Approach to the ‘repeat breeder mare’

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Introduction

Most clients expect or hope that their mares will conceive on their first breeding attempt. Failure to conceive loses time and incurs further charges for livery and veterinary management. In the case of chilled semen additional charges will likely be incurred for semen collection and shipping, in the case of frozen semen additional semen doses will be required which may have been purchased by the dose. If the clinician encounters a problem on the first cycle it is important to adapt the management for the second cycle to optimise conception rates however what does the clinician do when the mare fails to conceive after the second, third or fourth attempts at breeding? An understanding of the individual mare’s issues is necessary in order to formulate a management plan for her; if the mare’s issues are not readily apparent additional diagnostics may need to be performed.

Susceptibility to post breeding endometritis

Mares that are susceptible to post breeding endometritis which fail to conceive may become infected if not managed appropriately. In addition repeated breeding attempts will likely increase the degree of inflammatory change within the endometrium and, as is often encountered, the mares uterus appears to become more ‘reactive’ to the deposition of semen within. If the susceptible uterus is free from infection repeated breeding attempts need to be made with intensive management using uterine lavage, uterine ecbolics, with or without intrauterine antibiotics. In addition to the Author will use oral prednisolone (0.5mg/kg bwt twice daily) to dampen down the inevitable inflammatory reaction. What more can the clinician do?

If further attempts are to be made then a change to chilled from frozen semen may be advantageous. The relatively longer lifespan of chilled semen in the mares reproductive tract compared to frozen allows the clinician to inseminate the mare in advance of ovulation. By this same reasoning fresh semen would be superior to chilled, allowing insemination well ahead, perhaps days ahead of ovulation. This strategy allows the clinician time to treat the uterus in advance of ovulation whilst the mare is in oestrus rather in the post-ovulatory period when progesterone is rising. The more time the uterus has to settle down after breeding the more likely she is to conceive. The change in semen type will also likely increase the number of progressively motile morphologically normal spermatozoa in the insemination dose. In addition, in the Author’s experience, certain mares appear to be especially sensitive to semen extenders, especially those which are egg based; a change to fresh semen means that an extender may not be absolutely necessary.

Clinicians need to be careful not to imply that a change in semen type is because of an issue with the stallion. Assuming normal stallion fertility changing from frozen to chilled or from chilled to fresh from the same stallion will have the same effect. In the case of a naturally covering stallion, if the rules of the breed society allow, one could consider collecting the stallion and inseminating the mare. This will reduce the degree of uterine contamination that would occur at natural cover and reduce the overall volume of inseminate.

Uterine infection

If infection is suspected on the basis of the clinical gynaecological examination screening in the form of guarded endometrial swab is recommended. A low volume lavage (LeBlanc et al. 2007) is a preferable method in many cases, especially in cases of negative culture results in the face of infertility. To utilise this technique, a small volume (100 mL) of saline or lactated Ringers is introduced into the uterus in an aseptic manner with a large bore catheter. The lavage fluid is then massaged in the uterus per rectum in order to cover a greater percentage of the endometrial surface and
then retrieved into a sterile vessel such as a 50 mL centrifuge tube. More recently a double-guarded low volume lavage technique using 250 mls of fluid has been described (Christoffersen et al. 2015). Another option for the collection of cytology samples is to use a cytobrush. The low volume lavage and cytobrush are both superior to the use of the cotton swab (Overbeck et al. 2011; Cocchia et al. 2012) yet due to the fact that they are more sensitive more studies are needed to establish criteria for interpretation of inflammation (Cocchia et al. 2012). It has been suggested that culture and cytology (via histopathology) from an endometrial biopsy may be the ‘gold-standard’ as it is more sensitive with a greater positive predictive value when compared to cotton swabs (Nielsen, 2005). If significant bacterial or fungal growth is detected prior to breeding it is necessary to treat this infection prior to further attempts at breeding; it is often necessary to miss the cycle and breed on the next spontaneous or induced infection free oestrus.

Recurrent infections with the same bacterial or fungal agent are frustrating to deal with and it is common place for the clinician to treat one infection only to culture a different one at the next cycle. Of particular interest are agents which colonise in an extracellular matrix known as a biofilm. Biofilm formation is a highly complex process involving the switch from a free floating existence to a sessile (attached) mode of growth. The latter enables the biofilm community to be protected from adverse environmental conditions such as desiccation as well as antimicrobial agents (Clutterbuck et al. 2007). Approximately 80% of the Gram negative bacteria E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa cultured from the equine uterus are able to produce biofilm in vitro (Ferris et al. 2014), and although Candida spp. are usually referred to as fluid living they are known biofilm producers (Jabra-Rizk et al. 2004). Beehan and co-workers (2015) demonstrated that approximately 30% of E. coli cultured from the reproductive tracts of mares had biofilm-forming potential and subsequently demonstrated that these isolates were capable of forming a biofilm colony by scanning electron microscopy. In addition Ferris and co-workers (2016) demonstrated that P. aeruginosa was capable of producing biofilm-like material in vivo.

