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Vaginal Cytology in the Bitch and Queen

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Introduction

Vaginal cytology is an underused, simple diagnostic tool that, when coupled with a history, physical examination, and other appropriate diagnostics, enhances one's ability to diagnose and treat diseases of the genitourinary tract in female dogs and, to a lesser extent, female cats. Vaginal cytology allows one to assess character of discharge and to assess whether or not the bitch or queen is under the influence of estrogen. Vaginal cytology does not allow one to localize source of discharge, diagnose brucellosis or other infectious diseases, or determine date of ovulation during estrus. Vulvar discharge may be physiologic or pathologic; may arise from the urinary tract, uterus, or vagina; and may be a manifestation of a systemic condition, such as coagulopathy.[1] Details of diagnosis and treatments are not described; the reader is referred to specific theriogenology texts or sites for that information.[2,3]

Collection

In female dogs, collection of vaginal epithelial cells, inflammatory cells, and any discharge in the vaginal vault is done using a non-sterile cotton-tipped applicator, with or without some sort of speculum. While specula are designed for use in bitches, they are rarely used as they are not well tolerated by all bitches. Some bitches will tolerate a large otoscope cone, through which a cotton-tipped applicator can be passed. Flushing of saline into the vaginal vault with collection of fluid containing cells from the vagina also has been described.[4] To collect a sample using solely a cotton-tipped applicator, moisten the applicator with sterile saline or tap water. Introduce it at the dorsal commissure of the vulva and advance it upward at a 45-degree angle for about half of its length. Roll it and pull it straight out. Studies disagree regarding best site from which to collect a sample. Care should be taken not to sample the clitoral fossa, which always contains cornified cells, potentially confusing interpretation.[4,5]

Roll the swab several times against a clean glass slide and allow it to air dry. Care should be taken not to rub the swab against the slide, so as not to break the cells. The slide can be stained with new methylene blue, and Romanowsky stains, like Diff-Quik (Baxter Healthcare Corporation, Miami FL), often are used. Use of Papanicolaou stain is described but rarely used in clinical settings.[6] Cells are examined under 100× to 1000× magnification. If viewed under oil immersion, a cover slip should be applied first if slides are to be saved, as prolonged exposure to immersion oil will distort epithelial cells over time.[4]

Cats have a short vagina with a significant narrowing just cranial to the vulvar lips. To collect a cytology specimen, a moistened, non-sterile cotton-tipped applicator is introduced straight through the vulvar lips, rolled, and pulled straight out. Slides are prepared as in the dog.

Cell Types

Four types of vaginal epithelial cells commonly are identified. Parabasal cells lie against the basement membrane. They are small and round with a prominent nucleus and low cytoplasm/nucleus ratio. This cell type is always present in the canine vagina. Intermediate cells, when present, are superficial to the parabasal cells. Their morphology is similar to that of the parabasal cells, but they are slightly larger overall and have a higher cytoplasm/nucleus ratio. These two cell types commonly are referred to as non-cornified cells. Superficial cells, also sometimes called superficial intermediate cells, form when parabasal cells are induced to divide by elevation in serum estrogen concentration.[7] Superficial cells are large and often irregular in shape, with a high cytoplasm/nucleus ratio. The nucleus of some superficial cells will fail to take up stain. These large, irregular cells with no

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visible nucleus are anuclear squame cells. Superficial cells and anuclear squames are commonly referred to as cornified cells. Other cell types common on vaginal cytology specimens are polymorphonuclear cells (PMNs or neutrophils), red blood cells (RBCs), and bacteria.

