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Repro Hormones in Practice
Chaired by Madeleine Campbell

08.30–08.55
Manipulation of the oestrus cycle

*Peter Daels*
KEROS NV, Westrozebekestraat 23A, 8980 Passendale, Belgium.

No abstract submitted

NOTES
Mares with acute endometritis seldom conceive and those that do are at higher than normal risk for early pregnancy failure. Bacterial culture of the mare’s endometrium has been in use since the 1920s to diagnose endometritis, but interpretation of positive culture results is not always straightforward. In accordance with the HBBLB Code of Practice prebreeding endometrial cultures are performed to rule out the presence of venereal bacteria. Once venereal infection is ruled out, the endometrial culture results are used to assess the genital health of the uterus, in particular to rule out the presence of uterine infection. Certain nonvenereal bacterial species are more commonly associated with uterine infection than others, namely Streptococcus zooepidemicus, E. coli, Staphylococcus aureus (coagulate positive), Pseudomonas aeruginosa (nonvenereal strains), Klebsiella pneumoniae (nonvenereal capsule types), Enterobacter aerogenes, and Proteus spp. If a guarded technique has been used, finding a heavy growth in pure culture of one of these bacterial species is likely to indicate bacterial infection of the uterus. However, there are many cases in which the endometrial culture results are less straightforward to interpret. Such cases would include those with a mixed or scant growth of potential acute endometritis producing organisms, or a moderate-profuse growth of organisms normally considered to be contaminants. In these cases further factors must be considered to clarify the diagnosis of acute endometritis. Clinical factors that indicate an acute endometritis include: presence of vaginal or cervical discharge, shortened dioestrous phase (‘short-cycling’), presence of free uterine echico fluid on ultrasonography. Endometrial cytology (smear test) provides a rapid screening test for acute endometritis. A normal mares in oestrus should have plenty of normal, healthy-looking endometrial epithelial cells in her smear but no more than the occasional (+/-) polymorphonuclear leucocytes (PMN). The presence of PMNs on the smear suggests the presence of an acute endometritis and the smear can be assessed semi-quantitatively to give an idea of the severity of the inflammatory response. Smears can be made from vaginal discharges, uterine swab samples and/or uterine washings. 

**Endometrial cytology samples: Indications**

- Routine prebreeding screen for acute endometritis.
- Clarification of endometrial bacteriology culture results.
- Assessment of mares suspected to be susceptible to uterine infection.
- Decision-making for foal heat covering.
- Investigation and decision-making in mares with pooling of uterine fluid.

**Sampling: Timing**

As the most common indication for sampling is a prebreeding screen, the sample should be taken in early-mid oestrus. Sampling during anoestrus can be unreliable, and sampling during dioestrus increases the risk of introducing iatrogenic infection.

**Collection methods**

Various techniques for obtaining a cytology sample are described. The cell harvesting device (cotton swab, brush) may be passed through the cervix using a guarded or unguarded technique, and the device may be passed via a speculum or with a gloved hand. Guarded culture rods are readily available (Kallajan Industries Inc., Long Beach, California, USA), and comprise an outer guard tube with an inner rod holding the swab. After routine washing of the perineum the instrument is passed through the cervix either via a lighted speculum or guided along the index finger of a gloved hand. Once inside the uterus, the inner plastic rod is advanced and the swab tip or brush is rubbed/rolled along the surface of the endometrium. The swab is then withdrawn into its sleeve and the instrument is removed. Smears may also be made from the material harvested in the cups of guarded swabs. A more practical and economic collection technique is preferred by the author’s practice. The mare must be in early oestrus with a dilating and moist cervix. The cervix is visualised using a sterile disposable speculum illuminated with a pen torch. An extended swab is inserted through the speculum, through the relaxed cervix and is rotated against the endometrium where it collects lining (epithelial) cells, fluid secretions and inflammatory cells, if present.

**Preparation of the smear**

The smear can be kept in its dry case for up to 2 h, but if there is to be a longer delay before processing, the smear should be made at mare-side. The smear is made by carefully rolling the moist swab onto a prepared slide and spray fixing. The slide can then be submitted to the laboratory for staining and microscopic (cytological) examinations.

