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The role the immune system plays in the development and persistence of endometritis in the mare has been examined by many researchers, and over many years. Although much has been learned, much remains unknown. It must be accepted that infertility is a multifactorial disease. Some mares fail to conceive because of poor conformation, others because of pathology in the endometrium, and others from as yet undiscovered reasons. Still, in order to treat this variety of mares with various causes of infertility, we must continue to explore the reasons behind infertility. Whatever differences, if any, there may be in the immune system between susceptible and resistant mares, they do not appear to be systemic. Mares that are subfertile due to a susceptibility to endometritis are not more likely to become ill than are resistant mares.

Within the last thirty years, studies have examined factors such as differences in neutrophil function, immunoglobulins and opsonins between resistant and susceptible mares. Workers from Florida measured IgA, IgG, IgG(T), and IgM in uterine secretions from normal and susceptible mares at estrus and diestrus, then challenged the mares with *Streptococcus equi*, subsp. *zooepidemicus* and re-examined them post challenge. Higher concentrations of IgA, IgG, and IgG(T) were observed in susceptible mares\(^1\). Other workers subsequently examined immunoglobulins in uterine secretions and were unable to detect differences in IgA, IgG, and Complement factor C3 concentrations during the first 24 h after infusion of *Strep zoo*, concluding that susceptibility to endometritis is not likely due to local immunoglobulin deficiency\(^2\). However, they did find lower concentrations of C3 at 36 hours post inoculation. Using *Strep zoo* to recruit neutrophils in susceptible and resistant mares, the susceptibility to endometritis was not found to be associated with a defect in the phagocytic function of uterine neutrophils\(^3\). The same workers examined opsonins, molecules that enhance phagocytosis or activate complement. In resistant mares, opsonins were greater during diestrus than during estrus, yet for mares susceptible to endometritis the opposite was true. Overall, opsonins were higher in mares susceptible to endometritis than in resistant mares, but this difference was only apparent at estrus\(^4\). During this same era, treatment of post breeding endometritis with an intrauterine infusion of plasma was advocated as a means to enhance phagocytosis, boost uterine defense mechanisms and improve fertility\(^5\). Subsequently, attention turned to physical clearance mechanisms in the uterus. Eloquent studies looking at lymphatic drainage or expulsion of uterine contents through the cervix demonstrated the importance of physical clearance for normal fertility and the establishment of pregnancy\(^6,7\). Clearly, if excess sperm, bacteria, neutrophils, and other debris remain within the uterine lumen, they will continue to act as inflammatory stimulants and decrease the chances of establishing pregnancy. For a time, research focus seemed to turn away from the immune system towards why mares differed in their ability to physically clear debris from their uterus and what could be done to restore uterine clearance to normal.
normal. Factors such as myometrial function, uterine innervation, and uterine position within the abdomen have all been implicated as factors contributing to infertility. The uterus has been described as having a mucosal immune system and some have suggested a corollary with the respiratory system. Intersting studies have examined the cilia of the endometrial epithelial cells to determine if defects at this level may impact uterine clearance in addition to myometrial defects. Although biofilms have not been demonstrated unequivocally in the equine uterus, a recent paper has described evidence for biofilms in chronic equine wounds. Biofilms are complex structures formed by an aggregate of different populations of bacteria in which the bacteria adhere to each other on a surface. These cells are frequently embedded within a self-produced matrix, generally composed of extracellular DNA, proteins and polysaccharides. Biofilms may form on living or non-living surfaces and can be found in natural or man-made settings. The microbial cells growing in a biofilm are different from free floating cells of the same organism. Microbes form a biofilm in response to many factors, which may include cellular recognition of specific or non-specific attachment sites on a surface, nutritional cues, or in some cases, by exposure of bacteria to sub-inhibitory concentrations of antibiotics, such as aminoglycosides. When a cell switches to the biofilm mode of growth, it undergoes a shift in behavior and becomes more resistant to the immune system and many therapeutic modalities.

