

## 36

### Reproductive Evaluation of the Mare

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#### 36.1 Purpose

- To evaluate the mare for breeding soundness.
- Examination includes inspection of the perineal conformation and caudal reproductive tract, palpation per rectum and transrectal ultrasonography to examine the uterus and ovarian structures, and collection of samples for endometrial culture, cytology, and biopsy to determine the status of uterine health and formulate a prognosis for the potential for fertility.
- Reproductive examination of the mare is important for pre-breeding assessment, for pre-purchase examination if the mare is expected to be a broodmare, for investigation of causes for reproductive failure, and for identification of reasons for changes in behavior.
- To record the reproductive history and examination findings following a systematic approach (Figure 36.1, Table 36.1 and 36.2).
- Disposable vaginal speculum
- Pen light or any other light source suitable for illuminating the vaginal speculum
- Uterine culture swabs, double guarded
- Uterine cytology brushes
- Glass slides
- Portable ultrasound unit equipped with a 5 or 7.5 MHz linear transducer
- Large alligator-jaw forceps for endometrial biopsy
- Bouin's fixative solution or 10% phosphate-buffered formalin to preserve the biopsy specimen.

#### 36.4 Restraint and Positioning

#### 36.2 Complications

- Iatrogenic rectal tears (see Table 16.1, page 182).

#### 36.3 Equipment Required

- Non-sterile and sterile shoulder-length obstetrical sleeve for palpation per rectum and per vaginal, respectively.
- Non-sterile and sterile lubricants
- Roll cotton for perineal asepsis
- Betadine scrub
- Bucket with disposable liner to hold water for perineal asepsis.
- The reproductive examination should preferably be conducted with the mare restrained in stocks.
- The degree of restraint will depend on the disposition of the mare and whether the mare has been previously subjected to serial examinations per rectum.
- Application of a nose twitch might be indicated if the mare appears to resent the examination.
- Administration of alpha-2 agonists (xylazine, detomidine, etc.) or acepromazine are good options for sedating mares that are nervous or unfamiliar with the examination (see Chapter 9).
- For mares that strain excessively, the administration of an anti-spasmodic drug (Buscopan™, N-butylscopolammonium bromide at the label dose of 0.3 mg/kg body weight, slowly IV) may prove of benefit.

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**Table 36.1** Relevant history information prior to breeding soundness examination in the mare

General	Reproductive specific
Age	Duration of estrus
When and why termination of performance career	History of vulvar discharge
Medications received during performance career	History of behavior during anestrus
History of abdominal surgery	History of behavior during breeding season
History of hindlimb lameness	Method of breeding: natural cover, artificial insemination with fresh, cooled or frozen semen
History of trauma	Number of pregnancies
Vaccination history	Number of foals delivered
Diet	Number of abortions
Medical problems in general	Number of early embryonic death
	Number of stillbirths
	Number of twin pregnancies
	Number of neonatal deaths
	History of dystocia

**Table 36.2** Systematic approach to a complete breeding soundness examination in the mare

Steps	Purpose
Examination of the vulva and perineal region	Evaluation of anatomical relationships provides information concerning the risk of reproductive problems (see Table 36.3)
Clitoral swabbing	Required by regulatory bodies for screening for contagious equine metritis (CEM)
Manual examination of the internal genital tract per rectum	Rectal palpation of the ovaries and uterus provides important information concerning the mare's reproductive status Most importantly is the determination of whether or not the mare is pregnant
Transrectal ultrasonography	Ultrasonographic examination of the ovaries and uterus provides further refinement of the information obtained by rectal palpation Ultrasonographic confirmation that the mare is not pregnant prior to any vaginal, cervical or intrauterine procedures is critical
Vaginal examination	Useful in identifying the stage of the cycle of the mare, as well as the presence of anatomical, and possibly pathological, abnormalities.
Digital examination of the vagina and cervix	Allows detection of lesions in the vagina or cervix, including tears and adhesions
Endometrial culture swabbing	Obtain a sample for microbial culture and sensitivity Endometrial cytology should be performed concurrently to aid the interpretation of the culture results
Endometrial cytology	Detection and evaluation of uterine inflammation
Endometrial biopsy	Prognosticate the mare's reproductive potential