**Endometrial aspirates and washings**

A technique described for obtaining uterine cytoclogic samples involves flushing a small volume 10–20 ml saline into the uterus per vaginum using a syringe connected to a stiff plastic flushing catheter. The catheter is agitated against the uterine lining and the fluid re-aspirated. This technique undoubtedly results in superior cytological specimens, but is more time consuming, and more likely to cause irritation, than required when the diagnostic aim is purely detection of the presence or absence of neutrophils.

The use of uterine lavage has become more utilised in broodmare practice and it is possible to harvest good cytological specimens from the fluid flushed back out of the uterus. We use a ‘closed’ flushing system whereby the portal of the 3 l saline-for-irrigation bag is connected to the flushing catheter and once the fluid has been run into the uterus, the bag is lowered and the fluid is allowed to collected back into the bag by gravity. A sample of this fluid can be submitted for centrifugation and cytological processing.

**Staining methods**

Any routine staining method may be used for uterine smear preparations. The author favours a modified Papanicolaou stain, which gives excellent morphological detail but is more time consuming and therefore more suitable for batch use. Using this stain, samples should not be allowed to air-dry, but should be spray fixed at mare-side. Simple stains such as Leishman, Giemsa and Diff quick are also perfectly adequate, and smear preparations can be air-dried for subsequent staining with these stains. Testsmiels (Boehringer Corporation) are slides pre-stained with Romanowski-type stain. They provide excellent results, simply and rapidly, with minimal equipment. They are, however, comparatively expensive and are thus more useful for urgent/occasional smears or for small throughput situations.
1. Make smear by rolling (like paint onto a wall), to minimise artefacts, into the pre-stained blue area. Label with pencil with name of mare, date etc.
2. Leave on bench at room temperature for 2–3 min.
3. Wash off background stain under a gently running cold water tap.
4. Allow to dry and coverslip if required.
5. Panchromatic staining appearance as for all Romanowski-type stains.

Interpretation of results
A reasonable quality laboratory microscope is required.

Epithelial cells are by far the most common cells seen in the normal uterine smear. They usually appear columnar and nonciliated. The nuclei are round-ovoid. The swab collection procedure causes significant cell distortion and there is often mucus present. The presence of endometrial epithelial cells is used as a measure of smear quality confirming sampling from the uterus. Squamous epithelial cells are rare and usually represent vaginal contamination or urine pooling. If epithelial cells are exclusively squamous, the smear is considered nondiagnostic, indicating that the vagina as opposed to the uterus has been sampled.

Polymorphonuclear leucocytes
Polymorphonuclear leucocytes (PMNs) are readily recognised by their lobed nucleus, and forms showing various stages of maturity, or presence of toxic degeneration are noted. The presence of PMNs suggests uterine inflammation or acute endometritis. Acute endometritis is most frequently associated with bacterial infection, less commonly with fungal infection, and these can be defined with aerobic, microaerophilic and sometimes anaerobic or fungal culture. It is useful to employ a semi-quantitative system to grade the degree of inflammation (Table 1).

<table>
<thead>
<tr>
<th>PMN score</th>
<th>%PMN differential count</th>
<th>Degree of inflammation indicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>None seen</td>
<td>No evidence of inflammation</td>
</tr>
<tr>
<td>+/-</td>
<td>&lt;0.5%</td>
<td>Very mild inflammation</td>
</tr>
<tr>
<td>1</td>
<td>0.5–5%</td>
<td>Mild inflammation</td>
</tr>
<tr>
<td>2</td>
<td>5–30%</td>
<td>Moderate inflammation</td>
</tr>
<tr>
<td>3</td>
<td>&gt;30%</td>
<td>Severe inflammation</td>
</tr>
</tbody>
</table>

NOTES
Once the operator is experienced at looking at the smear preparations, it becomes unnecessary to count a differential because an “eyeball” estimate can be made for the semi-quantitative assessment. We find this method of quantification more convenient than quantitative bacteriological culture to estimate the degree of significance of the response.

Other inflammatory cells
Macrophages are sometimes encountered in foal-heat (post partum oestrus) smears. Lymphocytes are relatively rare and their presence is of uncertain significance. Eosinophils are also rare and their presence is reported (mainly from endometrial biopsy histology) to be associated with pneumovagina. These cells are more frequently recognised in wet-prep specimens obtained by flushing as opposed to the dry-swab technique.