Physiologic Conditions Managed or Diagnosed by Cytology

Breeding Management

Ascertaining the stage of the estrous cycle using vaginal cytology is somewhat subjective. Accuracy is increased by evaluation of the cytology specimen relative to history, physical examination findings, and gross assessment of vulvar discharge and by practice.[8]

Proestrus is the first stage of the canine estrous cycle. This is the follicular stage of the cycle. Estrogen concentrations rise during proestrus and peak at the end of this stage. Serum progesterone and luteinizing hormone (LH) concentrations are low. Vulvar discharge ranging in character from serous to serosanguinous is present. On vaginal cytology, RBCs can be present throughout proestrus. PMNs are present early in proestrus but disappear as estrus nears and the vaginal epithelium thickens.[9] Lymphocytes may rarely be seen. Early in proestrus, the vaginal epithelial cell population is heterogeneous, with great variation in size and shape of cells and their nuclei (Figure 42.1). The vaginal epithelial cell population gradually changes from completely non-cornified to completely cornified over this stage of the cycle (Figure 42.2).[10] On average, cornification is complete about two days before estrogen peaks and about four days before estrus begins.[11]

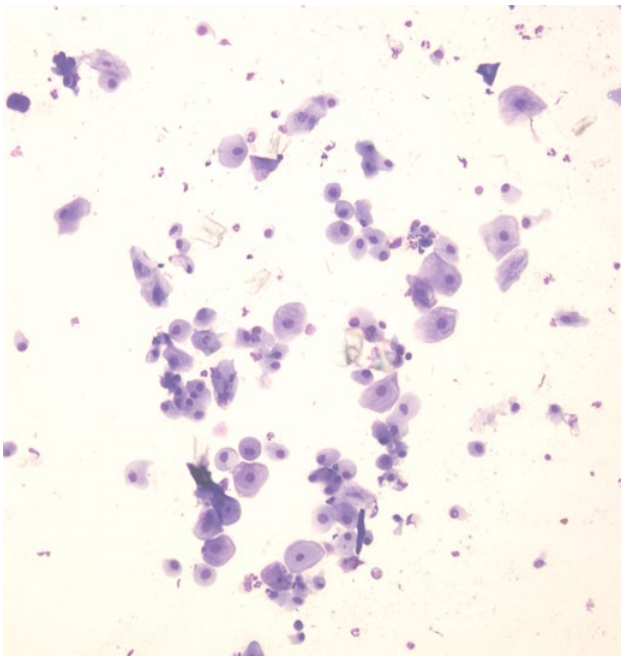

Figure 42.1 Early proestrous canine vaginal cytology (Wright's stain, 400×).

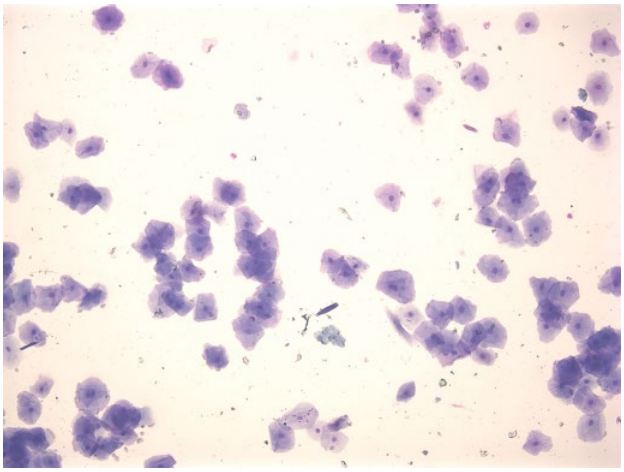

Figure 42.2 Mid to late proestrous canine vaginal cytology (Wright's stain, 400×).

Estrogen concentrations fall at the beginning of estrus. Decreasing estrogen, along with a preovulatory rise in progesterone, is necessary for the appearance of breeding behaviors in the bitch and presumably elicits the LH surge. In the average bitch, a surge of LH is released from the pituitary on or about the first day of estrus and causes ovulation of a primary oocyte two days later. After ovulation, corpora lutea (CLs) form, and progesterone production begins. During estrus, or standing heat, the vulvar discharge can become straw colored but can range from serous to serosanguinous in a normal bitch. The vaginal epithelial cell population is completely cornified throughout estrus, with greater than 50% of the cells anuclear squames (Figure 42.3).