## 36.5 Procedure: Breeding Soundness Examination of the Mare

Technical action	Rationale
Obtain a brief, but complete, history of the mare's general health and reproductive career. Make sure to include all relevant details.	<p>Important historical information is outlined in Table 36.1 and in the reproductive examination form in Figure 36.1.</p> <p>Proper identification of the mare is critical in cases of sale/purchase.</p>
Perform a brief physical examination, paying particular attention to the mare's general health appearance and body condition score.	Note any signs that might be indicative of overt medical abnormalities. Note any ambulatory/ locomotor problems that might impact the ability of the mare to sustain a pregnancy.
Perform a systematic breeding soundness examination following the sequential steps described in Table 36.2.	A systematic approach is important to avoid compromising the interpretation of a procedure because of interference from an earlier one. Considerations concerning reproductive procedures in the mare are listed in Table 36.3.
Perform an examination of the vulva and perineal region prior to administration of any sedative or spasmolytic drugs to the mare.	Sedation can lead to relaxation of the perineal area and interference with the functions of the vulvar lips and vaginovestibular fold.
Examine the vulva and perineal region to determine the mare's conformation.	See Table 36.4 for details on examination of the vulva and perineal region.
<ol style="list-style-type: none"> <li>1. Assess the integrity and seal of the vulvar lips (or labia) (Figure 36.2).</li> <li>2. Evaluate the vulvar vertical inclination (Figure 36.3).</li> <li>3. Evaluate the anatomical relation between vulva and pelvic brim (Figure 36.4).</li> <li>4. Evaluate the vestibule and the vaginovestibular fold function (Figure 36.5).</li> <li>5. Perform the "windsucker" test by parting the vulvar lips (labia) and listening for a sound of inrush of air.</li> <li>6. Evaluate the floor of the vagina for the presence and appearance of fluid.</li> </ol>	<ol style="list-style-type: none"> <li>1. The vulvar lips should be closely apposed to each other to minimize contamination of the vestibule and possibly the vagina and uterus.</li> <li>2. The vulva should be vertically straight and have a cranial to caudal slope of no more than 10°.</li> <li>3. At least two-thirds of the vulva should lie below the floor of the pelvis.</li> <li>4. The vaginovestibular fold functions as a sphincter (and is often referred as the "vaginal vestibular sphincter").</li> <li>5. The "windsucker" test evaluates the integrity of the vaginal vestibular sphincter.</li> <li>6. Accumulation of frothy fluid in the cranial vagina is indicative of pneumovagina.</li> </ol> <p>Older mares that are thin often have atrophy of vulvar lips and flat croup, making them more prone to pneumovagina.</p>
Before examining the mare per rectum, the tail should be bandaged or wrapped and tied around the mare's neck (Figure 36.6).	<p>The mare's tail hairs can irritate the rectum mucosa if inadvertently introduced into the anus during palpation.</p> <p>Never tie the tail to an object (e.g., the poll of the restraint stocks).</p>

Technical action	Rationale
If the mare is uncooperative and unfamiliar with reproductive examination, or if the mare is straining excessively, administration of sedative or spasmolytic drug is indicated.	Routinely, mares do not need to be given any drugs for breeding soundness examination. Exercise caution when working with maiden mares.
If indicated, a clitoral swab should be collected at this time.	The vulva should not be washed or scrubbed prior to collection of a clitoral swab.
1. Any gross contamination of the vulva must be wiped with a dry paper towel prior to clitoral swab.	Clitoral swabs are collected for the sole purpose of testing for <i>Taylorella equigenitalis</i> (causative agent of contagious equine metritis, CEM).
2. With a gloved hand, expose the clitoral area, parting the vulvar lips.	After collection of the clitoral swabs and placement into the transport medium, they should be kept at 4 °C.
3. Evert the clitoral area by placing the index finger below the vulvar lips (Figure 36.7).	The swab in transport medium should be submitted to an approved laboratory as soon as possible to ensure that it arrives within 48 hours from the time of collection.
4. Use a narrow-tipped swab to sample the central and, if present, the lateral sinuses.	
5. Use a standard-type swab to sample all other areas of the clitoral fossa.	
6. Place both swabs in Amies charcoal-based transport medium.	

**Table 36.3** Considerations concerning manipulations involving entry into the uterus

Consideration	Explanation
Perform thorough rectal palpation and ultrasonography of the uterus and ovaries prior to any procedure involving entry into the genital tract	Withhold any vaginal, cervical or intrauterine examination until it has been confirmed that the mare is not pregnant
Manipulations involving entry into the uterus are best performed during estrus	It is inevitable that resident populations of microorganisms from the vestibule and vagina will gain entry to the uterus Mares can best eliminate this contamination during estrus If a procedure involving entry into the uterus is performed during diestrus, the mare should be treated with a luteolytic dose of prostaglandin F <sub>2</sub> -alpha to minimize the chances of endometritis
Digital examination of the cervix during diestrus is only indicated if there is concern about cervical integrity	During diestrus the cervix should be tightly closed If it is not, then the cervical integrity is most likely compromised
If a procedure involving entry into the uterus is performed during diestrus, the mare should be treated	The mare should be given a luteolytic dose of prostaglandin F <sub>2</sub> -alpha This will bring the mare into estrus, which will help to eliminate any contamination