Red blood cells
Red blood cells (erythrocytes) and proteinaceous debris are often seen in smears taken at ‘foal heat’.

Crystals
Urine crystals (calcium carbonate) may indicate urine pooling. Other changes such as the presence of squamous epithelial cells and neutrophils are needed to clarify the significance.

Bacteria, yeasts and fungi
Bacteria are only rarely visible on routine cytological smears. Gram staining may be useful to visualise bacteria and hasten the initiation of antibacterial therapy whilst waiting for culture results. Fungal elements, (branching hyphae, spores) and conidiophores (yeasts bodies) are visible on routine Trichrome and Diffquick stains. Periodic acid Schiff (PAS) stain may be useful to confirm their presence.

Endometrial smears are not reliable for the diagnosis or investigation of chronic degenerative endometrial disease, for which biopsy techniques are used. Experience has shown that almost no PMNs should be seen in a smear taken from a healthy mare in oestrus. Up to 1%, may be acceptable in ‘foal heat’ and maiden (first oestrous period of the season) mares. In older mares, more than 1% PMNs may warn of deficient uterine immune responses, indicating the need for special prophylactic programmes to be used at mating to prevent post mating uterine infection. Used in conjunction with ultrasonography, uterine cytology is a particularly useful tool in the management of mares who are susceptible to uterine infection/fluid pooling.
How to: Obtain endometrial biopsies

Annalisa Barrelet
Rossdale and Partners, High Street, Newmarket, Suffolk, UK.

Introduction
Endometrial biopsy in an integral part of breeding soundness examinations in broodmares. Indications include diagnosis of specific endometrial abnormalities that will adversely affect fertility, monitoring response to specific intrauterine treatments, and (probably the main value) provision of a basis for assessing a breeding prognosis for the mare.

Safety
The procedure, in experienced hands, is simple, safe and nonpainful. The only contraindication is pregnancy, and it is essential to examine the mare and confirm that she is not pregnant before the biopsy is taken.

Timing
It is possible to pass a biopsy instrument safely through the mare’s cervix at any stage in her cycle, and indeed, during anoestrus, but the best time to perform barren mare examinations is in the autumn. This allows sufficient time to carry out treatments, assess response and enable a period of ‘rest’ before the breeding season commences. A mid dioestral biopsy has traditionally been recommended as routine, but taking a biopsy in dioestrus increases the possibility of iatrogenic infection due to reduced uterine defence during dioestrus compared with mid oestrus. This may be compounded by compromised uterine defence in the particular mares under examination. It is therefore better to take the biopsy when the mare is in oestrus, or at least induce oestrus (using prostaglandin) at the time of taking the sample.

Equipment
Various instruments have been used to obtain endometrial samples but it is imperative that the specimen obtained is of sufficient size to provide at least 1–2 cm of endometrium on the histological specimen. Long (65 cm) basket jawed forceps (Rocket of London) are the preferred biopsy instrument. Unfortunately, specimens obtained via endoscopic biopsy instruments are inadequate for histopathological appraisal. One biopsy taken from a mid-horn region is diagnostically representative unless there are palpable or endoscopically visible uterine abnormalities, when more than one sample should be taken.

Technique
A tail bandage should be applied, and the rectum should be emptied of faeces. The perineum should then be thoroughly washed with warm water and patted dry with paper towel. The biopsy instrument is passed into the vagina cupped in a gloved hand with the application of a small amount of sterile lubricant. The index finger is used to locate the external cervical os and the instrument is guided through the cervix and into one horn. The hand is then removed from the vagina and introduced into the rectum to palpate the biopsy instrument. The alligator jaws of the instrument are opened and gentle downward pressure is applied from the rectum to introduce a fold of endometrium into the open jaws. The jaws of the biopsy instrument are then closed firmly and the hand is removed from the rectum. The sample is taken by applying a short sharp tug to the biopsy instrument and withdrawing it via the vagina. The sample is retrieved carefully from the instrument with fine forceps or a needle to avoid artefactual damage.