Solutions of acetylcysteine (ACE), hydrogen peroxide, ethylene diamine tetra-acetic acid (EDTA)-Tris and dimethyl sulfoxide (DMSO) have been used clinically to treat uterine infections suspected to be complicated by biofilm presence but limited evidence from controlled studies is available. In vitro studies (Ferris et al. 2014; Ferris et al. 2016; Loncar et al. 2017) have assessed the effectiveness of ACE, EDTA-Tris, gallium nitrate, hydrogen peroxide, DMSO and antimicrobial peptide mimic (APM; Ceragyn) as anti-biofilm treatments. They have cumulatively demonstrated that no one treatment consistently prevents biofilm biomass in all bacterial isolates. These bacterial species-specific results stress the importance of acquiring organism identification when developing a treatment plan for bacterial endometritis because not all treatments are effective against every bacterial species (Loncar et al. 2017). It is the Author’s preference to use a 10-20% solution of DMSO in 1L lactated Ringer’s as an anti-biofilm agent; it also seems to be particularly useful in mares with a significant amount of mucus (Causey et al. 2000).

Recent work has indicated that Streptococcus equi subspecies zooepidemicus may reside deep in the endometrium in a dormant state (Petersen et al. 2009). Petersen an co-workers (2015) suggested that some strains of S. zooepidemicus were able to form ‘persister cells’ and acquired a tolerance to penicillin by means of their dormancy. They subsequently demonstrated in a population of sub-fertile mares that it was possible to activate thee dormant persister cells by means of an instillation of a bacterial growth medium (bActivate).

**Biological therapies**

Intrauterine infusion of platelet-rich plasma (PRP) causes down-regulation of mRNA expression of IL-1b, IL-6, IL-8 and iNOS when compared with untreated mares (Metcalf et al. 2012). Metcalfe (2014) went on to show that infusion of PRP in susceptible mares resulted in a lower incidence of delayed uterine clearance and higher pregnancy rates compared to controls (67 vs. 19%). A recent study by Segabinazzi and co-workers (2017) demonstrated that infusion of PRP into susceptible mares resulted in a decrease in mares positive for endometritis after breeding when compared to controls and significantly higher pregnancy rates in PRP treated mares. A study by Harnacke and co-workers (2013) demonstrated no difference in between pregnancy rates and PMN counts 48 hours after insemination in susceptible mares treated with autologous conditioned serum (IRAP) and controls. However, Ferris and co-workers (2014) IRAP into the uterus of mares and demonstrated that IRAP significantly reduced the number of neutrophils in the uterine lumen 6
hours after the sperm challenge. Immunomodulators have been used as treatment adjuncts for endometritis. Available products use a Mycobacterium cell wall extract or Propionobacter preparations. Although it is not yet clear the exact mechanism by which fertility is improved, there is some evidence that in some mares, treatment is helpful (Rogan et al. 2007; Rohrbach et al. 2007).

Uterine degeneration

Endometrial biopsies are classified into four categories (I, IIA, IIB, III) based on Kenney & Doig (1986). A mare with a Category I biopsy has an essentially normal endometrium. The likelihood of her becoming pregnant and carrying a foal to term, estimated at 80 to 90%, depends more on broodmare management than on the mare’s inherent fertility. Mares with a Category III biopsy have severe pathological changes in the endometrium and an estimated 10% chance of carrying a foal to term, even with good breeding management. Most mares will be classified as a Category IIA or IIB with an estimated 50 to 80% and 10 to 50% chance, respectively, of carrying a foal to term, reflective of a combination of management practices and the mare’s inherent fertility. When uterine biopsy findings indicate a severe degree of chronic endometrial degeneration (grade 3) it is expected that the chances of the patient carrying a pregnancy to term are significantly reduced (Kenny and Doig 1986). A recent study by Killisch and co-workers (2017) demonstrated that 22-30% of biopsies taken during the spring or autumn transition demonstrated ‘irregular’ endometrial differentiation and they concluded that diagnostic biopsy sampling should therefore be performed between late April and September.

