

## **Back to the Basics**

### **Importance and interpretation of a breeding soundness exam in the mare**

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Although it would be ideal, a complete breeding soundness examination does not need to be performed on every mare every time. It is nice to do one, and never turn down the opportunity, but you may not be spending the client's money effectively. Young mares that have never been bred (maiden) could probably skip the BSE and go right to breeding. The goal of a breeding soundness examination (BSE) is to identify any problems that would interfere with the mare's ability to get pregnant and carry a foal to term as well as identify any issues that may need management or treatment during the breeding or pregnancy. A BSE is an exam performed at a point in time and will not identify every cause for infertility in every mare. However, it is a good place to start.

As a general rule, the indications for a BSE are below.

#### **Indications:**

- **Prepurchase:** A full BSE should be done anytime the mare is being bought or sold, especially if her new role will be a brood mare, or if the new owner has intentions of breeding her in the future. Even in young mares, the BSE can rule out congenital problems (missing parts of the tract), and keep you out of trouble later.
- **Problem mares:** This is the second most common time you will perform a BSE. She becomes a problem mare if you have inseminated the mare several cycles with good quality semen, and she is not conceiving. Remember to rule out other causes for not getting pregnant (poor insemination timing, etc).
- **Pregnancy loss:** mares that have subclinical endometritis, placental insufficiency due to uterine pathology (fibrosis), or anatomical defects (incompetent cervix) may lose a pregnancy at any time during gestation. The most common time points due to these factors are 45-60 days and 6-7 months gestation.
- **Before the breeding season:** Mares may need a full or partial BSE prior to the breeding season, especially if they did not conceive last year (barren mares).
- **Old maiden mares:** This class of mares is unique. They are mares over the age of 12 that have not had a foal. Usually show horses that now the owner wants to breed. They can have all sorts of problems (uterine fibrosis, etc) and you benefit yourself and your client to do a BSE before hand so you know where you start.

#### **Components of Breeding Soundness Exam**

##### **History:**

A complete history of the reproductive performance of the mare prior to examination is essential, especially because improper management is often a cause for infertility. Reproductive history should include when the mare was first bred, number of years bred, number of foals delivered, any foaling problems, any abortions, placentitis, early embryonic death, and normal cyclicity for this mare (long indicating endocrine dysfunction, vs. short indicating endometritis). Also important is the time of year and if

the mare was exposed to lights to give you an idea of where in her cycle (anestrus, transition, full cyclicity) she should be based on the time of year.

Any information you can gather from the previous manager, veterinarian, or owner is valuable. They may be able to give you information on how well the mare shows estrus, any prior treatments she may have received, and current medications, and any personality quirks that may be present. Note any previous surgeries, especially abdominal surgeries that may have caused adhesions.

Also important is the history of the stallion to which this mare was bred. We like to blame the mare for any infertility problems, but we need to remember that it takes two to tango. Questions include previous fertility data for the stallion, has he settled other mares the same way this mare was bred? For example, he may be getting mares pregnant on the farm, but none with cooled-transported semen. Opportunity to get pregnant should also be considered. Just because the mare was bred once or twice and did not become pregnant, does not necessarily mean that she is subfertile.

#### Physical exam:

A full physical exam should precede any reproductive exam. You need to look for anything that could interfere with this mare's ability to carry a foal to term. There are a lot of crippled broodmares, but you need to consider the long term (11 month) potential of any health condition as well as the added weight of the foal. Some laminitic mares may not make the best broodmares because their laminitis is not well managed, etc. Extremes in body condition should also be noted. Mares that are too fat or too thin make poor reproductive candidates.

Examination of the external genitalia should include the entire perineal area. This should be done before the mare is sedated if possible. Any sedation will cause a relaxation of the vulvar lips and it will be difficult to assess the integrity of the vulva and vestibulo-vaginal sphincter following sedation. Look for any vaginal discharge that may be present either on the vulvar lips or the underside of the tail. The vulva should be vertical (or no more than 10° from vertical), the lips should meet evenly, and 2/3 should be below the pelvic brim. If you gently part the vulvar lips, the seal of the vulva and the vestibulovaginal sphincter should not allow air to enter the vaginal cavity. Also evaluate the perineal body, especially in older mares that have had many foals. This can be best done by inserting a finger into the rectum and the thumb through the dorsal vulva. The area between your fingers is the perineal body and it should be at least 3 cm thick. If it is less, surgical correction may be indicated.

The most common finding is a "sunken anus" or "tipped vulva". This does not necessarily indicate a reason to not purchase or breed the mare, but it does make you think about other problems that may occur with this. These mares are often old, pluiparous mares and may have other damage to their uterus or cervix. The sinking predisposes them to uterine contamination because the manure from the rectum will enter through this vulvar "shelf" along with all the bacteria it contains. The sinking is caused by the uterus being pulled forward which may predispose the mare to delayed uterine clearance of fluid, or urine pooling if the entire tract is pulled forward enough to allow the back flow of urine to accumulate in front of the cervix.

