
The bacteriological examination of endometrial swabs is one of the most common methods for diagnosing endometritis in the mare (Dimmock and Edwards 1923, Riddle et al. 2007). The vagina and particularly the vestibulum and clitoral fossa harbor a variety of facultative uterine pathogens (Hinrichs et al. 1988). These bacteria may be introduced into the uterine environment during the swabbing procedure, cause contamination of endometrial samples and endometritis. Therefore the technique and the type of the culture instrument are essential for the results of uterine culture swabs and reproductive health.

Guarded culture instruments reduce contamination of the swab itself during the most common transvaginal manual techniques.
swabbing technique (Allen and Newcombe 1979, Blanchard et al. 1981, Ricketts 1981), but endometrial contamination via the outside of the guard may still occur and lead to false-positive uterine culture results and uterine contamination. Traditional sampling by speculum and forceps allows introduction of the swab through the cervical opening without contact to the vulvovaginal area. This technique was recommended by Waelchli et al. (1992) and Handler (2005). However, swabbing with the speculum may show the risk of pneumovagina or air insufflation into the cervix, which may lead to uterine bacterial contamination and endometritis (Caslick 1937, Götz 1952, Thornbury 1975, Slusher 1986, Ricketts and Curnow 1988).

Few evidence-based information is available about the contamination risk of nowadays most commonly used double-guarded swabs taken manually transvaginal or by speculum and forceps. Guidelines published by the German Society for Equine Medicine prefer sampling by speculum and forceps (Bartmann et al. 2013). Täte (2011) compared the transvaginal manual sampling technique and the sampling with speculum and cervical forceps. The manual transvaginal sampling method revealed significantly more often bacterial growth than samples taken by speculum and forceps (p < 0.0001).

Recently, a rigid LED lightning endoscope equipped with a waterproof monitor and two working channels (6 and 10 mm diameter) was introduced to the bovine and equine practice (iVetscope®, Fa. QuIdee, Homberg/Ohm, Germany). It allows quick and easy to handle endoscopy of the reproductive tract, larynx and oral cavity. The iVetscope® has been used for uterine culture swabbing and introduction of a cytobrush through the 10 mm diameter working channel under visual control in bovine practice.

The objective of the present study was to compare uterine culture swabbing by transvaginal manual sampling, speculum and forceps and via iVetscope® with regard to bacterial growth, category of bacterial growth (no bacterial growth, apathogenic bacterial growth, facultative bacterial growth and samples with combined growth of apathogenic and pathogenic bacterial growth), number of different bacterial species in one sample and degree of bacterial growth in relation to uterine cytology.

A second double-guarded uterine culture swab and double-guarded cytobrush sample were taken 48 hours after the first sampling to assess the hygienic risk of uterine bacterial contamination by the three swabbing techniques.

The aim of the study was to identify the most reliable swabbing method which reduces the risk of sample and uterine contamination.

Materials and methods

Animals and sampling groups

Eighty-eight estrous mares of different age (11.6 ± 4.8 years) and reproductive history were included in the study in a commercial breeding program from March to July 2016. According to their reproductive history mares were divided in “assumed genitally healthy” (n= 37; maiden (n= 12), barren (n= 12), foaling mares (n= 12)) and “assumed subfertile” (n= 51; not pregnant in one cycle (n= 15), not pregnant in two cycles (n= 12), resorption (n= 12), genital catarrh (n= 12)). Mares from each group were assigned at random to one of three sampling groups. Following a gynecological examination double-guarded uterine culture swabs and double-guarded cytobrush samples to assess cytology were obtained “transvaginally” (MAN) (n= 29), by “speculum” (SPE) (n= 29) or via “iVetscope®” (SCOP) (n= 30) during estrous (estrus behaviour, > 30 mm follicle, uterine edema). A second double-guarded uterine culture swab and double-guarded cytobrush sample were taken 48 hours after the first sampling.

Equipment and procedures for sample collection

The mares were restrained in an examination stock. Following a transrectal ultrasonographic examination the tail was wrapped and covered by a glove (Fa. WDT, Garbsen). The vulva and perineal region was rinsed with warm water, scrubbed with Degraseptin (Degraseptin, Fa. Animedica, Senden), rinsed again three times and dried with paper towels.

A double-guarded uterine culture swab and a double-guarded cytobrush sample to assess cytology were obtained by three different sampling methods.