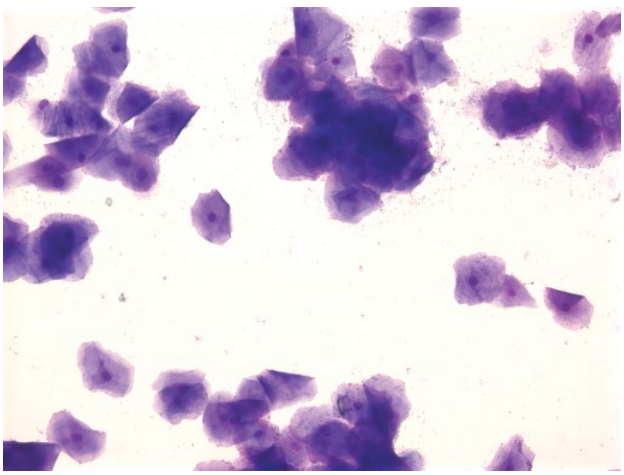

Figure 42.3 Estrous vaginal canine cytology (Wright's stain, 400×).

No PMNs or debris are present. Intra- and extracellular bacteria are commonly present. RBCs may or may not be seen.[4] Vaginal cytology cannot be used to predict ovulation time prospectively; that is best done using serum progesterone and/or LH concentrations.[12,13] Retrospective determination of ovulation date may be possible, as vaginal cytology changes abruptly as the bitch enters diestrus, with this change fairly consistently occurring six days after ovulation.[14]

Use of vaginal cytology to identify spermatozoa is not an accurate indicator of whether or not copulation occurred. Presence of intact spermatozoa or spermatozoal heads confirms breeding, but absence does not verify that breeding did not occur. Intact spermatozoa will be present for only hours after breeding.[15] Spermatozoal heads were identified in about 65% of vaginal cytology specimens collected from bitches one day after breeding.[16]

Diestrus is the luteal phase of the cycle and occurs in all bitches, regardless of breeding or pregnancy status. Small amounts of mucoid vulvar discharge can be present. On the first day of diestrus, the vaginal epithelial cell population abruptly shifts to complete non-cornification. Numerous PMNs can be present, and metestrum cells (non-cornified cells containing leukocytes) and/or foam cells (non-cornified cells containing vacuoles) can be present early in diestrus (Figure 42.4). By mid-diestrus, cytology is characterized by the presence of non-cornified vaginal epithelial cells, occasional PMNs, and some mucoid debris.[10]

Anestrus is a period of reproductive quiescence. Vaginal cytology reveals only a scant number of single cells or small groups of cells, all of which are non-cornified (Figure 42.5).[10]

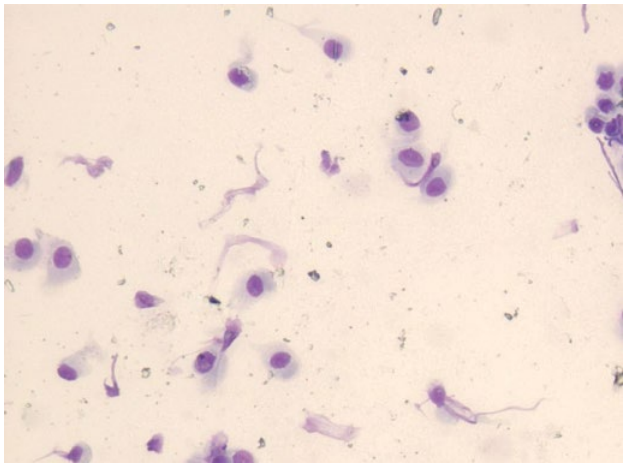

Figure 42.5 Anestrus vaginal canine cytology (Wright's stain, 400×).

Vaginal cytology also can be used to demonstrate stage of the estrous cycle in queens.[17–19] Changes are similar to those described for the bitch. Proestrus is rarely identified cytologically in queens as the first indications of estrus are behavioral and usually are not demonstrated by the queen until she is in cytologic estrus. In estrus, queens are less likely than bitches to demonstrate complete cornification of the vaginal epithelial cells and exfoliate fewer cells. Examples of predominantly cornified and predominantly non-cornified vaginal cytology from queens are described below (see Ovarian Remnant Syndrome Figures 42.6 and 42.7). Some queens do not show behavioral estrus and serial vaginal cytology can help identify estrus. In one study, cats with minimal behavioral signs of estrus were successfully bred using cytology alone.[20]