### Trans-rectal Palpation and Ultrasound:

Technique was discussed in a previous lecture. Remember, safety first for both you and the mare. Perform a standard, systematic trans-rectal palpation starting with the cervix. Determine where she is in her cycle (diestrus, estrus, transitional, etc), remembering that it may be difficult to determine with only one examination. Make sure the mare has a cervix, uterus, and 2 ovaries, and that there are no adhesions that may interfere with the ability of that uterus to carry a foal. With the ultrasound, look for any free fluid in the uterus indicating infection or delayed clearance, as well as any uterine cysts. These cysts don't usually cause a problem, but can interfere with embryo motility if they become large enough, or, more commonly, can be confused with an early embryo or diagnosed as a twin. A map of any cysts should be kept for each mare. If the mare is pregnant, be gentle in your palpation technique. This will be the end of the BSE for her. At this point, you already know that she is able to get pregnant.

### Wash:

The mare's perineal area should be thoroughly cleaned at this point of the exam. There are many techniques and preferences as far as what should be used, but as long as the mare is cleaned well and the substance is not irritating, almost anything can be used. I prefer betadine scrub or Ivory soap. The area should be scrubbed as for a sterile procedure. Cleanliness is vital, as the introduction of bacteria can interfere with the culture results (false positive with contamination), or worse, the beginning of an endometritis. When you clean the vulva, do not forget to also clean the clitoris and clitoral fossa. This area can harbor smegma and even contain a "bean" just like in the urethral fossa of males. This can be a reservoir for infection as your arm carries bacteria and debris from the sinus into the uterus.

### Culture

In order to identify the pathogens that may be present in the uterus, a swab of the endometrial layer is taken. Bacteria are common inhabitants of the vulva and vestibule, but the amount of "normal" bacteria decreases in the cranial vagina and are not present in the uterus. The uterine culture should be performed after the perineal wash and before anything else has contaminated the vagina (vaginal speculum, digital exam, etc). The culture should be taken using a double guarded swab to prevent contamination onto the culture swab from the vulva and vestibule. A sterile sleeve is worn, and a small amount of sterile lube is used. Too much lube will contaminate the culture and cytology. The swab is introduced into the uterus with the assistance of a finger inserted through the cervix. Once in place, the swab is pushed through the guard and allowed to come into contact with the uterus for about 30 seconds. Caution must be taken, however, as these swabs can break off in the uterus and cause chronic irritation and/or problems with cyclicity. It should be removed immediately either by manually dilating the cervix and removing it digitally, or with the help of a hysteroscope. The culture should be placed in culture medium and submitted or refrigerated until submission when in the field.

An alternative technique is a low volume lavage. This can be preferred when a traditional culture is negative despite problems within the mare's tract (persistent fluid, etc) indicating an issue. This technique must be done carefully as there is a greater chance of

contamination when performed in the field. A closed system should be used with a 250ml bag of saline and IV tubing attached to either a uterine catheter or an AI pipette. The saline is infused into the uterus, then the bag is dropped below the uterus to allow gravity flow of the fluid back into the bag. Manipulation of the uterus per rectum may be necessary to get enough of the fluid returned, however, this may increase the incidence of contamination. Once the fluid is collected, the fluid should be sterilely centrifuged or simply submitted to the lab for centrifugation there. The remaining fluid may be centrifuged and used for cytologic analysis (see below).

### Cytology

A cytology should always accompany a uterine culture but is often the most overlooked or neglected portion of the BSE. A cytology gives the clinician immediate feedback on active inflammation. If neutrophils are present on a slide, then there is inflammation present and treatment can begin while waiting on the culture (lavages). Also, a negative or positive cytology can help determine if a light positive culture is a contaminant or real. A cytology can be performed by either using a second double guarded swab to exfoliate the endometrial cells or using a brush to exfoliate cells. Either system is then rolled onto a slide, stained with diff quick stain and examined for inflammatory cells (neutrophils).

### Vaginal and Cervical Exam

Generally, I perform this step next since the mare's perineum is already washed. The biopsy can be done next, but you must rewash the mare following the biopsy (you go in rectally when taking a biopsy) and it is easier for me to just do the biopsy last. It is personal preference as to which you do first. The vaginal exam should be both digital (palpation) and visual with a speculum. The entire vaginal vault should be inspected through the speculum. The cervix should be inspected for shape and color, as well as any discharge (not normal). Tears and adhesions should be checked, but are usually easier to find on the digital palpation (see below). The rest of the vaginal vault should be checked for any abnormal or prominent vessels, areas of redness, or discharge, including urine pooling in front of the cervix. This is especially important if the mare has a tipped vulva or sunken anus. This tends to pull the entire tract cranially and allows urine to back flow over the urethral fold. The urine causes chronic irritation not only in the vaginal vault, but also in the uterus. As the speculum is removed, watch the vestibulovaginal sphincter close to assess integrity.