In the MAN-group the double-guarded uterine swab and cytobrush were passed manually with a sterile glove through the cervical channel into the uterine body. In the SPE-group a sterile spreadable Polskyspeculum was inserted into the vagina, the external cervical as fixed with forceps by Götzè modified by Albrechtsen and the double-guarded uterine swab and subsequent cytobrush introduced through the cervix under visual control with an electric torch. In the SCOP-group a rigid endoscope equipped with a monitor (iVetscope®, Fa. QuIdee, Homberg/Ohm, Germany) (Fig. 1) was introduced into the vagina, protected by a sterile sleeve (50 × 5 cm, Heißluft Rolle Typ HR, Fa. Steriking, Pattensen). When the external cervical os was displayed on the monitor, the sterile sleeve was pushed back and the double-guarded swab and subsequently the double-guarded cytobrush introduced through the working channel of the iVetscope®.

Fig. 1 iVetscope® Fa. QuIdee, Homberg/Ohm with a double guarded swabbing system Fa. Minitüb, Tiefenbach

In all three sampling groups the swab and the cytobrush were kept in contact with the endometrium for 30 secs. A second set of double-guarded uterine culture swab and cytobrush were taken in the same approach 48 hours after the first sampling to examine the effect of contamination of the three sampling methods.
**Microbiology**

The uterine culture swab was carefully removed from the double-guarded sampler and transferred into AMIES® transport medium and transported overnight to an internationally accredited laboratory (Labor Dr. Böse, Harsum, Germany). The swab samples were smeared on Columbia agar, Columbia agar with colistin and aztreonam and a Gasser agar (Oxoid, Deutschland GmbH Wesel). Incubation in an enrichment medium (Müller-Hinton Medium) followed. All samples were incubated for 24 hours at 37°C, evaluated for bacterial growth (mild, moderate, severe), incubated for another 24 hours at 37°C and re-examined. For further culture differentiation a MALDI-TOF microflex™ (Fa. Bruker, Bremen) was used, if necessary. The uterine culture swabs were analyzed with regard to bacterial growth, combination of different bacteria in one sample and degree of bacterial growth and bacterial categories (samples with no bacterial growth, samples with apathogenic bacteria, samples with facultative pathogenic bacteria, samples with apathogenic and facultative pathogenic bacteria). Facultative pathogenic bacteria included *Bacteroides fragilis* and *ureolyticus*, β-hemolytic Streptococcus, *Clostridium perfringens*, *Escherichia coli* hemolytic, *Klebsiella pneumoniae* spp. *pneumoniae*, Pseudomonas aeruginosa, *Staphylococcus aureus*, *Taylorella equigenitalis*, *Candida tropicalis*.

Apathogenic bacteria included α-hemolytic *Streptococcus*, nonhemolytic *Streptococcus*, *Aspergillus terreus*, aerobic spore-formers, *Corynebacterium* spp., *Coitrobacter farmeri*, *Enterobacter* spp., *Escherichia coli* nonhemolytic, *Flavobacterium* spp., *Proteus* spp., *Pseudomonas putida*, *Pseudomonas koreensis*, *Micrococcus*, *Candida pelliculosa*, *Pantoea agglomerans*, *Staphylococcus* spp. and S. *luteus*. Samples with facultative pathogenic bacteria – with or without combined apathogenic bacteria – were opposed to samples with no facultative pathogenic bacteria (including samples with no bacterial growth and samples with solely apathogenic bacteria).

**Cytology**

The Cytobrush was smeared on a microscopic glass slide (76 × 26 mm, Fa. Roth, Karlsruhe) which was dried at room temperature, fixed by M-FIX (Merckofix, Fa. Merck, Darmstadt) and stained by Diff-Quick® (Dip Quick Stein Fa. Jorgensen, Colorado). The slide was examined by light microscopy (400 × magnification) for the presence of PMN-cells. A minimum of 200 cells were counted and more than 5 PMN-cells per 10 high power fields was considered positive for endometritis (Dascanio 2003).

**Statistics**

Distributions of characteristic categories of the isolated bacteria, degree of bacterial growth and number of bacteria per sample were evaluated by Fisher-exact-Test/Chi-Square Test. McNemar-Test (2 × 2 table) or Bowker-Test (≥ 3 × 3 table) were used to proof paired samples of symmetry like types of bacteria and degree of bacterial growth for the time points 0 and 48 hours. To evaluate differences between the number of bacteria per sample at two time points Wilcoxon-Test was used. Kruskal-Wallis-Test calculated differences between methods used for endometrial sampling at different time points.