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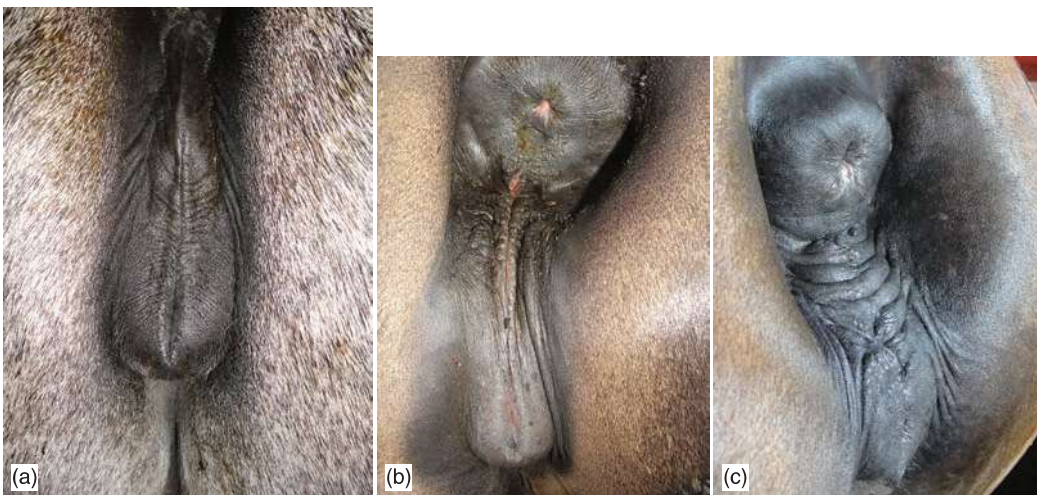
Consideration	Explanation
Do not collect an endometrial culture swab from mares without clinical signs of endometritis	The risk of a false positive culture is high Only after evidence of endometritis has been established by ultrasonography should an endometrial swab be collected because of the risk of contamination of the uterus during the collection of the endometrial culture swab.

**Table 36.4** Examination of the vulva and perineal region, and anatomical relationships

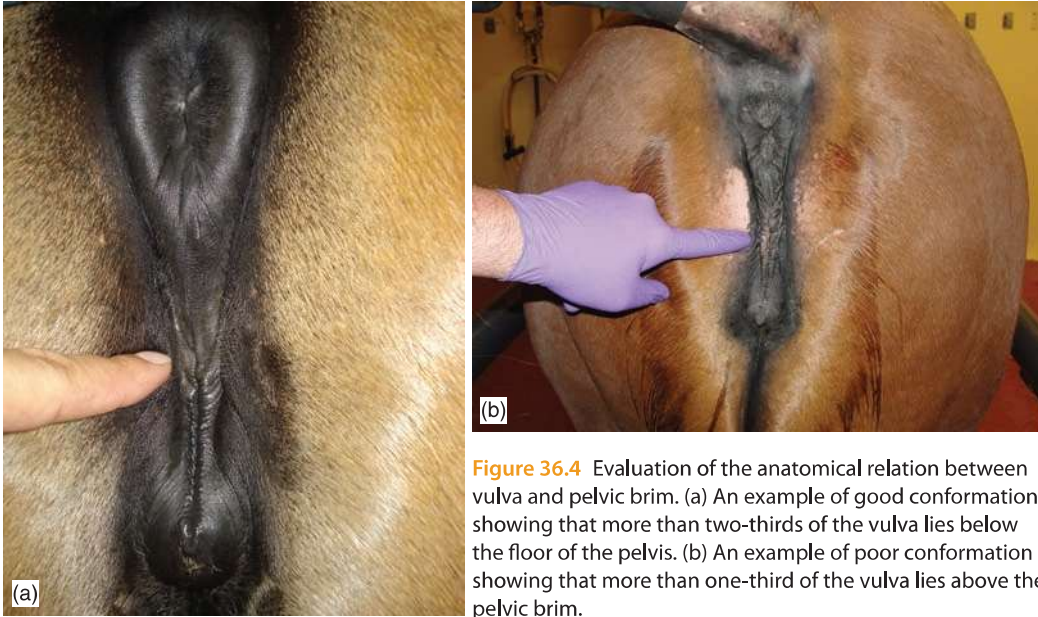
Anatomical relationships	Guidelines
Seal of the vulvar lips	Integrity of the vulvar lips and the proper anatomical relationship with the anus are essential because they provide the first barrier for contamination of external organisms to the uterus
Vulvar vertical inclination	The vulva should be vertical and the cranial to caudal slope should be no more than 10° Conformation that deviates from that is considered undesirable because it will predispose the mare to development of endometritis
Anatomical relation between vulva and pelvic brim	At least two-thirds of the vulva should lie below the pelvic brim (i.e., the floor of the pelvis) If not, the mare will be predisposed to contamination of the vagina with feces
Evaluate the vestibule (the area that separates the vulva and clitoris from the vagina proper)	At the cranial border of the vestibule is a folded muscular membrane, the vaginovevibular fold The vaginovevibular fold functions as a sphincter, acting as a second physical barrier between the uterus and the external environment A poor vaginovevibular seal will predispose the mare to development of endometritis
“Windsucker” test	To test the adequacy of the vaginovevibular fold as a physical barrier to external contaminants
Evaluate the vagina for the presence and appearance of fluid	Accumulation of frothy fluid in the cranial vagina is indicative of pneumovagina
Determine the presence of pneumovagina, which is a consequence of improper functioning of the first (vulva) and second (vaginovevibular fold) barriers	Constant or frequent entry of air into the vagina facilitates the entry of debris and contaminants into the caudal reproductive tract, leading to decreased fertility The presence of pneumovagina prompts the clinician to further evaluate the uterus to determine the presence of pneumouterus