Digital examination of the cervix is best performed by using the thumb on the external surface of the cervix and the forefinger inside the cervix and palpating around the cervix feeling for tears, thin areas, or adhesions. The cervix is best examined under the influence of progesterone when it is tightly closed. If the mare is post foaling or in estrus and a problem is suspected, re-evaluate her when she is in diestrus or under the influence of supplemental progesterone (Regumate) to assess the integrity of the cervix. Small tears usually do not cause problems, but tears that affect more than half of the cervix (from the external os to the internal os) may interfere with the functionality of the cervical seal.

### Endometrial Biopsy

A uterine biopsy is taken using a specialized instrument with a long handle and “alligator” jaws. The sample is usually taken at the base of one of the horns. The instrument is inserted vaginally, through the cervix, and into the uterus as was described for the uterine culture. Your arm is then removed from the vagina and inserted rectally. The instrument is palpated rectally and the uterine tissue is pushed into the biopsy jaws using rectal pressure. A piece of the uterine endometrium is then removed and submitted for analysis in either Bouin’s solution or formalin. The Bouin’s solution is a better fixative for the endometrial tissue than the formalin but is much less available and harder to work with.

Evaluation of the tissue includes assessment of inflammation, distribution of endometrial glands, periglandular fibrosis, dilated lymphatics, and cystic gland distension. The tissue samples are graded according to the severity of each problem. There are four categories of endometrial biopsies in the grading system used most often. **Category I** is a normal endometrium with minimal pathology. These mares have an 80% or better chance of carrying a foal to term. **Category IIA** has slightly more changes and has a 50-80% chance of carrying a foal to term. **Category IIB** is associated with a 10-50% chance of carrying a foal to term, and **Category III** is an endometrium with severe changes and has a less than 10% chance of carrying a foal to term. Mares can be different at different times of the year or from year to year. A mare can be in a category IIB due to severe acute inflammation or cystic gland distension. If these problems are corrected, she may upgrade to a normal mare (Category I). However, periglandular fibrosis is permanent and will not improve with treatment. The categories give you an idea about the mare’s ability to carry her own foal. It does not account for embryo transfer. There are many category IIB and III mares that produce embryos for other mares to carry and do great. As part of the BSE, you must educate the owner. Even with a “bad” biopsy, if they are willing to spend money, ET is a viable option for obtaining foals from this mare.

Elements of the biopsy also indicate potential issues with the mare. The type and degree of inflammation observed on a biopsy may indicate how long and what type of treatment should be performed. Cystic glands and lymphatic lacunae indicate poor contractility of the mare’s uterus. If these are observed in the biopsy, it can be assumed that the mare’s uterus may have problems eliminating fluid post breeding. In these cases, proactive treatment such as oxytocin or uterine lavage may be indicated earlier rather than later.

### Anatomical Barriers

There are three main barriers to prevent contamination from entering the uterus. The first is the **vulvar lips**. They should close evenly and tightly. The second is the **vestibulovaginal sphincter or ring**. This is the area where the hymen once was (or still is in young mares). This sphincter is more important than once thought. The bacterial flora is dramatically different between the vestibule and the cranial vagina and it is due to the sphincter. The third barrier is the **cervix**. Although not as much of a barrier as in the cow, it is extremely effective in acting like a gatekeeper between the uterus and vagina. It must be tight to prevent contamination from entering, but also loosen to allow fluid and debris to exit the uterus.

Identifying susceptible mares:

Normal mares have a mechanism of defense in place that enables them to eliminate bacteria and debris from their uterus making them resistant to endometritis. However, there is a class of mares, called **Susceptible Mares** that have a breakdown in this defense mechanism. By identifying these mares using a BSE, fertility can be improved by early treatment.

Susceptible mares are usually a class of mares that are older, have had multiple foals, poor perineal conformation, poor uterine tone, and poor uterine clearance. These mares tend to have free fluid in their uterus, but may or may not have bacteria present. These mares simply have a flabby, saggy, stretched out uteri, which means they need some assistance to clear debris and fluid associated with breeding or infection could occur. It is far easier to be proactive when dealing with these mares than have to deal with the fluid accumulation and inflammatory response after.

***The key to improving fertility is to identify these susceptible mares before breeding and manage them appropriately.***

Biopsy submission to Auburn:

Auburn University Large Animal Hospital  
Equine Theriogenology Service  
Attention: Dr. Aime Johnson  
1500 Wire Road  
Auburn, AL 36849  
(334)-844-4490



## Equine Theriogenology

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Equine Endometrial Biopsy Submission Form

### ***PLEASE PRINT ALL INFORMATION***

***Date:***

Owner Name:	Referring Veterinarian:
<b>Patient Information:</b>	Clinic Name:
Name:	Address:
Age:	City, State, Zip:
Breed:	Phone Number:
	Fax Number:
	E-mail address:

Brief History, Clinical, and Physical Findings:

**Have you submitted a biopsy to Auburn before?** Yes / No

**Have you submitted a previous biopsy on this mare?** Yes / No

**If so, when?** \_\_\_\_\_

**Is a check included with specimen?** Yes / No

**(please bill me)**

**Fee - \$60.00 per biopsy submission**  
**Please make checks payable to Auburn University**