Fixation
Bouin’s fixative is the preferred histological fixative for reproductive tissues in general and endometrial tissues in particular because their high water content leads to excessive artefactual shrinkage in more conventional fixatives, i.e. formol saline. If there is a delay in processing, samples should be transferred to formol saline after 24 h, as excessive fixation in Bouin’s fixative can result in dense specimens which do not stain well. Samples are processed and sectioned using standard methods, and stained with haematoxylin and eosin.

Microscopic examination
Normal endometrial anatomy
Cyclic changes
The histological (microscopic) appearance of the endometrium changes with the stage of cycle and this should be compared with teasing behaviour and the results of vaginal and rectal palpation examinations. Characteristics that should be noted include height of luminal epithelium, configuration of glands and amount of stromal oedema. During anoestrus, endometrial glands are inactive, luminal and glandular epithelial cells are cuboidal and stromal oedema is minimal. Glands appear mostly in cross section as circular, and occasionally pinkish proteinaceous material is seen within the glandular lumena. During oestrus, luminal and glandular epithelial cells are taller columnar and pale staining, often with basal vacuoles. There is considerable oedema in the lamina propria and glands appear more straight (less tortuous) on longitudinal section. During dioestrus, epithelial cells are low columnar to cuboidal, and glandular branches are tortuous, and form a ‘string of pearls’ appearance on longitudinal section. In transitional phase histological characteristics are less clearly defined and considerable variations can occur among different sections of the biopsy.

Pathological abnormalities
Mixed pathology is common, but specific changes are classified and their significance is assessed in terms of degree:

Acute endometritis
Polymorphonuclear leucocytes (PMNs) are seen under the epithelial cell lining and, in severe cases, degeneration of the epithelial lining may be seen. In cases with pyometra, PMNs are seen coursing through the luminal epithelium and accumulations of PMNs are seen at the luminal surface. Eosinophils may be seen in mares with pneumovagina, delayed recovery after foaling or following the use of some types of intrauterine medication. Acute endometritis indicates an inflammatory response, most commonly associated with bacterial infection.

Chronic infiltrative endometritis
Mononuclear cells, e.g. lymphocytes and plasma cells are seen, either diffusely throughout the endometrial tissues (stroma) and/ or in focal aggregations. The presence of these cells indicates a local immune response to challenge with foreign protein/material. This is a normal response and therefore no specific treatment is...
Degenerative changes are inevitable to a degree and thus each biopsy specimen is assessed in terms of the mare’s age and her foaling history. In general terms, mares aged ≥13 years should have no more than mild signs involving 1–3 layers of fibrosis, nests at a rate of <2 per 5 mm linear field in at least 4 fields. Mares aged ≥15 years should have no more than moderate signs, with nests/cysts at a rate of 2–4 per 5 mm linear field, and fibrosis up to 4 layers. Mares aged ≥17 years are likely to have advanced signs of CDED with cysts/nests averaging more than 5 per 5 mm linear field and widespread fibrosis. Paradoxically, more extensive CDED is often seen in mares who have had fewer pregnancies in relation to their age, i.e. excessive CDED changes are often seen in aged mares presented for breeding after a career outside the breeding shed. A study by Waelchli showed that the number of years barren has a significant effect on foaling probability. Unfortunately, no treatment for CDE is particularly effective, especially in older (≥17 years) mares.

In some cases, recurrent uterine infection may produce diffuse stromal fibrosis, possibly associated with recurrent shortened dioestrous periods and therefore relatively persistent oestrogen levels. Successful treatment of the acute endometritis, followed by normal periods and then pregnancy, can sometimes reduce the signs of diffuse stromal fibrosis.

**Endometrial hypoplasia**

Usually a feature of immaturity which resolves in time. (In the case of barren maiden mares, usually by the following breeding season). Where the degree of hypoplasia is extreme and the ovaries are abnormally small, or where the condition persists, the possibility of a basic genetic abnormality (gonadal dysgenesis) should be considered.

**Endometrial hyperplasia**

Diffuse glandular over-development (hyperplasia) with thickening of the endometrium and excessive secretory activity of the glands is a normal feature of the post-foaling or post abortion period. Glandular architecture and secretory activity usually returns to normal by 10–12 days but occasionally may persist for weeks if not months, when it is considered pathological. Acute endometritis is frequently seen as a complicating feature.

