Approach to the ‘repeat breeder mare’

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Topics For Discussion

- Susceptibility to PBIE
- Semen Choice
- Uterine Infection
- Biofilm
- Hysteroscopy
- Chronic Uterine Degeneration
- Buserelin
- Progestagen Supplementation
- Autologous Plasma
- Oviductal Blockage
- Lymphocyte Immunisation
- Test Breeding

Susceptibility to PBIE

- Intensive management
- Uterine lavage, uterine ecbolics, with or without intrauterine antibiotics
- Oral prednisolone (0.5mg/kg bwt twice daily) to dampen down the inevitable inflammatory reaction

Delayed Uterine Clearance

- Impaired uterine contractility
- Impaired lymphatic drainage
- Compounded by anatomical deficiencies:
  - Pendulous uterus
  - Breakdown in the vulvo-vestibular or cervical seals
  - Failure of cervical relaxation inhibits fluid clearance (older maiden mare syndrome)
- Retention of fluid, inflammatory cells and inflammatory by-products +/- bacterial colonisation is not conducive with pregnancy

Semen Choice

Consider advantages of changing from frozen to chilled or even better to fresh semen

Consider changing from natural cover to fresh semen insemination

Change of semen extender (away from egg based extender)

Change stallion; MHC incompatibility??
Uterine Infection

- Trans-cervical swab
- Guarded swabs/brushes
  - Separate swabs for bacteriology & cytology (with & without media)
- Uterine flush/lavage
- Low volume lavage
- Uterine biopsy

Endometrial Cytology

- Endometrial cells, inflammatory cells (PMN's), bacteria, fungi
- 1 or 2 PMN/hpf (x400) = active inflammation
- >5 per hpf = more severe inflammation
- More sensitive than culture
- Used in conjunction with culture

Uterine Biofilm

- Extracellular matrix
- Approximately 80% of E. coli, Klebsiella pneumoniae and Pseudomonas aeruginosa isolates cultured from the equine uterus are able to produce biofilm in vitro.
- Candida spp. are also known biofilm producers

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Anti-Biofilm therapy

- 10 to 20% in 1L Dimethylsulfoxide (DMSO)
- 20% (150 ml of 200mg/ml) Acetylcysteine (ACE)
- 3% (20 to 120ml) Hydrogen Peroxide
- Tris-EDTA and Tricide (250 to 500ml)
- Ceragyn 60ml infusion

Table 1. Anti-Biofilm therapy

<table>
<thead>
<tr>
<th>Anti-Biofilm therapy</th>
<th>Concentration</th>
<th>Volume</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>10 to 20%</td>
<td>1L</td>
<td></td>
</tr>
<tr>
<td>Acetylcysteine (ACE)</td>
<td>20% (150 ml</td>
<td>200mg/ml</td>
<td>150 ml</td>
</tr>
<tr>
<td>Hydrogen Peroxide</td>
<td>3% (20 to 120ml)</td>
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Table 1. Antibiotic and Non-Antibiotic Combinations For The Treatment Of Biofilm Associated Endometritis In Horses

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<tr>
<td>Ceragen (50mg/ml)</td>
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<tr>
<td>Hydrogen Peroxide (3%)</td>
<td></td>
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<td>Acetylcysteine (ACE) (200mg/ml)</td>
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Jabra-Rizk et al. (2004), Ferris et al. (2014)
**Hysteroscopy**
- At least 1.3 metre long
- Outer diameter >10 mm (rigidity)
- High-intensity light source
  - Uterine lumen large
  - Ideally 350 watts
- Videoendoscope makes it easier for both examiners to negotiate and evaluate the reproductive tract

**Endometrial Cysts**
- If large/numerous may affect placentation and placental nutrient supply
- May be obstructive and inhibit early embryonic mobility or fixation
- Make life difficult when it comes to diagnosis of pregnancy

**Hysteroscopy**

**Endometrial Biopsy**
- Safe, practical technique
- Aid to diagnosis of endometrial pathology
- Guide to treatment
- Prognostic information
- Management to maximise breeding potential for individual mares
- Culture and cytology from biopsy provides the most accurate results wrt sensitivity and positive predictive value

**Endometrial Histology**
- Cyclic cellular changes
  - Anoestrus
  - Transitional
  - Oestrus
  - Dioestrus
- Endometrial cells
- Glandular system
- Stromal infiltrates
- Fibrosis
- Lymphatic lacunae (cysts)

**Chronic Uterine Degeneration**
- Inflammatory & degenerative changes
- Inflammation
- acute (neutrophils), chronic (lymphocytes)
- Endometrial glandular degeneration
- Fibrosis, gland nests/cysts, inspissation
- Degenerative changes with age
  - 4 Grades I, IIa, IIb, III
- Breeding prognosis
Endometrial Biopsy

- Chronic Infiltrative Endometritis (CIE)
- Chronic Degenerative Endometrial disease (CDE) ‘Endometrosis’
  - Grade I (1) – No histopathological abnormality
  - Grade IIa (2) – Mares up to 9 years < grade 1
  - Grade IIb (3) – Mares up to 13 years < grade 2
  - Grade III (4) – Mares over 17 years likely grade 4
- Age related degeneration
- CDE more effect of age than parity
- Mild abnormality
- Moderate abnormality
- Severe abnormality
- Breeding prognosis
- Paired biopsy

