent creation of a septic tract, there are numerous reports in the veterinary literature documenting FNA for diagnosis or treatment of prostatic disease with no complications [31,32]. Several techniques have been described in the literature, including blind transrectal and perirectal techniques [29]. The author prefers ultrasound-guided transabdominal FNA of the prostate. Place the dog in lateral recumbency and shave the caudal abdominal area. Sedation usually is not necessary. Evaluate the prostate by ultrasound. Prep the area with sterile solution and place a sterile sleeve over the ultrasound probe. While viewing the area of interest by ultrasound, pass a sterile needle through the port on the side of the transducer. The needle is visible as it passes into the prostate. Attach a sterile syringe to the needle and use it to exert negative pressure several times. Release pressure and withdraw the needle. More than one sample may be taken. A diagnostic sample was retrieved 47.0% of the time by FNA in dogs [33]. One report described hematuria, usually of less than 4 days duration, after this procedure [33]. The FNA was positively correlated with histopathologic diagnosis in 80% of dogs with prostatic adenocarcinoma [34] and 75% of dogs with prostatitis [34,35]. Prostatic FNA samples should be submitted to a pathologist for interpretation.

10. Prostatic biopsy

Indications for prostatic biopsy are as for prostatic FNA. Oftentimes, biopsy is used to verify a tentative diagnosis that was not upheld by FNA or to definitively diagnose prostatic adenocarcinoma, which is associated with a grave prognosis. Sedate the dog, place him in lateral recumbency and shave the caudal abdominal region. Evaluate the prostate by ultrasound. Prepare the area with sterile solution and place a sterile sleeve over the ultrasound probe. While viewing the area of interest by ultrasound, pass a triggered biopsy instrument into the prostate. The instrument is visible as it passes into the prostate. Trigger the biopsy instrument and retrieve the sample. Ensure the sample appears to be adequate before reversing sedation; more than one biopsy sample may be taken. A diagnostic sample was retrieved 66.7% of the time with prostatic biopsy in dogs [33]. As with FNA, hematuria, usually of less than 4 days duration, has been reported as a negative side-effect of ultrasound-guided prostatic biopsy [33]. Hemospermia also may be seen. Prostatic biopsy was reported to be a technique with greater diagnostic accuracy than FNA for dogs with prostatic adenocarcinoma. A positive correlation between prostatic biopsy result and histopathologic diagnosis was reported in 89.0% of dogs with prostatic adenocarcinoma [34]. Prostatic biopsy samples should be submitted to a pathologist for interpretation.

11. Prostatic fluid culture

Because the seminal fluid is prostatic in origin, culture of prostatic fluid will be discussed with culture of semen, below. The first and third fractions of the ejaculate are prostatic fluid [28]; the third fraction is less likely to be adulterated with normal flora from the distal urethra. Therefore, collection and submission of the third fraction of the ejaculate may facilitate localization of the infection to the prostate.

12. Prostatic tissue culture

Indications for culture of prostatic tissue are as those for prostatic FNA. Culture samples can be collected by FNA or biopsy. Direct culture of prostatic tissue was 25% more accurate than culture of prostatic fluid alone [30]. In one study comparing prostatic fluid culture to culture of prostatic biopsy specimens, only 11 of 18 dogs with positive cultures from prostatic tissue had positive cultures from prostatic fluid [33]. Finally, histopathologic assessment for presence of infection on biopsy specimens was well correlated with culture results from those tissue specimens [36]. Although more invasive, tissue culture was a more accurate technique for diagnosis of prostate infection.

13. Testicular cytology

Indications for testicular cytology include poor semen quality, infertility, orchitis or epididymitis and assessment of lesions viewed on ultrasound. If orchitis is present and the dog is in great pain, aspiration of the affected testis may not require sedation. However, in most dogs, aspiration of the testis is extremely painful and heavy sedation or general anesthesia is recommended. Place the dog in lateral recumbency or in dorsal recumbency if ultrasound guidance is to be used. Insert a 20 gauge needle attached to a 5 or 10 mL syringe on the midline and aspirate, releasing pressure and redirecting the needle three of four times before withdrawing. Control any hemorrhage with digital pressure. Smear the collected material onto a glass slide, air dry it, fix it with methanol and stain it as for a blood

smear [37,38]. This technique does not allow one to assess normal testicular architecture or progression of spermatogenesis, and number of cells retrieved is low if the testes are softer than normal [37].

Reported negative side-effects include minimal scrotal swelling and erythema in the first 72 h, with histologic evidence of hemorrhage and necrosis followed by focal fibrosis and tubular atrophy by 14 days after sample collection [38]. Libido and semen quality did not change, even in dogs that underwent the procedure at monthly intervals for 7 months [37,38]. One dog (of eight) developed testicular atrophy [38]. Creation of anti-sperm antibodies by penetration of the testicular capsule and subsequent exposure of immunologically privileged tissue to the animal's immune system is a reported concern with this technique, as is formation of sperm granulomas if the epididymis is aspirated [39]. Of three dogs that underwent epididymal aspiration, only one developed anti-sperm antibodies and those were no longer detectable 35 days after sample collection. There was no long-term effect on total sperm output or progressive motility of spermatozoa in dogs in that study [40].

Leydig cells were not identified using this technique. However, Sertoli cells and developing sperm cells were seen. Reported cell types seen in various disease states include PMNs and bacteria in dogs with orchitis, atypical spermatogenic cells in dogs with seminoma and Sertoli cells with hyperchromatic nuclei in dogs with Sertoli cell tumor [37]. Unless one is skilled in cytologic evaluation, testicular aspirate samples should be submitted to a pathologist for interpretation.