### Grading systems

Kenney originally proposed a classification system based on 3 categories: an essentially normal group (Category 1), a severely

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td><strong>Prognosis 1: After first examination, before treatment</strong></td>
</tr>
<tr>
<td>Category 1</td>
</tr>
<tr>
<td>Category 2</td>
</tr>
<tr>
<td>Category 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Prognosis 2: After treatment and the second (follow-up) examination</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
</tr>
<tr>
<td>Category 2</td>
</tr>
<tr>
<td>Category 3</td>
</tr>
</tbody>
</table>
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affected group (Category III) and an in-between group (Category II). Foaling rates of Category I are considered to reflect overall breeding management (>80%), whereas foaling rates in Category III are considered to reflect the severe changes in the endometrium (<10%). Foaling rates in Category II are considered to reflect endometrial changes but improvement could be obtained with careful gynaecological management and veterinary intervention. A refined 4 category grading system was subsequently developed.

Mare owners are keen to obtain a prognosis for the chance of successful future breeding to help with their mating plans or to help with the decision to retire the mare. Category I and III mares are relatively easy to provide breeding prognoses on the basis of one biopsy. However the majority of mares fall into Category II and prognoses are most usefully and more accurately made in 2 stages, after the initial evaluation and after repeat biopsy to check for response to treatment applied (Table 1).

It is also essential to take into account the mare’s age when classifying her endometrial changes. Part of the decrease in fertility in older mares is undoubtedly the result of increased severity of endometrial fibrosis, however, an adverse effect of age that is not related to endometrial changes also seems to exist.

An assessment of the mare’s future breeding prospects (prognosis) may be offered in 2 stages:
1. After the first complete diagnostic evaluation.
2. After treatment and follow-up examinations, assessing the ability of the mare to respond to treatment and/or compensate for the abnormalities diagnosed. Failure to respond to specific treatment clearly confers a poor prognosis.

NOTES
How to: Collect, process and certify equine semen

Madeleine Campbell
Hobgoblins Stud Reproduction Centre, Dudleswell, Ashdown Forest, East Sussex TN22 3BH, UK.

Health tests
No semen should be collected nor used until the stallion has been tested for the venereal diseases of equine viral arteritis (EVA); contagious equine metritis organism (CEMO); Klebsiella pneumoniae; and Pseudomonas aeruginosa. Details of testing protocols can be found in the HBLB Codes of Practice: email equine.grants@hblb.org.uk

Semen collection
The equine ejaculate consists of spermatozoa (80% of which are contained in the first 3 jets of the ejaculate); seminal plasma i.e. the fluids provided by the accessory sex glands, which suspends the sperm cells and provides a temporary energy source; and the gel fraction, which is ejaculated last and is spermicidal. Semen may be collected using an artificial vagina (AV), a condom or by catching a ‘dismount sample’. The latter gives a highly misleading picture of semen quality. Use of an AV is the most common and easiest method. There are various models of AV. The AV is used in conjunction with either a ‘jump’ mare, or a dummy mare. A ‘jump’ mare may be a mare in oestru, or an ovariectomised mare injected with oestrogen . A ‘dummy’ mare is a structure like a pommel horse that stallions are trained to mount. Though some investment of time is needed in training the stallion to use a dummy mare, this method of collection is vastly preferable since it greatly reduces the risk of injury to stallion, personnel and mares, and means that semen can be collected upon demand without the need for a mare in season. Some stallions will also tolerate ground collection, and this can be a useful technique for example if a stallion has an orthopaedic problem.

Semen analysis
The motility, concentration and morphology of the semen should be analysed. The following tests may be easily performed using equipment which might reasonably expected to be available in general practice (normal values are given in brackets).

(i) Total volume (15–100 ml)
(ii) Total motility (>50%)
(iii) Progressive motility (>50%)
(iv) Morphology (minimum 50% morphologically normal live sperm cells; no more than 15% of any one deficit)
(v) Concentration (100–800 million sperm cells/ml of semen)

All motility analyses should be made at 37–38°C to avoid heat shock, which would affect motility.

