Equine subclinical endometritis caused by dormant beta-hemolytic streptococci

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Bacterial endometritis as a cause of sub/infertility in the mare has been known for almost a century.[1] Over the years reproductive efficiency has increased due to improved management and treatment protocols and in well-managed breeding farms the number of open mares by the end of the breeding season is expected to be less than 10%.[2] Despite these improvements, and in some settings routine use of antibiotics, infectious endometritis maintains to be a significant problem in the mare.

Beta-hemolytic streptococci (beta strep), predominantly *Streptococcus* equi subspecies *zooepidemicus* (*Strep zoo*) and *E. coli* are the most commonly isolated pathogens from the uterus of the mare, accounting for approximately 80% of cases.[3-5] Despite no reports of bacterial resistance and high *in vitro* sensitivity to the most commonly used intra-uterine antibiotics, beta-strep infections have been identified as the uterine infection correlating with the lowest fertility in well-managed Thoroughbred mares.[6]

Inspired by the work of Jesper Nielsen demonstrating improved diagnostic sensitivity and specificity when using the uterine biopsy as a diagnostic tool compared to the swab,[4] we decided to investigate the localization of *Strep zoo* in the endometrium of different types of infected mares including experimentally infected and chronically infected mares. In the experimentally infected mares (young healthy research mares, which all cultured negative prior bacterial challenge), *Strep zoo* could be found only on the endometrial surface following uterine infusion, whereas no bacteria could be re-isolated 48 h after bacterial challenge. In the chronically infected mares (mares with a history of repeated uterine infections) *Strep zoo* was consistently found to have a multifocal distribution, located within the endometrium, thus markedly different from the experimentally infected mares. In some mares, *Strep zoo* could be visualized several millimeters into the endometrium. These findings support previous notions by Nielsen who found a significantly higher proportion of endometrial biopsies culture positive when compared to endometrial swabs from the same mare[4].

Despite a monoclonal genetic backbone, bacteria can be phenotypically heterogeneous.[7] This apparent survival mechanism was suggested in the early days of antibiotics, were it was demonstrated that despite full sensitivity to the antibiotic tested, a small fraction of the monoclonal bacteria was able to survive.[7] These cells were accordingly named persisters, as they did not grow nor die in the presence of microbicidal antibiotics.[7,8] One suggested survival mechanism used by persisters is to enter a non-dividing, or dormant state.[9] An example of such a mechanism is the ability to shut down protein synthesis and DNA replication and by doing so eliminating the mechanism of action of the antibiotic present.[10] Within the group of persisters toxin-antitoxin systems are often used to regulate metabolic activity.[8,11] Essentially, the toxin inhibits basic cellular processes, such as DNA replication or protein synthesis, whereas the anti-toxin reverses this effect. Hence, the bacterial activity level is governed by the toxin/anti-toxin equilibrium.[12]

Based on previous reports and our own clinical observations on intensely managed barren mares unable to get in foal, in spite of repeated antibiotic treatments, inability to isolate bacteria or other explanatory factors stimulated further research in this area.

Resuscitating promoting factors are molecules demonstrated to override the toxin effect mentioned above and induce active growth of otherwise inactive/dormant bacteria.[13] We recently discovered bActivate* (proprietary substance) that acts a resuscitating factor, which upon uterine instillation in subclinically infected mares induced growth of beta strep in a high proportion of mares.[14] A vehicle-control study in which problem mares were infused with either bActivate or phosphate buffered saline (vehicle), supported that growth of resident streptococci and not contamination was induced by bActivate.[15] In other words bActivate is a growth medium capable of re-activating dormant streptococci residing deep in the subclinically infected endometrium. Based on this assumption we initiated a clinical study aiming at demonstrating that subclinically infected (culture-negative) barren mares instilled with bActivate and, if strep positive, treated with systemic and intrauterine antibiotics, would have a better treatment success. The study included a group of client owned subfertile barren Thoroughbred mares identified by the theriogenology group of Hagyard, Davidson and McGee in a project led by Dr. Kristina Lu. The majority of mares had a

history of repeated uterine infections and treatments. To enter the study mares had to be barren for a minimum of three cycles, despite breeding to stallions with proven fertility and high quality veterinary management. A uterine culture was collected before and 24 hours after uterine instillation of bActivate, and only culture positive mares were treated with uterine lavage, with or without mucolytics and local and systemic antibiotics. A total of 64 problem mares were included and subsequently bred. Pregnancy was established in 53 (83%) mares. Of the 53 pregnant mares, seven were lost for follow-up and a live foal was produced by 32 mares (foaling rate 70%). In this selected group of problem mares active growth of beta strep was induced in 30/64 (47%), underlining the high frequency of subclinical endometrial beta strep infections. When evaluating a group of mares not selected by fertility or history of repeated infections, only one out of twenty was found to be activation positive (unpublished data), demonstrating a strong correlation between reduced fertility and chronic dormant uterine beta strep infections.

As no placebo group was included in the clinical study, it is not possible to determine whether fertility was significantly increased following activation and treatment. Historic data demonstrate that expected fertility in this group of mares is reduced compared to the general population (foaling rate 15-50% vs. 80-85%[16]). These data show that activation and subsequent antimicrobial treatment likely improves foaling rate, almost to the level of the normal fertile population.

Activation of dormant streptococci has made it possible to diagnose subclinical chronically infected mares in the field using standard diagnostic methods. If mares are strictly selected based on fertility and reproductive history, a group of high-risk mares can be identified in which improved treatment outcome and fertility following activation and treatment can be expected.

Further research in the chronic dormantly infected mare and in the microbiology laboratory will increase knowledge of this important disease in the barren mare.

Keywords: Beta-hemolytic streptococci, mare, endometritis, dormancy, subfertility

*bActivate is produced by Bojesen and Petersen Biotech, (www.bactivate.eu)

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