14. Testicular biopsy

Indications for testicular biopsy are azoospermia associated with high concentrations of alkaline phosphatase in seminal fluid and identification of lesions visible by ultrasound. In human medicine, testicular biopsy is not considered a high-yield diagnostic procedure if testicular size is significantly decreased [41]. Total scrotal width, as an indicator of testicular size, is correlated with body weight in dogs and can be used as an objective measure [42]. Several techniques for testicular biopsy have been described. Place the dog under general anesthesia and prepare as for castration. Expose the testis of interest by incising through the skin, subcutaneous tissue and tunica vaginalis. Stab the testis through the tunica albuginea with a #10 scalpel blade. Testicular tissue will swell through the incision; shave off this bulging tissue as your sample [43]. An alternative is to introduce a triggered biopsy instrument through the incision and trigger it to retrieve a sample from within the testis [44]. The sample should be placed in fixative; Zenker's or modified Bouin's are preferred to formalin [43].

Incisional biopsy, performed in 39 dogs, and biopsy with a triggered instrument, performed in 36 dogs, were compared by gross and histologic examination of testes removed by castration 1–36 days after biopsy. Overall, incisional biopsy induced gross and microscopic lesions more commonly than biopsy with a triggered instrument [44]. Gross lesions described included hemorrhage (28% of dogs after incisional biopsy, 33% after biopsy with a triggered instrument), and adhesions between the tunica vaginalis and tunica albuginea (44% after incisional biopsy, 3% after biopsy with a triggered instrument). Microscopic lesions were described in 90% of dogs after incisional biopsy, with 51% of these changes described as severe, and in 61% of dogs after biopsy with a triggered instrument, with 5% described as severe. 26% of dogs had maturation arrest at the biopsy site after incisional biopsy, whereas only 2.6% of dogs had maturation arrest at the biopsy site after biopsy with a triggered instrument [44]. The ability of biopsy with a triggered instrument versus incisional biopsy to identify specific disease processes in the testis has not been reported. As with testicular aspirate, formation of anti-sperm antibodies is a concern with disruption of the testicular capsule. Of three dogs undergoing testicular biopsy, two developed anti-sperm antibodies but they were undetectable by 7 and 9 days after biopsy and no long-term effect on total sperm output or progressive motility of spermatozoa was noted [40]. Testicular biopsy samples should be submitted to a pathologist for interpretation. It is recommended by one authority that a pathologist with specific knowledge of canine testicular architecture be consulted.

15. Seminal fluid culture

Indications for seminal fluid culture include evidence of prostate disease, poor semen quality or infertility, recurrent urinary tract infections and recurrent infections of the penis or prepuce. Semen can be fractionated or submitted neat; fractionation may permit localization of infection as described above. Organisms commonly cultured from semen of reproductively normal male dogs are *E. coli*, *Pasteurella multocida*, beta-hemolytic *Streptococcus*, coag-negative *Staphylococcus*, *Staphylococcus intermedius*, , mycoplasma and ureaplasma [17,26,45]. Because a normal bacterial population is present in the distal urethra, quantitative culture should be requested of the

laboratory. Reported significant concentrations of bacteria in semen are greater than 10^5 colony-forming units/mL [46] or greater than 2 $\log_{10}$ than concentration of bacteria from the urinary bladder [collected by antepubic cystocentesis] or the urethra [47]. Cytologic assessment for presence of inflammation should not be used to guide suitability of submission of samples for culture. Although presence of inflammatory cells on cytology was positively correlated with significant growth of bacteria from semen [48], lack of inflammatory cytology did not exclude the possibility of infection. Interpretation of mycoplasma and ureaplasma culture results are complicated by inability of laboratories to provide a quantitative result, as described in the bitch. Mycoplasma was recovered from 72% of semen samples and was not more commonly isolated from infertile male dogs or dogs with reproductive tract disease than from clinically normal dogs [26]. Mycoplasma has been implicated in canine orchitis [49–51]. Ureaplasma was isolated significantly more commonly from male dogs with reproductive tract disease or infertility (69% of samples) than from normal dogs (0%) in one study [26].

In dogs with prostate disease, culture of tissue samples was more definitive than culture of seminal fluid. In one survey of 18 dogs with prostate infection, culture of seminal fluid was positive in only 11 (61.1%) [47]. In another survey, culture of ejaculated prostatic fluid was positively correlated with culture of prostatic tissue in 80% of samples [30]. As stated previously, although more invasive, tissue culture was more accurate than culture of seminal fluid for diagnosis of prostate infection.

16. Penile/preputial cytology

Indications for collection of penile/preputial cytology specimens include presence of discharge or masses on the penile or preputial mucosa and suspicion of an estrogen-secreting tumor. Normal penile and preputial epithelial cells are cuboidal to columnar and contain a healthy nucleus. Occasional PMNs and macrophages normally are present. Round cell tumors, such as TVT, exfoliate easily as described above. Elevated concentrations of estrogen in serum, as may be associated with a functional Sertoli cell tumor in male dogs, cause cornification of preputial epithelial cells, which may be evaluated as for a bitch in estrus.

17. Penile/preputial culture

The most common indication for culture of the penis or prepuce is balanoposthitis, associated with discharge. The normal bacterial population of these mucosal surfaces must be taken into consideration when interpreting culture results. As in the bitch, culture of any single organism with moderate to heavy growth, or 3+ to 4+ growth on a four-point scale, is considered relevant.

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