Morphology is assessed using a live:dead stain, e.g. eosin nigrosin stain. The principle of this stain is that it will cause red cells pink/purple. It is important to distinguish genuine abnormalities that relate to spermatogenesis or sperm maturation from abnormalities caused by the handling of the semen.

Semen processing
Fresh semen can be used if the mares to be inseminated are on-site. If the mares are at a different location, or if the semen needs to be held for more than an hour, semen should be extended and chilled. Extension is necessary because sperm cells, being highly specialised, have a high metabolic rate and produce waste products, which will kill off the cells if they are not diluted in extender. An extender typically contains nutrients (most commonly skimmed milk); antibiotics, and is buffered for pH and for osmolarity. Semen should be extended to 25–50 million sperm/ml and a minimum of 500 million progressively motile sperm should be provided in each insemination dose.

How to calculate this dose and process the semen to provide it will be discussed in the lecture, using this example (Table 1):

<table>
<thead>
<tr>
<th>Sperm count (million/ml)</th>
<th>Dilution rate (Semen:Extender)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100–250</td>
<td>1 : 3</td>
</tr>
<tr>
<td>250–300</td>
<td>1 : 4</td>
</tr>
<tr>
<td>300–350</td>
<td>1 : 5</td>
</tr>
<tr>
<td>350–400</td>
<td>1 : 6</td>
</tr>
<tr>
<td>400–450</td>
<td>1 : 7</td>
</tr>
<tr>
<td>450–500</td>
<td>1 : 8</td>
</tr>
<tr>
<td>500–550</td>
<td>1 : 9</td>
</tr>
<tr>
<td>550+</td>
<td>1 : 10</td>
</tr>
</tbody>
</table>

• If the concentration of the raw semen is 300 million/ml, and the initial volume of the raw semen was 25 ml.
• The chart tells you that for concentrations of 300–350 million/ml you need to add 5 parts extender to 1 part semen.
• 1 part = the initial volume of the semen.
• You have already added 1 part extender (25 ml). So add 4 parts more extender (5-1 parts).
• e.g. The initial volume of the raw semen was 25 ml, so you would have added 25 ml of extender to create a dilution of 1:1. You now need to add another 4 parts extender i.e. 4 x 25 ml = 100 ml more of extender. This creates a final dilution of 50 million/ml, and a final volume of 150 ml.

BUT
• Not all of the sperm will be progressively motile and not all of them will have normal morphology. Therefore sending 10 ml of 50 million sperm/ml semen does NOT give you the 500 million normal sperm the mare needs.
• Suppose that progressive motility was 60%.That means that in each ml of 50 million sperm there are 30 (i.e. 60/100 x 50) million normally motile sperm.
• Therefore to send 500 million progressively motile sperm you need 16 (i.e. 500/30) ml, not 10 ml.

Various containers are available that have been developed for semen shipment. The ‘gold standard’ is the Hamilton-Thorne Equitainer, as these cool semen at a very convenient rate and withstand changes in environmental temperature better than other models. However, Equitainers are expensive to buy and heavy to ship, and for this reason disposable polystyrene models have become popular.
All semen shipments must be accompanied by paperwork certifying that the stallion has been tested free of venereal diseases. Exact details of the information which must be provided are given in the ‘Checklist for the use of artificial insemination’ included in the HBLB Codes of Practice (p 30 in the 2009 version). If semen is being exported, additional health tests as defined by Defra and an export health certificate are necessary.
11.10–11.30

Induction of lactation in mares and adoption of an orphan foal

Peter F. Daels
Keros Equine Insemination and Embryo Transfer Center, Westrozebekestraat 232A, 8980 Passendale, Belgium.

When facing an orphan foal due to the death of the mare or unwillingness to accept her foal, it is critical to find an alternative to feed the foal and create a suitable social environment for the upbringing of the foal. There are excellent milk replacers on the market that enable us to feed an orphan foal up to weaning age with excellent body development. However, prolonged bottle feeding of an orphan foal often results in a horse with poor social skills. This aberrant behaviour may interfere with the training of the horse as an adult and may make it unsuitable even dangerous for normal equitation.