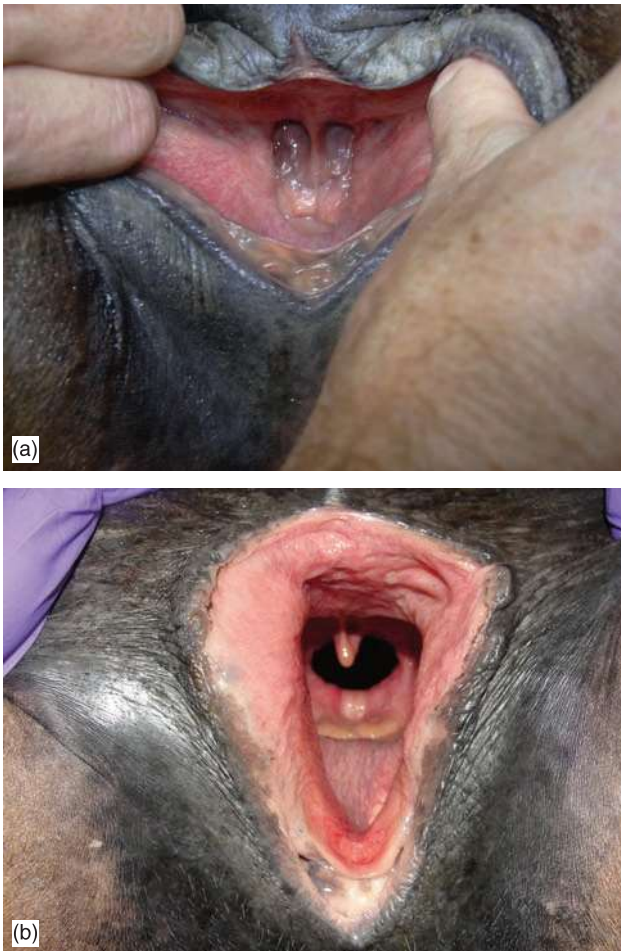
**Figure 36.2** Assessment of the integrity and seal of the vulvar lips. (a) Good seal of the vulvar lips is noted when pulling one of the sides away. (b) An example of good perineal conformation showing the vulvar lips closely apposed to each other. (c) Poor vulvar lip integrity noted by the presence of visible mucosa (pink), indicating poor vulvar lip seal.



**Figure 36.3** Evaluation of vulvar vertical inclination. (a) An example of good conformation showing that the vulva is vertically straight and has a cranial to caudal slope of no more than  $10^{\circ}$ . (b) An example of poor vulvar inclination showing a curved/crooked vulva. (c) Another example of poor vulvar inclination showing cranial to caudal slope greater than  $10^{\circ}$ .



**Figure 36.4** Evaluation of the anatomical relation between vulva and pelvic brim. (a) An example of good conformation showing that more than two-thirds of the vulva lies below the floor of the pelvis. (b) An example of poor conformation showing that more than one-third of the vulva lies above the pelvic brim.



**Figure 36.5** Evaluation of the vestibule and the vaginovesibular fold seal: (a) a normal vaginovesibular fold seal and (b) an inadequate vaginovesibular fold seal.





**Figure 36.6** Wrap the tail with neoprene tail wrap (or brown gauze) and tie it around the mare's neck before proceeding with rectal examination of the mare.



**Figure 36.7** Evert the clitoral area to perform the clitoral swabbing. The clitoral area is exposed by parting the vulvar lips and placing the index finger below them, as shown. This procedure requires sterile gloves to be worn.

#### Technical action

The rectal examination should be done before proceeding with aseptic procedures. Aseptic procedures include vaginal examination and intra-uterine collection of endometrial samples.

Wearing a non-sterile sleeve, adequately lubricated, the arm is introduced into the rectum and any fecal material within arm's reach should be removed prior to attempting to palpate the uterus and ovaries.

#### Rationale

Pregnancy should always be ruled out before performing procedures that will invade the cervix and uterus.