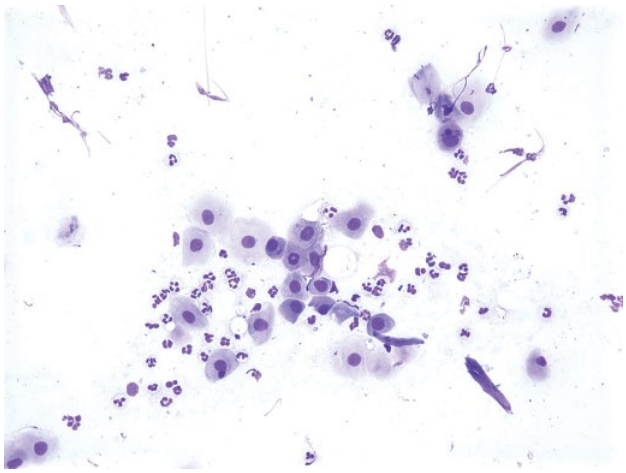

Figure 42.4 Diestrous vaginal canine cytology (Wright's stain, 400×).

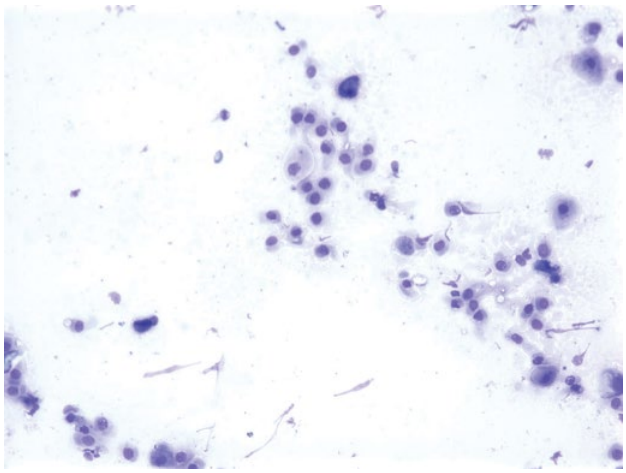

Figure 42.6 Non-cornified feline vaginal cytology (Wright's stain, 400×).

Postpartum

Lochia is the normal postpartum discharge of the bitch. It may be present for up to three weeks after parturition. The discharge varies in color from green to black to red and should decrease in volume over time.[21] It should not be frankly hemorrhagic nor be foul smelling. All vaginal epithelial cells should be non-cornified, and the number of PMNs and bacteria present should not be excessive.

Queens are reported to have a serosanguinous discharge for two to six days. The amount produced is scant, and the queen removes it through normal grooming. RBCs are reported to constitute 75–80% and PMNs 15–20% of cells postpartum, and both diminish to 0 and 1%, respectively, over 20–25 days.[22,23] One study found superficial cells increase to 70% after day 5 postpartum, suggesting an influence of estrogen.[23]

Pathologic Conditions Associated with Cornified Vaginal Epithelium

Ovarian Remnant Syndrome

Ovarian remnant syndrome is the appearance of estrous behavior in previously ovariectomized dogs and cats. The syndrome is more common in cats than in dogs. In cats, vaginal cytology specimens should be collected when the owner perceives the cat to be showing signs of behavioral estrus, including lordosis and repetitive vocalization. Cornified vaginal epithelial cells indicate elevated serum concentrations of estrogen and likely the presence of functional ovarian follicular tissue (Figures 42.6 and 42.7).[24–26] Ovarian remnant tissue can be present at one or both ovarian pedicles or, rarely, elsewhere in the abdomen. Identification of remnants during abdominal exploratory is facilitated by the presence of follicles or CLs, the latter induced to form from follicular tissue by administration of gonadotropin-releasing hormone (GnRH; 25 µg/cat IM).[27]

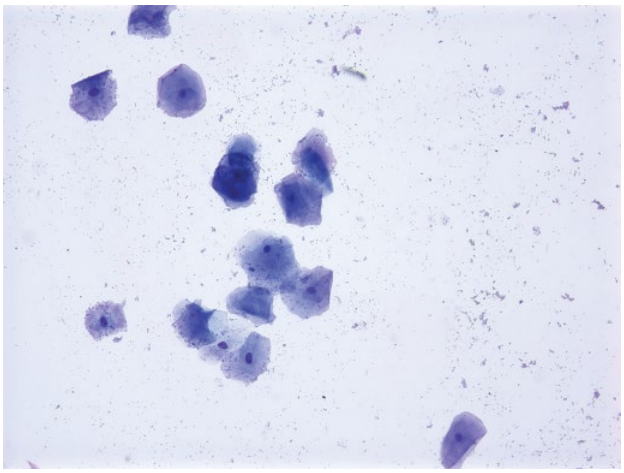

Figure 42.7 Cornified feline vaginal cytology (Wright's stain, 400×).