Lactation can be induced in nonparturient mares that have foaled in previous years using a treatment that includes oestradiol, progesterone and a dopamine D2 antagonist (sulpiride or domperidone). The quality of the milk produced during these induced lactations appears similar to a normal post parturient lactation even though production of colostrum was only observed occasionally.

The most impressive aspect of our studies has been the ability of these mares to adopt the foal that is presented to them (even though these mares have not foaled since at least one year) and to develop a maternal bond with this adopted foal that is identical to the behaviour displayed by mares nursing their own foal. These observations are important because it is the establishment of this mare-foal bond that makes it possible to raise the orphan foal in a normal social environment and develop the social and learning skills of a normal foal.

Induction of milk production
Mares must have given birth and successfully nursed a foal at least once in their life. Mares must be willing to stand confined behind a contention bar for prolonged periods of time and have a history of being a ‘good mother’.

We have been using 2 treatment protocols that have both worked well in our experience.

Protocol 1
Once the mare has been approved, she receives a single i.m. injection of oestradiol-benzoate (50 mg/500 kg mare) and the same day she is started on daily altrrenoestrogen administration (22 mg q. 24 h i.m.) and twice a day injections of sulpiride (1 mg/kg bwt q. 12 h i.m.). Sulpiride is available in an aqueous solution for injection for human use. Human preparations are available for injection in Europe. When the mammary gland is significantly increased in size, has a ‘filled’ appearance and/or milk drops are present at the teat, milking can be started. Generally, milking is started on Day 4–7 of treatment. Once milking has started, it is important to continue milking 5–7 times per day.

Protocol 2
This protocol starts on Day 1 with a single administration of dinoprost (5 mg i.m.), oestradiol benzoate (50 mg i.m.), altrrenoestrogen (44 mg q. 24 h per os) and domperidone (1.1 mg/kg bwt q. 12 h per os). On Days 2–15, mares receive oestradiol benzoate (10 mg q. 24 h i.m.), altrrenoestrogen (44 mg q. 24 h per os) and domperidone (1.1 mg/kg bwt q. 12 h per os). Mares are not milked until adoption.

After 3–4 days of milking, the production should have reached 3–5 l per day for a 500 kg horse. At this point, the mare is ready for adoption. In our hands, mares that are not producing a significant amount of milk on Day 7 (>3 l) are not suitable adoption candidates. Altrrenoestrogen treatment is generally stopped on Day 7 and sulpiride (or domperidone) treatment is continued until several days after adoption is completed. Continuation of treatment for more than 7 days after adoption is likely inefficient because the effect of these dopamine antagonists on prolactin secretion tends to decrease over time.

Introduction of the foal
Mares were only used for adoption when they were producing sufficient amounts of milk (3–5 l per day). During the induction of lactation mares were kept side by side at all times and allowed to interact through a grilled window (or mesh fence placed in front of open window). Adoption was performed only when the adopting mare had accepted the presence of the foal in the adjacent stall. Once or twice a day the foal was taken away, out of the mare’s site and the adopting mare’s behaviour was evaluated. A few days into the induction protocol the mares would start to show signs of separation anxiety and they would begin to whinny and look for the foal. In general, the adoption was performed when mares had received 5 days of treatment. Mares were administered dinoprost (5–15 mg i.m.). When the mare started to sweat from the progstaglandin administration, the foal was introduced into the mare’s stall and allowed to nurse. Every effort was made to keep the foal nursing during the phase that the mare displays the typical colic-like symptoms induced by prostaglandin. When doing so, the mare usually starts to lick and nuzzle the foal. During this time, the foal was encouraged to continue to nurse the foal and the mare was being restrained sufficiently to allow the foal to nurse but not inhibit her desire to lick and nuzzle the foal. Maternal behaviour has been initiated when the mare tries to follow the foal around the stall, vocalises to communicate with the foal and licks the foal. When the maternal behaviour was established, both mare and foal were allowed to circulate freely in the stall and to continue the bonding process. At this point, the mare-foal bond was generally established and no further intervention was needed. Once the foal was adopted by the mare, the domperidone or sulpiride treatment was continued for a total of 30 days. Foals were allowed free access to a creep feeder or supplemented 3–4 times per day with milk replacer as mares to compensate for the slowly increasing milk production after induction and adoption.