The presence of fecal balls can make palpation of reproductive structures difficult while also increasing intra-rectal pressure.

*(continued)*





**Figure 36.8** Transrectal ultrasonography is an important part of the reproductive examination of the mare. While wearing a non-sterile sleeve, adequately lubricated, introduce the arm with the rectal probe into the rectum after the rectum has been emptied of feces. The reproductive organs (uterus and ovaries) are scanned. (a) Ultrasonographic image of the uterus showing persistent post mating accumulation of fluid in a case of endometritis. (b) Ultrasonographic image during estrus showing the ovary with mature preovulatory follicle (left) and the uterus with normal edematous walls (right). (c) Ultrasonographic image of the ovary during estrus (note the mature preovulatory follicle measuring more than 25 mm in diameter).



**Figure 36.9** The sequence of steps for cleaning the mare's perineum thoroughly. (a) Place warm water into a bucket with a disposable plastic liner. Rip large tufts of cotton from the rolled cotton and place them in the warm water. (b) Use the clean, non-dominant hand to pick up the cotton from the warm water bucket and squeeze out the excess water. (c) Transfer the wet cotton tufts to the dominant hand. (d) Have an assistant pour about a tablespoon of betadine scrub onto the center of the cotton. (e) Scrub the perineum firmly (but not forcefully), starting at the vulva and working around in a circle towards the periphery. Discard the used piece of cotton in the garbage. Repeat these steps several times until the perineum is visibly and thoroughly clean. Courtesy of Dr. Lais R.R. Costa.

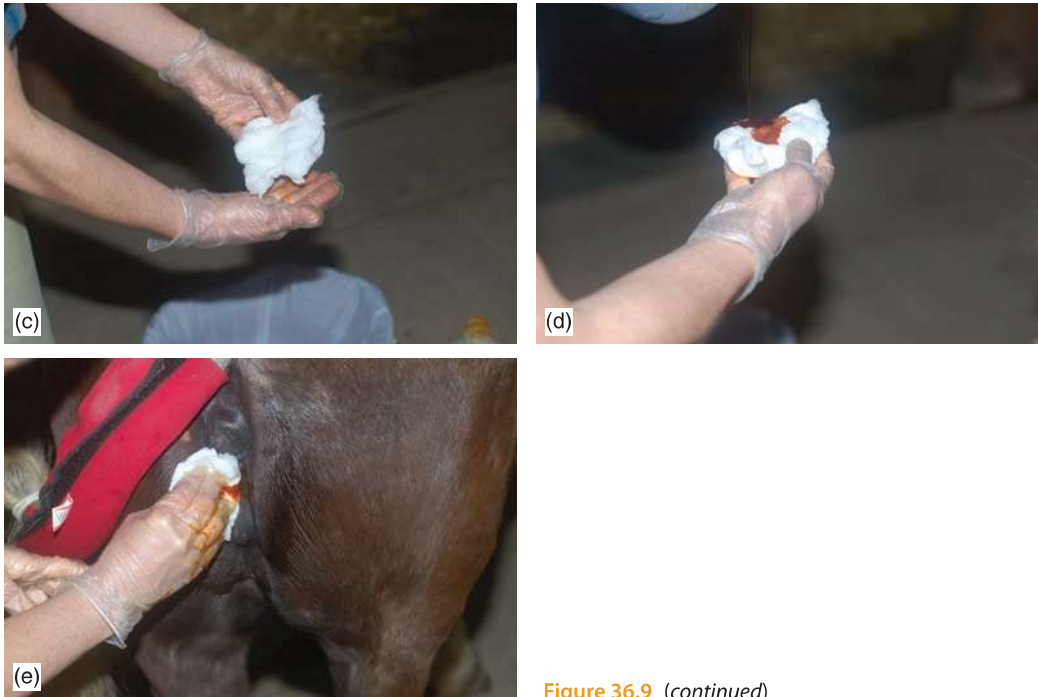


Figure 36.9 (continued)

**Technical action**

Perform a vaginal examination.

- Apply sterile lubricant onto the disposable vaginal speculum (Figure 36.10a)
- Part the labia and introduce the speculum initially at a 30° angle (Figure 36.10b).
- Once passed the vestibular area, advance the speculum horizontally (Figure 36.10c).
- Use the light source to inspect the vaginal mucosa and floor, and the cervix.
- Observe the appearance of the cervix (and interpret your findings considering the normal cyclical changes), gathering information concerning the mare's current reproductive status.

Perform a digital examination of the vagina and cervix using a sterile shoulder length glove and water-soluble sterile lubricant..

Palpate all around the vagina and cervix, feeling for the presence of lesions such as tears and adhesions.

If endometrial culture and endometrial cytology samples are to be collected, they should be collected sequentially right after vaginal examination.