Ovarian Follicular Cysts

This is an uncommon disorder in bitches and queens. It is most common in young bitches and middle-aged to older queens. Combined length of proestrus and estrus in affected bitches is greater than six weeks, with typical vulvar discharge and behavioral changes. In queens, it may be difficult to identify prolonged signs of estrus as these changes are behavioral and not physical. Vaginal epithelial cells are completely cornified and may appear ragged. PMNs are not present. Bacteria may be visible, adhered to the surface of the epithelial cells.[15] Treatment is by ovariohysterectomy or induction of ovulation (GnRH; 50 µg/dog IM or 25 µg/cat IM).[27,28]

Ovarian Neoplasia

Ovarian tumors are uncommon in bitches and extremely uncommon in queens, most often occurring in older animals (see Chapter 40).[27,28] Granulosa cell tumor is most commonly described, with changes referable to hormone production by the tumor. Combined length of proestrus and estrus in affected bitches is greater than six weeks, with typical vulvar discharge and behavioral changes. In queens, it may be difficult to identify prolonged signs of estrus as these changes are behavioral. Vaginal epithelial cells are completely cornified and may appear ragged. PMNs are not present. Bacteria may be visible, adhered to the surface of the epithelial cells.[28] The ovary usually is grossly enlarged, may be palpable per abdomen, and is visible using ultrasound.

Pathologic Conditions Associated with Non-cornified Vaginal Epithelium and Hemorrhagic Vulvar Discharge

Subinvolution of Placental Sites

Subinvolution of placental sites (SIPS) is an uncommon disorder in dogs. SIPS is most common in young bitches after whelping their first litter. The bitch appears normal, but postpartum discharge continues beyond three weeks. This is a noninflammatory discharge. RBCs usually are

present. All vaginal epithelial cells are non-cornified. Trophoblastic cells with abundant, vacuolated cytoplasm can be shed from the endometrium.[29–32] Diagnosis requires collection of samples from the uterus, and so is more commonly done by excluding metritis and brucellosis. Most cases are self-resolving.

Neoplasia of the Genital Tract

Neoplasia of the genital tract is uncommon (see Chapter 43). Masses may or may not be visible. Perineal swelling can occur. The discharge usually is hemorrhagic with no foul odor. Many RBCs and occasional PMNs are present, and vaginal epithelial cells are non-cornified.[17] Finding of neoplastic cells on vaginal smears is uncommon. Tumors that have been observed with vaginal cytology in dogs include transmissible venereal tumor, transitional cell carcinoma, and squamous cell carcinoma.[31,33–35] Leiomyoma is the most common genital tumor and occurs in aged intact or spayed bitches with finding of atypical mesenchymal cells rarely reported.[36]

Coagulopathies and Blood Parasites

Vulvar discharge associated with coagulopathy may occur in intact or spayed bitches of any age. Reported causes include hereditary (von Willebrand's disease, Factor VII deficiency) and acquired (anticoagulant rodenticide toxicity) coagulopathies.[37–39] Babesiosis and ehrlichiosis are reported to be associated with sanguinous vulvar discharge.[1] Dogs present with a frankly hemorrhagic discharge that often waxes and wanes in volume. The microscopic appearance is of peripheral blood. All vaginal epithelial cells are non-cornified.

Pathologic Conditions Associated with Non-cornified Vaginal Epithelium and Mucoid or Mucopurulent Vulvar Discharge

Metritis

Metritis occurs in postpartum bitches and, rarely, in postpartum queens. Often there is a history of dystocia or retained fetuses or placentas. The vulvar discharge is purulent and foul smelling. This is an inflammatory discharge, with full fields of degenerate PMNs. All vaginal epithelial cells are non-cornified.[32] Diagnosis is straightforward based on the history of recent parturition and presence of purulent vulvar discharge.