Chronic Uterine Degeneration

MARE ENDOMETRIAL BIOPSY REPORT
Lotter (1996)

Pathological findings
There is a full thickness focal polymorphonuclear and mononuclear cell infiltration of the lamina epithelialis and superficial stromal layers. The occasional plasma cell is seen. There are signs of diffuse stromal oedema and some lymphatic dilatation are seen. There is a sign of widespread moderate chronic granulocellular degenerative changes with well formed giant cells and some giant cells, the more superficial of which contain granulated secretion and degenerative leucocytes.

Comments
The pathological findings indicate the presence of an acute endometritis superimposed upon a degree of chronic infiltrative endometritis consistent with some local immune disturbance. The degree of chronic endometrial degeneration disease seems to be excessive for a 14 year old mare.

In the basis of these findings, you may wish to consider treatment with endometrial curettage followed by a course of intravaginal antibiotic irrigations, guided by the results of sensitivity tests performed on significant pathogens isolated from the mare’s uterus. I am not sure that Kerosene therapy was isolated from the mare’s uterus in May (our reference 1983 to 1985) but not subsequently (1947 to 1997). I recommend that this is rechecked.

I recommend that you resample approximately 3 to 4 weeks after the end of treatment to help assess progress and to provide a more accurate breeding prognosis for next season.

Curettage

Physical

Chemical curettage

Kerosene protocol

- 60 ml commercial kerosene
- Rubber free syringe
- Infuse mid to late luteal phase (>6 days post ovulation)
- Retention for 24-72 hours
- Lavage (+/- prostaglandin)
- Infuse b/s antibiotics (+/- lavage) for 3-5 days
- Long follicular phase
- Miss a cycle before re-breeding

Buserelin

- GnRH agonist (Buserelin)
- Field trials conducted by Newcombe and co-workers (2001) on 2,346 mares determined that treatment of mares with 20 to 40 µg buserelin between Days 8 and 12 significantly increased pregnancy rates by approximately 10%
- 40 µg buserelin (Veterelin; Laboratorios Calier, S.A.) 10 days post ovulation
- The result of this treatment will therefore be to prevent pregnancy loss before day 14

Newcombe et al. (2001)
Progestin Supplementation

- General lack of evidence justifying the use
- Pregnancy loss is rarely caused by a fall in plasma progesterone
- No published evidence that progestagen supplementation will improve conception or 14 day pregnancy rates
- Progesterone essential for maintenance of early pregnancy
- Oral vs. injectable supplementation
- Suppression of maternal luteal function

DeLuca et al. (2001)

Altrenogest supplementation

- Suppression of endogenous P4
- Altrenogest 0.044mg/kg from no earlier than day 5 post ovulation
- Cessation of treatment options:
  - When endogenous P4 demonstrated as being adequate
  - When 2°/accessory corpora lutea formed (42 days)
  - From 100 days gestation

Intrauterine Plasma

- Supplement the intrauterine environment with complement and immunoglobulins, both of which are key components for bacterial opsonisation (Asbury 1984)
- Pascoe (1995) reported the results of a large field study where the addition of autologous plasma to a post-breeding infusion of antibiotics significantly improved pregnancy rate per cycle in lactating mares, tended to improve pregnancy rate per cycle in barren mares, and had no effect on maiden mares

Pascoe 1995

Oviductal Occlusion

- Candidates have encountered multiple episodes of inexplicable failure of conception to the same or multiple stallions
- Laparoscopically guided administration of PGE2 gel directly onto the surface of the oviducts
- Sixty to 70 % conception rate post treatment

Bennett et al. (2002), Allen et al. (2006)

Hysteroscopic Hydrotubation

- 200-cm polyethylene tube (1.7 mm outer diameter) with a 22-G 1.75-in. injection catheter attached at one end and guide wire (0.46 mm diameter, 220 cm long)
- N=28 barren mares (2-20 cycles)
- Mean number of cycles for each mare prior to treatment was 6.5 +/- 4.5 cycles
- Saline was successfully infused into 89% (50/56) oviducts
- 26 of 28 mares conceived after the treatment
- Mean number of cycles to conception was 1.8 +/- 0.8.

Inoue, 2013; Inoue Y. and Sekiguchi, 2017

Lymphocyte Immunisation

- Controversial human therapy
- Immunisation of women who have a history of recurrent spontaneous abortion with lymphocytes isolated from their husband or a third party donor
- LIT alters the proportions and functions of most peripheral blood lymphocyte subsets
- Requires access to stallion blood
- Change stallion!!!

Mathias & Allen, 2000; Gatenby et al. 1989
Test Breeding

- Fresh semen from a stallion of known fertility
- Fresh AI in advance of ovulation
- Embryo flush at day 7
- Pregnancy scan day 12/13
- Termination of pregnancy

Thank you
Thank you for your attention