A complete histopathological description is usually provided by the pathologist. However, of primary concern to the practitioner is the severity and distribution of inflammation and degenerative changes including periglandular fibrosis, angiosis and lymphatic lacunae. Degenerative changes carry a worse prognosis than inflammatory changes because they are considered to be permanent and progressive. Attempts have been made to improve the ‘quality’ of the endometrium and improve breeding prognosis by physical (Ricketts 1985), or chemical curettage (Bracher et al. 1991). Ricketts (1985) demonstrated that mechanical endometrial curettage resulted in an overall improvement in the extent and degree of chronic degenerative endometritis in 82% of cases. Fifty eight percent of the mares produced live foals following curettage. Bracher and co-workers (1991) reported on the use of kerosene as a chemical agent for endometrial curettage. An infusion of 50ml of commercial kerosene in dioestrus induced a severe acute inflammation of the endometrium of short duration with mild to severe necrosis of the luminal epithelium. Pregnancy was established in 65% of treated mares, half of which had grade 3 histological changes. No adhesions developed in any mares treated. Although these studies suggest an improvement in biopsy grade and pregnancy outcome the techniques have not been widely adopted. The use of kerosene however remains controversial and there is a lack of information from controlled, randomised clinical trials. Despite this the Author does use intrauterine infusion of kerosene in selected mares with repeated conception failure and evidence of severe endometrial degeneration.

Stem Cells

The development of stem cells for ‘regenerative’ applications in musculoskeletal injury has led to research into stem cells for uterine applications. With particular interest is the potential of stem cells to reverse the process of endometrial degeneration and modulate the inflammatory response of the uterus to breeding. Mesenchymal stem cells (MSCs) for uterine use have been derived from adipose (Mambelli et al. 2013; Falamo et al. 2015), equine umbilical cord (Casarini et al. 2016), bone marrow (Ferris et al. 2014; Alvarenga et al. 2016) and endometrium (Rink et al. 2017; Esteves et al. 2017). MSCs have been safely applied by infusion (Mambelli et al. 2013; Ferris et al. 2014; Casarini et al. 2016) trans endoscopic injection (Alvarenga et al. 2016) and in vivo (Mambelli et al. 2013) and in vitro cell presence has been demonstrated post application (Falamo et al. 2015).

Mambelli and co-workers (2014) demonstrated that MSCs were able to positively modulate the expression pattern of secretory proteins as well as promote the induction of glandular epithelial cell proliferation. In addition Ferris and co-workers (2014) infused bone derived MSCs into the uterus of mares and demonstrated that they significantly reduced the number of neutrophils in the uterine lumen 6 hours after the sperm challenge and an increase in the anti-inflammatory cytokine IL-1Ra was observed after treatment.
Acupuncture

A retrospective study involving 44 broodmares showed a reduction in post-mating fluid and improved pregnancy rates in mares with a history of susceptibility (Rathgeber, 2000). It is thought to induce uterine contractions and improving tone to improve uterine clearance.

Repeated failure to conceive without fluid accumulation

Excluding mares which have ovarian pathology or failure of ovulation, those that repeatedly fail to conceive without fluid accumulation are a challenge as the clinician has very limited evidence to guide appropriate treatment. A small number of mares that have normal uterine clearance mechanisms may still develop significant uterine inflammation. If in doubt regarding the presence of inflammation a single uterine lavage >6 hours post breeding may be both diagnostic and therapeutic. Beyond this what can one do?

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%. The Author uses 40 µg buserelin 10 days post ovulation. Given the timing of this therapy it is not doing anything to improve conception rates rather improvements will be 14 day pregnancy rates and/or pregnancy loss rates beyond 14 days (Pycock and Newcombe 1996). The result of this treatment will therefore be to prevent pregnancy loss before day 14. The mechanism for this remains to be determined however increases in circulating LH concentrations have been demonstrated in buserelin treated mares (Kanitz et al. 2007).

Altrenogest supplementation

There is a general lack of evidence justifying the use of synthetic progestagen therapy to reduce pregnancy loss rates in mares. Pregnancy loss is rarely caused by a fall in plasma progesterone (Irvine et al. 1990) although individual cases may present with premature luteal regression (Canisso et al. 2013) necessitating supplementation if the pregnancy is to be maintained. To the Author’s knowledge there is no published evidence that progestagen supplementation will improve conception or 14 day pregnancy rates yet anecdotally it is used in cases of repeated conception failure. There is a general notion that altrenogest therapy will do no harm however it should be recognised that altrenogest may suppress endogenous progesterone levels. A study by DeLuca and co-workers (2011) demonstrated that administration of altrenogest to pregnant mares was associated with lower concentrations of endogenous progesterone from days 14 to 18 and on day 21 compared with endogenous progesterone levels in pregnant mares not administered altrenogest. This effect was presumed by the authors to be mediated by a reduction in pituitary luteinizing hormone (LH) release and a decrease in luteotropic support.