For endometrial culture collection, wear a sterile shoulder-length sleeve lubricated with sterile lubricant gel and introduce the arm into the mare's vagina holding the endometrial double-guarded culture swab (Figure 36.12). With the index finger,

**Rationale**

Alternatively a sterile, reusable autoclavable speculum can be used (Figure 36.11).

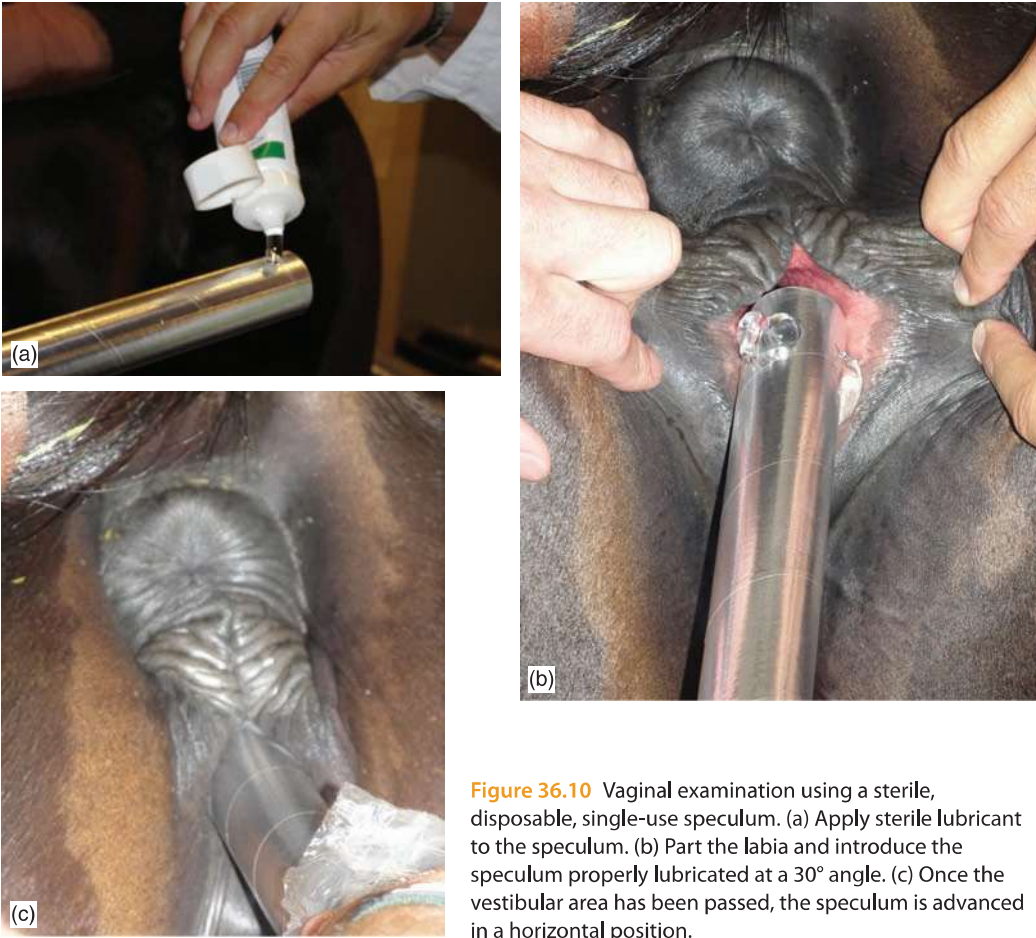
A resistance to advancing the speculum at the level of the vaginovebicular fold should be felt. This is normal as the fold works as a second physical anatomical barrier to protect the uterus from contaminants. The cervix position in relation to the vaginal floor changes significantly according to the stage of the estrous cycle.

See Table 36.6 for cyclical changes in the cervix observed during speculum or digital examination.

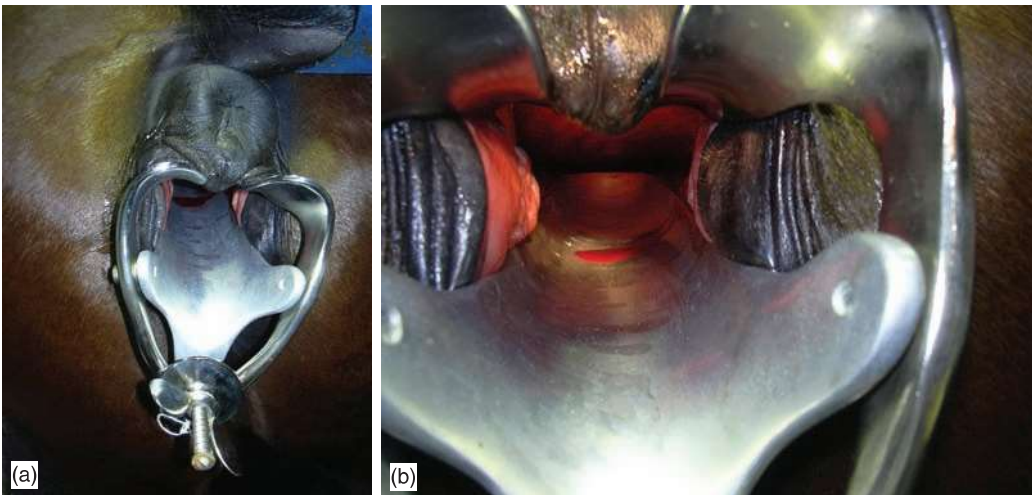
See Table 36.3 for important considerations concerning manipulations involving entry into the uterus.