Further reading
11.30–11.40
Marbles and vaccination against GnRH

Peter Daels
KEROS NV, Westrozebekestraat 23A, 8980 Passendale, Belgium.

No abstract submitted
11.40–11.50

How to: Deal with rectal tears

Oliver Crowe
Willesley Equine Clinic, Byams Farm, Willesley, Nr Tetbury, Glooucestershire GL8 8QU, UK.

No abstract submitted

NOTES
Thursday 10th September 2009

11.50–12.00

How to: Deal with kicks to the penis

Oliver Crowe
Willesley Equine Clinic, Byams Farm, Willesley, Nr Tetbury, Glooucestershire GL8 8QU, UK.

No abstract submitted
How to examine a mare’s placenta

Katherine Whitwell
Katherine Whitwell Equine Consultancy, Newmarket, Suffolk, UK.

Placental morphology is the culmination of 11 months of in utero development, hopefully resulting in the birth of a healthy foal. Its examination after birth provides a small window of opportunity to look for established or recent variations and abnormalities that may range from the incidental, to those which significantly influence the foal’s health or very survival. For full-term foalings placental inspection should be carried out as a routine by stud staff, referring any that they are concerned about to their vet for assessment. If the pregnancy results in abortion, stillbirth or a neonatally sick foal, the placenta can be referred to an equine diagnostic lab for examination and appropriate sampling. This communication is intended as a guide for practitioners undertaking a placental inspection and is presented in procedure form.

Note which side is outermost: and inspect that side first (red villous side or pale, smooth avillous allantoic side). Normally it is dropped mainly avillous side outermost.

Spread the chorion out into an ‘F’ shape identifying both horns (smoother wider pregnant horn, PH, and more wrinkled narrower nonpregnant horn, NPH) and the body. Identify if any part is missing (it is usually the NPH tip that may get retained). Confirm it is a singleton placenta and not twins. Note hippocorne and fragments.

Microbiology check
Take samples for EHV screening if required (star, body, PH and NPH into viral transport medium). Take bacteriology swab if suspect an infected site (usually cervical pole).

Examination of the paler avillous side

Amnion
First check the amniotic sac for abnormal contents (excessive blood, meconium). Look for tears, lesions. ‘Cigarette burn’ foci are normal. Remove amnion

Umbilical cord
Extend it and assess if excessive twisting present. Undo twists, then measure total and amniotic cord lengths. Identify any urachal dilations in amniotic part. Check end (cord rupture site) - 2 arteries protrude normally: assess their size ratio.

Check size and location of yolk sac remnant (normally hidden in infundibular cavity near the cord attachment site to the chorion.

Chorion
Inspect allantoic surfaces in all parts of the chorion. Check for tears, haemorrhages, focal areas of oedema or necrosis, adherent hippocorne debris, sites of allantoic calcification, allantoic ‘cysts’, and invaginations from the villus surface (endometrial cup sites sometimes invaginate debris).

Identify if the cord attachment site is normally located - at bifurcation of the horns on the larger PH or a short distance up the NPH. (Body attachment is not normal.)

Examination of the red villous side

• Reverse the placenta so it is red, villous side outermost, and spread it out completely.
• Confirm if any is missing, possibly retained in utero (rather than just torn.)
• Identify the radiating folds (the star) at the cervical pole: check for sign of infection
• Note any villous pallor sites at the cervical pole - slight villus atrophy is common.
• Check size and integrity of the 2 horns and body.
• Note sites with focal oedema or thickening and the state of overlying villi. (At term it is ‘normal’ to find one or 2 oedema plaques at the tip of the PH and associated villus discoloration - possibly caused by foal foot pressure points)
• Note any extra wide or extra narrow dimensions - normal published data is available.
• Identify any avillous areas: if they are linear check if they merely reflect folding over large blood vessels present on the allantoic side.
• Take an overview of the villus density and quality - they reflect endometrial health.
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• Suspend the placenta by grasping a horn in each hand and attempt to determine which are the left and right horns. (The presence of a ‘convexity’ bulge below the PH aids this determination)
• Record findings on placenta diagram - take written and photographic evidence.

Much potentially useful information can be derived from a conscientious placental inspection. The message in this presentation is ‘Check it before you chuck it!’.

NOTES