**Results**

**Bacterial growth**

Including all sampling methods and mares, bacteria were isolated in 63/88, 71.6% mares at the first endometrial sampling. 48 hours after the first endometrial swabbing growth in swabs taken by the iVetscope® (16/30, 53.3%) was significantly more often detected in swabs taken by the iVetscope® (16/30, 53.3%) in the first sampling (p = 0.047). 48 hours later bacterial growth was significantly more often detected in the MAN-group compared to the SPEC-group (22/29, 75.9%; p = 0.02), and SCOP-group (20/30, 66.7%; p = 0.03)

Facultative pathogenic bacteria – with or without combined apathogenic bacteria – were obtained from 11/88 mares 12.5% and 25/88, 27.3% mares at the first and the second sampling, respectively (p = 0.008). Comparing the three different sampling methods, bacterial growth was detected in 24/29, 82.8% and 28/29, 96.6% mares in the MAN-group at the first and second sampling, respectively. Significantly fewer mares showed bacterial growth in swabs taken by the iVetscope® (16/30, 53.3%) in the first sampling (p = 0.047). 48 hours later bacterial growth was significantly more often detected in the MAN-group compared to the SPEC-group (22/29, 75.9%; p = 0.02), and SCOP-group (20/30, 66.7%; p = 0.03)

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**Table 1**

Number of swabs with 0-3 and 4-6 bacteria isolated per sample for each sampling method manually (MAN) (n=29), by iVetscope (SCOP) (n=30) and by speculum (SPEC) (n=29) at the first (0h-Sample 1) and the second sampling (48h-Sample 2) *p< 0.05. *statistically significant difference between swabbing techniques within time of swabbing

<table>
<thead>
<tr>
<th>Number of isolated bacteria</th>
<th>MAN n=29</th>
<th>SCOP n=30</th>
<th>SPEC n=29</th>
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<tbody>
<tr>
<td></td>
<td>Sample 1</td>
<td>Sample 2</td>
<td>Sample 1</td>
</tr>
<tr>
<td>0-3</td>
<td>20 (69%)</td>
<td>18 (62.1%)</td>
<td>29 (96.7%)</td>
</tr>
<tr>
<td>3-6</td>
<td>9 (31%)a</td>
<td>11 (37.9%)a</td>
<td>1 (3.2%)b</td>
</tr>
</tbody>
</table>

a:b statistically significant difference between swabbing techniques within time of swabbing
Most commonly isolated were Staphylococcus species (25.3%), α-hemolytic Streptococci (14.6%), β-hemolytic Streptococci (11.7%) and non-hemolytic Escherichia coli (9%). If more than three different bacteria were present, the result was recorded as contamination (8%).

Combination of different bacteria

Differentiation of bacterial growth revealed differences in the variety of bacteria in one sample (Figure 3).

As more than three different bacteria in one sample was defined as contamination, two groups were formed (0–3 vs 4–6 bacteria) to analyze the number of bacteria isolated per sample.

From samples taken manually significantly more samples 9/29, 31% and 11/29, 37.9% showed bacterial growth of more than three bacterial species at the first and second sampling respectively than in samples in the SCOP-group (1/30, 3.3%, and 0/30, 0%), and SPEC-group (0/29, 0%, and 2/29, 6.9%) (Table 2).

Degree of bacterial growth

Growth of each bacterium was differentiated in mild, moderate and severe. 7/147, 4.8% bacterial species isolated in all samples and mares at the first sampling showed severe bacterial growth, 9/147, 6.1% showed moderate and 131/147, 89.1% mild growth. 48 hours after the first sampling the bacterial growth generally increased to 21/159, 13.2% bacteria with severe growth, 24/147, 15.1% with moderate and 114/147, 71.7% with mild bacterial growth.

Comparing the three sampling techniques, significantly more results of mild bacterial growth were isolated from swabs taken manually 63/131, 48.1% compared to the SCOP-group (33/131, 25.2%; p=0.0022) and the SPEC-group (35/131, 26.7%; p=0.0047) at the first sampling.

48 hours after the first sampling moderate bacterial growth was significantly more often observed in samples taken manually 17/24, 70.8% than in the SCOP-group (3/24, 12.5%; p=0.0017) and SPEC-group (4/24, 16.7%; p=0.0046). Furthermore severe bacterial growth resulted more often from swabs taken manually (18/21, 85.7%) than taken by iVetscope® (0/21, 0%; p<0.0001) or speculum (3/21, 14.3%; p=0.0011) at the second sampling.

![Fig. 2](image1)

*Number of swabs with the number of bacteria isolated per sample (0-6) regarding sampling method manually (MAN) (n=29), by iVetscope (SCOP) (n=30) and by speculum (SPEC) (n=29) at the first (0h - Sample 1) and the second sampling (48h - Sample 2).