If altrenogest is to be administered to the mare with conception failure the Author would suggest that it is administered at a rate of 0.044mg/kg from no earlier than day 5 post ovulation. If the mare becomes pregnant one should continue therapy until either, it can be demonstrated that endogenous progesterone levels are adequate (> 3ng/ml; assay dependant), the mare has secondary ovulation(s) that will boost endogenous progesterone production, or until the fetoplacental unit takes over progesterone production at around 100 days of gestation. The Author recommends that mares are weaned off altrenogest by halving the dose for at least one week.

Intrauterine autologous plasma

Autologous plasma has been suggested as a method to 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. The fashion has changed and plasma is now rarely used however in a mare that fails to conceive without explanation it provides another option for the clinician to try.
Blocked oviducts

Bennett and co-workers (2002) investigated 31 mares with histories of prolonged infertility for 2–4 breeding seasons. Evaluation of the reproductive tract using trans-rectal palpation, ultrasound, culture, cytology, biopsy, and hysteroscopy was performed and failed to provide a definitive diagnosis for infertility. The oviducts of these mares were assessed under general anaesthesia demonstrating 42% were bilaterally occluded and 19% were unilaterally occluded. Twelve mares that were bilaterally blocked had their oviducts flushed in orthograde direction and were subsequently bred with a 67% conception rate. Recent work by Köllmann and co-workers (2011) demonstrated that the oviducts can be successfully observed and flushed using a laparoscopic flank-approach.

In the Author’s practice mares which are candidates for oviductal blockage have PGEs gel applied to their oviducts under laparoscopic guidance. Allen and colleagues (2006) demonstrated that laparoscopically guided administration of PGE2 gel (Dinoprostin) directly onto the surface of the oviducts was successful at restoring fertility in mares assumed to have oviductal blockage based on their reproductive histories. Fifteen mares that had exhibited failure to conceive during 1–4 years were treated, of which 93% conceived within the same or subsequent breeding season. Sixty percent of mares conceived to their first mating (Allen et al. 2006). If oviductal flushing by the application of PGE2 is best performed within the physiological breeding season (Martynski et al. 2015). More recently, uterine tube catheterization has been described by Inoue (2013) via hysteroscopy and demonstrated that the technique is applicable to retrograde flushing of suspect blocked oviducts (Inoue and Sekiguchi 2017).

Lymphocyte Immunisation

The abstract from Mathias and Allen (2000) summarises this therapy: ‘The incidence of recurrent spontaneous abortion within the human population in the Western world is low (2-5%) but significant, and a proportion of these pregnancy losses are thought to have an underlying immunological cause. Immunisation of women who have a history of recurrent spontaneous abortion with lymphocytes isolated from their husband or a third party donor is one of several forms of immunotherapy used to treat the problem. Early pregnancy loss in Thoroughbred mares is also significant and, as in women, a small number of mares undergo repeated pregnancy losses. Two trials have been performed in which Thoroughbred mares suffering from recurrent spontaneous abortion before day 150 of gestation were immunised with lymphocytes isolated from the mating stallion or from an unrelated stallion. The first trial, which was conducted without control mares, resulted in a very high live birth rate (97%) for the mares (n=24) treated. Therefore, a second controlled double-blinded trial was established in which randomly selected mares (n=17) were treated with stallion (heterologous) lymphocytes and control mares (n=13) were injected with their own (autologous) lymphocytes. The live birth rates after these treatments were 88 and 77%, respectively. Thus, immunisation with stallion lymphocytes had no effect on the incidence of abortion in mares suffering from recurrent spontaneous abortion.’

Test Breeding

In the Author’s practice mares which have encountered multiple episodes of inexplicable failure of conception to the same or multiple stallions are candidates for test breeding to a stallion with excellent fertility by fresh semen insemination. This can be coupled with a test flush on day 7 or the mare left until 13 days for pregnancy determination. A good example is that of a mare that had six cycles of chilled AI with a stallion based in Germany. The mare had a test breeding with fresh semen and produced a Grade 1 expanded blastocyst on day 7 after ovulation. The recommendation to the mare owner was to either change stallion or take the mare to Germany where she could have fresh semen from the previously used stallion. The owner opted for the latter and the mare conceived on the first cycle. In this case it is difficult to give precise reasoning as to why the mare failed to conceive to chilled semen given the intensive management she received however the result guided the decision whether the mare had her oviducts treated with PGE2 or further breeding attempts made.
References


Ferris R. A., Frisbee D. A. and McCue P. M. (2014) Use of mesenchymal stem cells or autologous conditioned serum to modulate the inflammatory response to spermatozoa in mares. Theriogenology. 82, 36-42