Pyometra

Pyometra occurs during or after diestrus and is most common in aged, nulliparous bitches and queens. Animals with closed-cervix pyometra do not have vulvar discharge. Bitches and queens with open-cervix pyometra may or may not show signs of systemic disease (anorexia, polyuria, and polydipsia). The vulvar discharge is purulent and foul smelling, often with the appearance of cream of tomato soup. This is an inflammatory discharge, with full fields of degenerate PMNs. All vaginal epithelial cells are non-cornified.[37] Most bitches and queens also will have an elevated total white blood cell count with a left shift. Diagnosis is by demonstration of uterine enlargement due to accumulation of intrauterine fluid.

Vaginitis

This can occur in prepubertal bitches ("puppy" vaginitis) or in spayed, adult bitches. Puppies rarely show evidence of vaginitis, other than sticky discharge gluing together their vulvar lips. Adult dogs may show evidence of vulvar licking and perivulvar dermatitis. The occasional bitch will have traumatized her perivulvar region. The discharge may be scant, mucoid, and sticky (puppy vaginitis), or can vary from mucoid to purulent with tremendous variation in amount present (adult-onset vaginitis). The discharge rarely is bloody. Cytology varies from mucoid (lacking neutrophils) to purulent.[40] Nuclear streaming and proteinaceous debris often are present. Lymphocytes and macrophages can be seen with chronic vaginitis.[31] Vaginal epithelial cells all are non-cornified (Figure 42.8). If cornified cells are present, especially if the discharge appears bloody, ovarian remnant syndrome should be considered.[41]

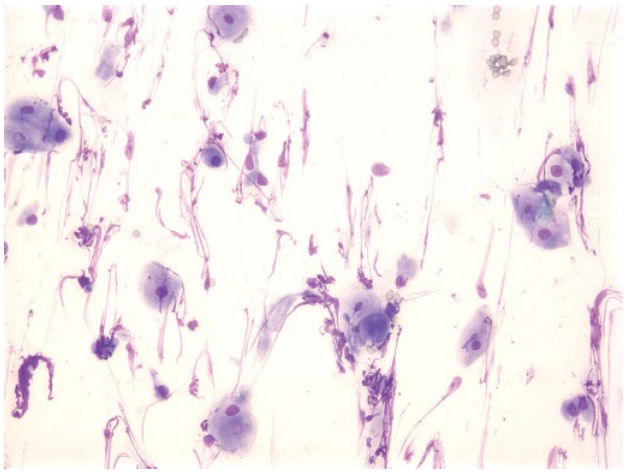

Figure 42.8 Vaginal cytology from a dog with vaginitis (Wright's stain, 400×).

Puppy vaginitis is diagnosed on inspection and usually resolves spontaneously. Adult-onset vaginitis is diagnosed by vaginoscopy and investigation for underlying urinary tract disease or vaginal anatomic anomalies.

Brucellosis

Canine brucellosis usually presents as late-term abortion. Bitches also may present with persistent vulvar discharge that varies from mucopurulent to purulent and may be blood-tinged. All vaginal epithelial cells are non-cornified.[42] Brucellosis is reportable and has zoonotic potential. The rapid slide agglutination test (RSAT) is a good screening test for suspect cases. Animals that test negative are free of infection, provided testing is performed 8–12 weeks after exposure and the animal has not received antibiotics. Animals positive with the RSAT should have the result verified by agarose gel immunodiffusion (AGID) or PCR testing.

References

1 Root Kustritz, M.V. (2006). Collection of tissue and culture samples from the canine reproductive tract. *Theriogenology* 66: 567–574.

2 Root Kustritz, M.V. (2010). Clinical Canine and Feline Reproduction: Evidence-Based Answers. Ames, IA: Wiley-Blackwell.

3 Root Kustritz, M.V. (2016). Books and articles. https://sites.google.com/a/umn.edu/margaret-v-peggy-root-kustritz/home (accessed 9 May 2016).