![Fig. 3](image2)

*Number of bacteria isolated with a degree of mild, moderate and severe growth for each sampling method manually (MAN) (a number of 151 bacteria isolated), by iVetscope (SCOP) (a number of 76 bacteria isolated) and by speculum (SPEC) (a number of 79 bacteria isolated) at the first (0h - Sample 1) and the second sampling (48h - Sample 2). p<0.05; *statistically significant difference between swabbing techniques within time of swabbing.

Table 2

<table>
<thead>
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</tr>
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<tbody>
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<td>0-3</td>
<td>20 (69%)</td>
<td>29 (96.7%)</td>
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</tr>
<tr>
<td></td>
<td>18 (62.1%)</td>
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<td>27 (93.1%)</td>
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</tr>
<tr>
<td></td>
<td>11 (37.9%)</td>
<td>0 (0%)</td>
<td>2 (6.9%)</td>
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</table>
The purpose of this study was to compare uterine culture swabbing by transvaginal manual sampling, speculum and forceps and via iVetscope® to identify the most reliable swabbing method which reduces the risk of sample and uterine contamination.

All three sampling techniques were performed with a double-guarded swab and cytobrush, which reduces the risk of contamination (Allen and Newcombe 1979, Blanchard et al. 1981, Aguilar et al. 2006). However, Täte (2011) isolated bacterial growth from a double-guarded swab, which was introduced transvaginally manual into the mare’s uterus without opening of the inner sheath. The same experiment performed by swabbing with a speculum and forceps did not lead to bacterial growth. Furthermore, the perineal region and vulva has been rinsed with warm water, scrubbed with Degraseptin, rinsed again three times and dried with paper towels before the swabbing techniques were performed to reduce contamination.

Including all sampling methods and mares, bacteria were isolated in 71.6% mares at the first endometrial sampling. This high rate of positive uterine cultures is in accordance to Neuberg (2009) who observed 74% positive culture samples by double-guarded, transvaginal manual swabbing. Other authors report bacterial growth in 10–50% of endometrial swabs (Leidl et al. 1976, Merkt et al. 1987, Ricketts and Makintosh 1987, Hinrichs et al. 1988, Huchzermeyer 2003, Nielsen 2005, Riddle et al. 2007). These divergent observations might result from different microbiological tests and evaluation criteria (Albihn et al. 2003). Furthermore most studies involved genetically healthy, fertile mares whereas the present studies included mares of different reproductive history (37 “assumed genitally healthy” maiden (n=13), barren (n=12) and foaling mares (n=12) and 51 “assumed subfertile” mares, which were not pregnant in one cycle (n=15) or two cycles (n=12), had a previous resorption (n=12) or genital catarrh (n=12).

The three different sampling methods showed significant differences in bacterial growth, especially in the second sampling after 48 hours. While 97% of MAN-samples showed bacterial growth, bacteria were isolated from 76% of SPEC- and 67% of SCOP-samples (p < 0.05). Waelchli et al. (1992) observed 92% positive samples after transvaginal, manual sampling and 25% positive samples by speculum in the contamination experiment.

Even more striking were the differences with regard to the facultative pathogenic bacteria. Especially at the second sampling significantly more facultative pathogens were isolated from swabs taken manually (55.2%) compared to swabbing by iVetscope® (13.3%) or speculum (17.2%) (p < 0.05).

But not only the increase in bacterial growth and growth of facultative pathogenic bacteria in swabs taken at the second sampling support the hypothesis of bacterial contamination through the different swabbing techniques. Furthermore the variety of bacterial species and the degree of bacterial growth underline the relevance of the swabbing method.

Some authors define mixed cultures of different bacterial species as contamination and exclude them from microbiological diagnosis (Ricketts 1981, Nielsen 2005). Others consider mixed cultures relevant (Albihn et al. 2003, Leblanc et al. 2007, Wittenbrink et al. 2008). A mixed culture of two bacteria was isolated from mares, which showed a positive cytology (Riddle et al. 2007). Recently bacterial growth of more than two bacteria was considered contamination (Nielsen 2005, Riddle et al. 2007, Christoffersen et al. 2015). The internationally accepted laboratory which analyzed the culture swabs in the present study defines more than three bacteria in one sample as contamination.
From samples taken manually significantly more samples (31% and 37.9%) showed growth of more than three bacterial species at the first and second sampling respectively than in samples in the SCOF-group (3.3% and 0%) and SPEC-group (0% and 6.9%), which strongly supports contamination of transvaginally taken manual swabs (p < 0.01).