The double-guarded swab minimizes the risk of contamination with flora from the caudal reproductive tract. The sample may be taken from the uterine body or from the base of one of the uterine horns.

Technical action	Rationale
<p>locate the caudal cervical os and introduce the double-guarded uterine culture swab into the uterus. Once inside the uterus, the inner protective casing is pushed through the outer casing, then the cotton swab is pushed into the uterus. Expose the swab for at least 30 seconds to allow contact with the endometrium. Once uterine sampling has been performed, the cotton swab is retracted into the inner casing, and the inner casing is retracted into the outer casing. Then, the double-guarded swab is withdrawn through the vagina. Once out of the mare, carefully expose the cotton tip swab without contaminating it, and place it in a tube with transport media. Break off the hand-contaminated end of the swab rod and discard it.</p>	<p>There are many options of transport media for endometrial culture swabs, BBL™ Port-A-Cul™ tubes work very well in preserving the samples up to 72 hours at room temperature.</p>
<p>For collection of an endometrial cytology sample use a cytology brush guided through the double-guarded endometrial swab (Figure 36.13). Place a double-guarded culture swab into the uterus as described above. Remove the cotton swab itself, leaving the outer guard of the culture swab in the mare's uterus. Carefully introduce the cytologic brush into the guard until it reaches the uterus. Rotate the shaft of the cytology brush a couple of times to ensure the brush bristles harvest endometrial epithelial cells. Prepare a smear using the sample collected from the cytology brush. Air dry and heat fix the smear, then stain with Romanowski, Giemsa or Diff-Quik (Baxter Healthcare Ltd, Thetford, UK) staining techniques.</p>	<p>The cytology brush is a very effective method for collecting sufficient epithelia cells for endometrial cytology. Care should be taken to not vigorously rotate the brush inside the mare's uterus as the 10 cm long cytobrush that is glued to an 18" long plastic shaft may break off within the mare's uterus.</p> <p>Alternatively, the endometrial cytology sample may be collected using a standard single or double-guarded endometrial swab. There are some instruments available for collection of both culture swab and cytology all in one.</p> <p>Endometrial cytology should be collected during early and mid-estrus. The presence of neutrophils during the postpartum period is considered normal.</p>
<p>Evaluate the endometrial cytology under a light microscope.</p>	<p>Figure 36.14 depicts examples of endometrial cytologic findings.</p>
<p>Finally, if obtaining an endometrial biopsy is indicated, this procedure would be performed last.</p>	<p>See Table 36.3 for important considerations concerning endometrial biopsy.</p>
<p>An endometrial biopsy can be obtained using a long large alligator-jaw biopsy forceps (Figure 36.15). The collection procedure is illustrated in Figure 36.16. Introduce the arm wearing a lubricated sterile shoulder-length sleeve into the mare's uterus while holding the biopsy forceps, making sure to protect the forceps within the palm of your hand. Once in the vagina the forceps are guided through the cervix by the index finger and advanced until they reach the body of the uterus. The hand is then withdrawn and inserted into the rectum. The jaws of the forceps are kept closed until they are located in the uterus by the hand in the rectum. The jaws should be positioned sideways (horizontally) and then opened. The uterine endometrium is pushed down into the jaws, which are then closed. Withdraw the forceps and place the specimen into a tube or jar with a fixative solution at a ratio of at least 1:10 (specimen:fixative solution).</p>	<p>The specimen should be carefully removed from the forceps jaws with a fine needle (25-gauge) to avoid tissue damage and potential artifacts. Check in advance with the pathology laboratory you are using to find out what fixative solution they prefer or recommend. Tissue in Bouin's should be processed within 24 hours of collection. If the biopsy is not likely to reach the laboratory within 24 hours, it should be transferred to 70% alcohol on the day after collection. Alternatively, the specimen may be placed in 10% phosphate-buffered formalin, which is the preferred fixative of several histopathology laboratories.</p>



