Equine endometrial streptococcal infections
Prevalence of isolates from sexually rested and sexually active mares

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Introduction
A recent study has shown that the Streptococcus equi subspecies zooepidemicus isolated from the endometrium of mares is predominantly belonging to a distinct genetic group [1]. Harvesting material from the uterine lumen and the endometrium for further diagnostic procedures in the laboratory has been performed for nearly 100 years. These techniques have over the years been refined from a simple sterile cotton swab to more invasive techniques such as endometrial biopsy, uterine lavage and a cytobrush. Several studies have shown that especially beta hemolytic streptococcal species is a common isolate to be found in the endometrium, as well as the harvested tissue has been used for the diagnosis of an infection by cyto logical and histological examination[2,3]. The negative influence of streptococcal infection on the chances of pregnancy and the ability of carrying a foal to term has been described [4] in a paper also showing the need to use more invasive techniques such as endometrial biopsy for improving the sensibility of the diagnosis in practice. The more invasive techniques needed for diagnosis of streptococcal infections in endometrium has produced evidence by FISH technique and confocal microscopy, that the infection resides deeper in the tissue, as well as the ability of beta hemolytical streptococci to enter a dormant stage in the endometrium has been hypothesised. [5]. Potential activation of a dormant streptococcal infection in the endometrium of the mare with a proprietary substance has been described [6]. Whether this activation is due to a characteristic of the streptococci themselves or to external factors in the uterus and the endometrium however has to be further investigated.

To further examine the hypothesis of activation of streptococci in the endometrium of the mare, it was decided to investigate, if a difference in the prevalence of beta hemolytic streptococci isolated from sexually active and sexually rested mares could be shown.

Materials and methods
The prevalence of beta hemolytic streptococci and other bacteria isolated from endometrial biopsies at an equine practice laboratory (Ansager Dyrehospital, Denmark) during a four year period (2012-2015) was retrospectively recorded. All specimens from endometrial biopsies examined in the laboratory during this period were included in the study. The prevalence of different isolates were recorded in two different periods of isolation, April to September and October to March respectively. Because of the breeding season in the northern hemisphere, the isolates from mares obtained from October until March would typically originate from mares, that had not been breed for several cycles. These mares was in this study recorded as sexually inactive. In the other period April to September the mares would typically have been bred or manipulated in their reproductive organs in the breeding cycles before the endometrial biopsy was obtained. These mares were in this study recorded as sexually active. Statistics was done by Chi Square and significance was set at P < 0.05.

Results
Bacterial isolates from endometrial biopsies at the laboratory at Ansager Equine Hospital during 2012 to 2015. *P < 0.5, **P < 0.01.

<table>
<thead>
<tr>
<th>Season</th>
<th>April – September</th>
<th>October - March</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile</td>
<td>167 (40%)</td>
<td>42 (45%)</td>
</tr>
<tr>
<td>Culture-positive</td>
<td>253 (60%)</td>
<td>52 (55%)</td>
</tr>
<tr>
<td>Beta-hemolytic streptococci</td>
<td>103 (24%)</td>
<td>9 (10%)</td>
</tr>
<tr>
<td>Other organisms</td>
<td>150 (36%)</td>
<td>43 (45 %)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>24 (6%)</td>
<td>9 (10%)</td>
</tr>
<tr>
<td>E. coli</td>
<td>40 (10%)</td>
<td>16 (17%)</td>
</tr>
<tr>
<td>Yeast</td>
<td>41 (10%)</td>
<td>10 (11%)</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>18 (4%)</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>2 (1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Mixed growth</td>
<td>25 (6%)</td>
<td>6 (6%)</td>
</tr>
<tr>
<td>Total</td>
<td>420 (100%)</td>
<td>94 (100%)</td>
</tr>
</tbody>
</table>

Conclusions
• The prevalence of sterile and positive samples were not statically different (P > 0.5) between the two periods evaluated (April-September vs. October-March).
• The prevalence of beta-hemolytical streptococci and other cultures were statistically different (P < 0.01) between the two periods evaluated (April-September vs. October-March).

Discussion
This study showed that a significantly higher proportion of mares were positive for beta hemolytical streptococci from the endometrium during the breeding season (April-September) compared to the off-season winter period (October-March). The hypothesis that it is more likely to isolate beta hemolytical streptococci from sexually active mares than from sexually inactive mares were therefore supported.

Several possible scenarios explaining these findings could be suggested:
• Beta hemolytic infections is not detected during the winter period because of dormancy and inability to be detected with present diagnostic procedures.
• The manipulation and breeding of the mares during the breeding season (April-September) activates streptococcal infections, then possible to detect with present diagnostic procedures (culture on blood agar).
• The theory of dormant streptococcal infections in the endometrium and the possibility that activation of these infections from chemical or mechanical stimuli is supported by the papers of Petersen et al. [5,6]
• The manipulation of the cervix and the breeding of the mares results in more ascending infections with beta hemolytical streptococci during the breeding season.
• The theory of ascending infections however is not supported by the findings by Rasmussen et al. [1], who found, that the streptococcal infections isolated form the endometrium predominantly was of a single bacterial strain, that could not be isolated from the vagina, clitoris or surface of the mares.

Most mares with a confirmed infection with beta hemolytical streptococci during the breeding season will receive treatment. The low proportion of beta hemolytical streptococci isolated in the off season could be because the infections were treated and eradicated during the breeding season. This theory is supported by the paper of Nielsen et al. [7]. In this paper mares with a detected and treated bacterial infection in the endometrium had the same pregnancy percent per cycle as mares in general in the study.

With the design of this retrospective study it is however not possible to prove or make any of these theories more probable than the other. For this further studies has to be performed.

References

Beta-hemolytic Streptococci isolated from an endometrial biopsy using blood agar and chrome agar, respectively.