Although comparisons with previous studies regarding degree of bacterial growth is difficult due to different laboratory tests and evaluation criteria, the significantly increased number of samples with moderate and severe bacterial growth in the MAN-group, especially 48 hours after the first sampling, confirms bacterial diversion from the caudal reproductive tract and contamination. Hinrichs et al. (1988) demonstrated that endometrial swabbing can lead to bacterial contamination of the uterus, which might be caused by air insufflation or bacterial contamination from the caudal reproductive tract. The results of the present study strongly confirm increased contamination in the MAN-group despite of intensive cleaning of the vulva and perineal area, application of double-guarded swabbing systems and sterile gloves.

Regarding endometrial cytology, the number of mares with > 5 PMNs/10 HPF did not increase between the first and second sampling. To examine the relevance of bacterial contamination of the uterus by different swabbing techniques during oestrus, it would be interesting to further examine the bacterial growth and cytology at the end of oestrus. In resistant oestrus mares (oestrus behaviour, >30 mm follicle, uterine edema) a persistent endometritis due to the bacterial contamination is unlikely. However, susceptible mares with a cervical incompetence, reduced myometrial activity and uterine immune response might respond to a uterine contamination with persistent endometritis.

In conclusion, the results of the present study strongly recommend endometrial swabbing by instrumental techniques, speculum and forceps, or iVetscope® in our study. Although these swabbing techniques might be more time consuming in handling and cleaning and require more material, the significantly reduced bacterial contamination is worth the trouble.

Authors’ contributions

HS, RB, and KS designed the study. KS, GM, SP, WH, and KR performed the research. KS, JS and HS wrote the manuscript. All authors were involved in discussing and interpreting the data described in this study, critically read the manuscript, and approved the final version of the manuscript.

Acknowledgments

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Animal Welfare Statement

The study was approved by the Lower Saxony State Office for Consumer Protection and Food Safety Hannover (reference number 33.19-42502-05-15A586). Furthermore, procedures and maintenance of animals was according to German animal welfare legislation.

Conflict of interest statement

The authors exclude any possible conflict of interest in regard to the medical products or technology used in the study.

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Erweiterte Zusammenfassung

Vergleichende Genauigkeit von Entnahmetechniken zur Gewinnung eines Endometriumabstrichs bei der Stute


Zwischen den drei Entnahmetechniken konnten insbesondere zum Zeitpunkt der zweiten Probenentnahme (nach 48h), signifikante Unterschiede im bakteriellen Wachstum ermittelnd werden. Während 28/29, 97% Proben in der Gruppe MAN bakterielles Wachstum zeigten, wurden in der Gruppe SPEC lediglich von 22/29, 76% Tupfern und in der Gruppe SCOP von 20/30, 67% Proben Bakterien isoliert (p<0.05). Zum Zeitpunkt der zweiten Probenentnahme wurden fakultativ pathogene Erreger signifikant häufiger aus transvaginal-manuell entnommenen Proben isoliert (16/29, 55.2%), als aus der Gruppe SPEC (5/29, 17.2%) und SCOP (4/30, 13.3%) (p<0.05). Die Gruppe MAN wies zudem sowohl bei der ersten als auch bei der zweiten Tupferprobenentnahme eine signifikant höhere Anzahl von Proben mit mehr als drei Bakterienarten je Probe auf (31% bzw. 37.9% in der Gruppe MAN versus 3.3% bzw. 0% in der Gruppe SCOP und 0% bzw. 6.9% in der Gruppe SPEC bei der ersten bzw. zweiten Probenentnahme (p<0.01)). Die erhöhte Anzahl an Mischkulturen mit mehr als 3 Bakterienspezies deutet auf eine bakterielle Kontamination der manuell entnommenen Tupfer aus kaudalen Abschnitten des Reproduktionstrakts hin.

Anhand der Ergebnisse der vorliegenden Studie wird die instrumentelle Entnahme eines Endometriumabstrichs (Spekulum und Zervix-faßzange oder iVetscope®) empfohlen. Auch wenn diese Methoden zeitaufwendiger sind und mehr Equipment benötigen, zeigt die signifikant geringere bakterielle Kontamination die Relevanz der instrumentellen Entnahme eines Endometriumabstrichs in der Pferdepraxis.

Schüsselwörter: Stute, Uterus, Endometriumabstrich, bakterielle Kontamination, Mikrobiologie, Zytologie, Reproduktion