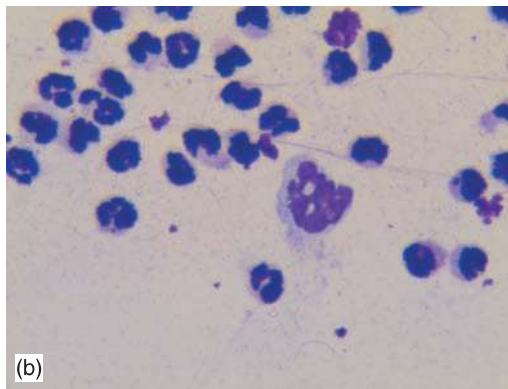
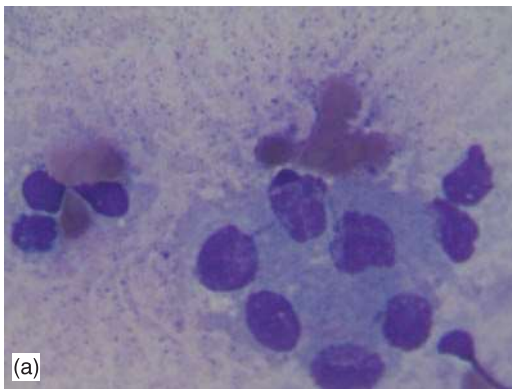
**Figure 36.10** Vaginal examination using a sterile, disposable, single-use speculum. (a) Apply sterile lubricant to the speculum. (b) Part the labia and introduce the speculum properly lubricated at a 30° angle. (c) Once the vestibular area has been passed, the speculum is advanced in a horizontal position.



**Figure 36.11** Vaginal examination using a sterile, reusable, autoclavable metal speculum, also known as Caskick's speculum or Polanki's speculum. This speculum is especially useful during post-foaling evaluations of vagina and cervix for detection of lacerations or tears that may have occurred during foaling. (a) Apply the speculum in the retracted position separating the labia. (b) Crank the knob to widen the speculum and expand the opening to allow examination of the vaginal mucosa and floor, and the cervix.

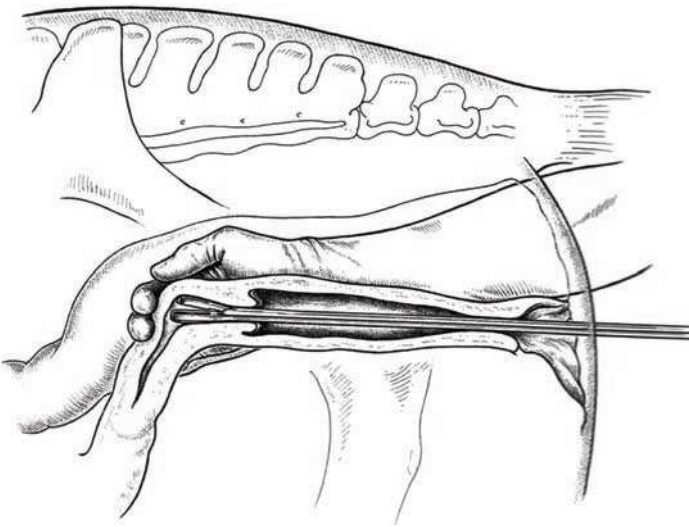
**Table 36.6** Cyclical changes in the cervix visualized during speculum or digital examination

	Estrus	Diestrus	Anestrus	Pregnancy
Diameter of the cervix (number of fingers)	≥3	1	1–3	1
Color of the cervical mucosa	Red	Pale grey or yellow	Pale white	White
Overall appearance of the cervical os	Glistening, edematous, slit-like	Dry, closed	Dry, atonic, possibly open	Dry, closed
Position of the cervix	On the floor of the vagina	Midway in rostral wall of vagina	Midway in rostral wall of vagina	Midway in rostral wall of vagina

**Figure 36.12** Instrument for the collection of endometrial swabs. Close-up of the tip of the double-guarded uterine culture swab showing the outer and inner protective layers and the cotton tip exposed.**Figure 36.13** Instrument for the collection of the endometrial cytologic sample. The endometrial cytology brush is inserted through the outer guard of the culture swab.**Figure 36.14** Example of endometrial cytology findings: (a) endometrial cells and (b) numerous degenerative polymorph nuclear cells/neutrophils in endometritis.



**Figure 36.15** Large alligator-jaw forceps.



**Figure 36.16** Schematic drawing demonstrating how the endometrial biopsy is collected. Wearing a lubricated sterile shoulder-length sleeve, protect the biopsy forceps when introducing them into the mare's uterus. Once the biopsy forceps is in the uterus, the hand is then inserted into the rectum. The alligator-jaw forceps is kept closed until it is located by the hand in the rectum. The jaws are positioned horizontally (sideways), and opened while the hand in the rectum applies gentle pressure on the uterine wall, pushing the endometrium tissue to be biopsied. The endometrial biopsy is obtained as shown. LeBlanc *et al.* (2000). Reproduced with permission of Elsevier.

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