4 Johnston, S.D., Root Kustritz, M.V., and Olson, P.N. (eds.) (2001). Vaginal cytology. In: Canine and Feline Theriogenology, 32–40. New York, NY: W.B. Saunders.

5 Hiemstra, M., Schaefers-Okkens, A.C., Teske, E., and Kooistra, H.S. (2001). The reliability of vaginal cytology in determining the optimal mating time in the bitch. *Tijdschr Diergeneeskd* 126: 685–689.

6 Perez, C.C., Rodriguez, I., Dorado, J., and Hidalgo, M. (2005). Use of ultrafast Papanicolaou stain for exfoliative vaginal cytology in bitches. *Vet Rec* 156: 648–650.

7 Bell, E.T., Christie, D.W., and Younglai, E.V. (1971). Plasma oestrogen levels during the canine oestrous cycle. *J Endocrinol* 51: 225–226.

8 Moxon, R., Copley, D., and England, G.C.W. (2010). Quality assurance of canine vaginal cytology: a preliminary study. *Theriogenology* 74: 479–485.

9 Groppetti, D., Pecile, A., Barbero, C., and Martino, P.A. (2012). Vaginal bacterial flora and cytology in proestrous bitches: role on fertility. *Theriogenology* 77: 1549–1556.

10 Groppetti, D., Aralla, M., Bronzo, V. et al. (2015). Periovulatory time in the bitch; what's new to know? Comparison between ovarian histology and clinical features. *Anim Reprod Sci* 152: 108–116.

11 Concannon, P.W. (2011). Reproductive cycles of the domestic bitch. *Anim Reprod Sci* 124: 200–210.

12 Holst, P.A. and Phemister, R.D. (1975). Temporal sequence of events in the estrous cycle of the bitch. *Am J Vet Res* 36: 705–706.

13 Bouchard, G.F., Solorzano, N., Concannon, P.W. et al. (1991). Determination of ovulation time in bitches based on teasing, vaginal cytology, and ELISA for progesterone. *Theriogenology* 35: 603–611.

14 Holst, P.A. and Phemister, R.D. (1974). Onset of diestrus in the beagle bitch: definition and significance. *Am J Vet Res* 35: 401–406.

15 Whitacre, M.D., Yates, D.J., VanCamp, S.D., and Meuten, D.J. (1992). Detection of intravaginal spermatozoa after natural mating in the bitch. *Vet Clin Pathol* 21: 85–87.

16 Olson, P.N. (1989). Exfoliative cytology of the canine reproductive tract. *Proceedings of the Annual Meeting of the Society for Theriogenology*, Coeur d'Alene, ID (29–30 September 1989).

17 Mills, J.N., Valli, V.E., and Lumsden, J.H. (1979). Cyclical changes of vaginal cytology in the cat. *Can Vet J* 20: 95–101.

18 Kanca, H., Karakas, K., Dalgic, M.A. et al. (2014). Vaginal cytology after induction of ovulation in the queen: comparison of postoestrus and dioestrus. *Aus Vet J* 92: 65–70.

19 Shille, V.M., Lundström, K.E., and Stabenfeldt, G.H. (1979). Follicular function in the domestic cat as determined by estradiol-17 beta concentrations in plasma: relation to estrous behavior and cornification of exfoliated vaginal epithelium. *Biol Reprod* 21: 953–963.

20 Root, M.V., Johnston, S.D., and Olson, P.N. (1995). Estrous length, pregnancy rate, gestation and parturition lengths, litter size, and pre-weaning mortality in the domestic cat. *J Am Anim Hosp Assoc* 31: 429–433.

21 Johnston, S.D., Root Kustritz, M.V., and Olson, P.N. (eds.) (2001). Canine parturition: eutocia and dystocia. In: Canine and Feline Theriogenology, 105–128. New York, NY: W.B. Saunders.

22 Blanco, P.G., Rodríguez, R., Batista, P.R. et al. (2015). Bidimensional and Doppler ultrasonographic evaluation of postpartum uterine involution in the queen. *Theriogenology* 84: 82–85.