Most of the earlier work in endometritis used Streptococcus zooepidemicus in experimental models of endometritis. However, recent reports indicate that the uterus may react differently to gram negative bacteria such as E coli than they do to gram positive cocci such as Strep zoo. When the response of susceptible and resistant mares to Strep vs. E coli was compared, differences in the uterine response were observed and may be related to induction of different immune responses. Susceptible mares exhibited a more vigorous exudative response. In a field study investigating ultrasound findings and cytology in relation to culture results, uterine fluid was associated with moderate to severe inflammatory cytology and not with a specific organism. However, fluid and a positive cytology were more common with B Strep than with E coli. More recently, as our knowledge of the inflammatory response and the immune system in general has grown, this knowledge has been applied to aid us in further understanding what role these processes might have in the mare’s uterus. Numerous studies have investigated cytokines, toll-like receptors and other mediators of the inflammatory response. In a study examining cytokines in the equine uterus, IL-1B, IL-6 and TNF-α were higher in susceptible mares than in resistant mares during estrus and IL-1B and TNF were higher in susceptible mares during diestrus. No differences were found in resistant mares between estrus and diestrus, but in susceptible mares IL-6 and TNF were higher during estrus. Compared to “baseline”, 24 h after artificial insemination (AI) all 3 cytokines were significantly increased in resistant mares, but not in susceptible mares. After AI, there was no difference between susceptible and resistant mares at 24 hours, but 7 days later, IL-1B and TNF were higher in susceptible mares. Toll-like receptors (TLR) are part of the innate immune system, and are activated by bacteria, yeasts, and viruses. Binding of TLR initiates a cytokine cascade that furthers the inflammatory response. The TLR are a key link between the innate and humoral immune responses. Resistant mares increase expression of TLR4 after insemination, susceptible mares do not, and this may contribute to a delay in clearance in susceptible mares. In an attempt to identify putative markers of mating induced endometritis (MIE) and persistent mating induced endometritis (PMIE), uterine cytology, histology, and bacteriology, along with uterine PGF2α, serum amyloid A, IL-8, and TLR4 were examined prior to and at various time points after AI. Interestingly, of the parameters tested, neutrophil numbers by cytological analysis and PGF2α were regarded as the most accurate markers of inflammation during MIE. Immunostimulants have generated interest for...
their potential to improve fertility and are commercially available for this purpose. In a follow-up to the cytokine study previously mentioned, mares were treated with Mycobacterium cell wall extract (MCWE), IV, at the time of AI. After treatment, there were no differences between susceptible and resistant mares in the cytokines studied and the increases in the cytokines seen after AI without MCWE were not observed. Treatment with MCWE at the time of AI downregulated the mRNA expression of IL-1 with significant effects observed in susceptible mares which then responded like resistant mares. In another study, MCWE was used either IV or IU in mares 48 h after the intrauterine infusion of Strep zoo. Treatment with MCWE resulted in the elimination of endometritis in 7 of 20 mares by the time of ovulation, and 14 of 20 mares by 7 days post-ovulation.

Another immunomodulator, Propionibacterium acnes, has also been studied for the treatment of PMIE. The report was a blinded, placebo-controlled field study in which the P acnes treatment was administered IV on days 0, 2, and 6. The authors, using multivariate logistic regression, concluded that P acnes, used as an adjunct to other conventional treatments for PMIE, improved pregnancy rates and live foal rates. Optimal effect was observed in mares that were bred during an interval spanning from 2 days before to 8 days after the first P acnes treatment.

Various studies have examined the use of glucocorticoids for the treatment of MIE. In a sequence of studies, the effect of a single dose of dexamethasone (50 mg, IV) administered to mares at the time of breeding was investigated. In an initial experiment, the safety of treatment was monitored, had no negative impact on the mare’s medical or reproductive parameters. In a second experiment, the effect of treatment on the uterine environment, fetal development and pregnancy outcome was examined, and a reduced inflammatory response was observed after mating in treated mares. Finally, mares susceptible to persistent mating induced endometritis were treated. Mares with multiple risk factors for susceptibility to persistent mating induced endometritis showed improved pregnancy rates following treatment (20). In another study, mares received 0, 10 or 20 mg dexamethasone, IV, 6 to 12 h post AI. Dexamethasone treatment did not affect pregnancy rates in any group, even in mares with fluid pre-breeding. In another report, susceptible and resistant mares were treated once with 40 mg dexamethasone, 2 h prior to breeding. Treatment did not reduce uterine fluid accumulation.

Concern is often raised regarding the use of corticosteroids and the risk of laminitis. However a recent publication reviewed the evidence in this regard and found that in most cases, treatment is safe. The authors did recommend caution in horses that are overweight or insulin resistant, or have had a recent case of laminitis. They deemed the risk acceptable if the therapy could be expected to be of benefit.

Because of concerns with glucocorticoids, some researchers have investigated the use of non-steroidal anti-inflammatories (NSAIDs). One report used prednisolone acetate (0.1 mg/kg, IV, q 12 h until ovulation) rather than dexamethasone, and found a significant positive effect on pregnancy rate using the corticosteroid. Another study examined the development of PMIE in mares treated with oxytocin only, vedaprofen (initially 2 mg/kg, PO, q 12 h) beginning on the day of hCG administration (24 h pre-AI) and oxytocin, or vedaprofen beginning 1 day after AI and oxytocin. Treatment continued until 1 day after ovulation. The group that received vedaprofen beginning post-AI had fewer neutrophils on uterine cytology than either of the other groups. Both groups receiving vedaprofen had fewer Cox-2 positive endometrial cells compared with mares receiving only oxytocin, leading the authors to conclude that anti-inflammatory treatment in conjunction with uterine ecbolics may prevent development of PMIE.

A similar study by the same group used barren mares with a history of repeated PMIE. Mares were treated with vedaprofen (initially 2 mg/kg, followed by 1 mg/kg, PO q 12 h) from 1 day before the first insemination to 1 day after ovulation. All mares, including controls,
received oxytocin (20 I.E., SC, q 8 h). There was no difference in the amount of fluid in the uterus the day after ovulation between groups. Pregnancy was confirmed in two of nine control mares and seven of eight mares treated with vedaprofen. Without doubt, further studies are needed to more clearly define the role the immune system of the equine uterus plays in the development and resolution of mating induced endometritis and how susceptible mares differ from resistant mares. It is likely that many differences exist as infertility is of course a multifactorial disease. Therefore, no single treatment can be expected to successfully cure all cases. With increasing knowledge of the interplay of the immune system and fertility, better options will hopefully be available to the practitioner to diagnose and treat equine endometritis.

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