23 Sendag, S., Alan, M., Eski, F. et al. (2016). Postpartum uterus involution observed by real-time ultrasound scanning and vaginal cytology in Van cats. *J Feline Med Surg* 18: 954–958.

24 Miller, D.M. (1995). Ovarian remnant syndrome in dogs and cats: 46 cases (1988–1992). *J Vet Diagn Invest* 7: 572–574.

25 Wallace, M.S. (1991). The ovarian remnant syndrome in the bitch and queen. *Vet Clin North Am Small Anim Pract* 21: 501–507.

26 Demirel, M.A. and Acar, D.B. (2012). Ovarian remnant syndrome and uterine stump pyometra in three queens. *J Feline Med Surg* 14: 913–918.

27 Johnston, S.D., Root Kustritz, M.V., and Olson, P.N. (eds.) (2001). Disorders of the feline ovaries. In: Canine and Feline Theriogenology, 453–462. New York, NY: W.B. Saunders.

28 Johnston, S.D., Root Kustritz, M.V., and Olson, P.N. (eds.) (2001). Disorders of the canine ovary. In: Canine and Feline Theriogenology, 193–205. New York, NY: W.B. Saunders.

29 Sontas, H.B., Stelletta, C., Milani, C. et al. (2011). Full recovery of subinvolution of placental sites in an American Staffordshire terrier bitch. *J Small Anim Pract* 52: 42–45.

30 Dickie, M.B. and Arbeiter, K. (1993). Diagnosis and therapy of the subinvolution of placental sites in the bitch. *J Reprod Fertil Suppl* 47: 471–475.

31 Olson, P.N., Thrall, M.A., Wykes, P.M. et al. (1984). Vaginal cytology. I. A useful tool for staging the canine estrous cycle. II. Its use in diagnosing canine reproductive disorders. *Compend Contin Educ Dent* 6: 288–390.

32 Orfanou, D.C., Ververidis, H.N., Boscos, C.M., and Fthenakis, G.C. (2010). Post-partum pathological conditions in the bitch-part II. *Eur J Compan Anim Pract* 20: 119–135.

33 Johnston, S.D., Root Kustritz, M.V., and Olson, P.N. (eds.) (2001). Disorders of the canine vagina, vestibule, and vulva. In: Canine and Feline Theriogenology, 225–242. New York, NY: W.B. Saunders.

34 Erünal-Maral, N., Findik, N., and Aslan, S. (2000). Use of exfoliative cytology for diagnosis of transmissible venereal tumour and controlling the recovery period in the bitch. *Dtsch Tierarztl Wochenschr* 107: 175–180.

35 Magne, M.L., Hoopes, P.J., Kainer, R.A. et al. (1985). Urinary tract carcinomas involving the canine vagina and vestibule. *J Am Anim Hosp Assoc* 216: 767–772.

36 Ozmen, O., Haligur, M., and Kocamuftuoglu, M. (2008). Clinocopathologic and immunohistochemical findings of multiple genital leiomyomas and mammary adenocarcinomas in a bitch. *Reprod Domest Anim* 43: 377–381.

37 Johnston, S.D., Root Kustritz, M.V., and Olson, P.N. (eds.) (2001). Disorders of the canine uterus and uterine tubes (oviducts). In: Canine and Feline Theriogenology, 206–224. New York, NY: W.B. Saunders.

38 Hamilton, H., Olsen, P.N., and Jonas, L. (1985). Von Willebrand's disease manifested by hemorrhage from the reproductive tract: two case reports. *J Am Anim Hosp Assoc* 21: 637–641.

39 Wheeler, S.L., Weingand, K.W., Thrall, M.A. et al. (1984). Persistent uterine and vaginal hemorrhage in a beagle with factor VII deficiency. *J Am Vet Med Assoc* 185: 447–448.

40 Root Kustritz, M.V. (2008). Vaginitis in dogs: a simple approach to a complex condition. *Vet Med* 103: 562–567.

41 Johnson, C.A. (1991). Diagnosis and treatment of chronic vaginitis in the bitch. *Vet Clin North Am Small Anim Pract* 21: 523–531.

42 Root Kustritz, M.V. (2005). Pregnancy diagnosis and abnormalities of pregnancy in the dog. *Theriogenology* 64: 755–765.