Detection of bacterial populations colonizing the genital tracts of jennies and their changes during the oestrous cycle

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Abstract

The aim of this study was to help improve the management of assisted reproduction of donkeys and to extend the existing information on the fertility of donkeys by qualitative and quantitative assessment of bacterial contamination of the jennies' genital tract in connection with the perineum formation and oestrous cycle phases. Ten female donkeys were included in the study and were repeatedly rectally palpated and sonographically examined during the oestrous cycle. Samples were taken from the fossa clitoridis and around the cervix for microbiological examination. Each jenny was sampled twice, always at different stages of the cycle, both in the oestrus and in the dioestrus. In addition, samples from the endometrium were taken in oestrus for both microbiological and cytological examination. After collection, the samples were examined in a microbiological laboratory. There were 62 different species of microorganisms found belonging to 19 different bacterial genera. The presence of agents of mares' infectious metritis *Taylorella equigenitalis* was not found in any of the samples. The outcome of our study is an initial mapping of the microbial colonization of the endometrium and the genital tract in jennies with correct formation of the perineal region. The hypothesis that the level of bacterial contamination was related to the oestrous cycle was not confirmed. The practical significance of the study lies mainly in the description of the composition, amount and changes of microbiota in healthy individuals during their oestrous cycle, which allows the evaluation of the risk of developing infection during the active oestrous cycle in connection with mating.

Donkeys, reproduction, endometritis, microbiota

Until recently, because of the lack of specific information, donkeys were treated as small horses. The rapidly growing interest in their breeding as hobby animals and companions, and the increasing need for a sophisticated approach to reproduction of this species encourages new studies and brings added knowledge. The major differences revealed between these two species must be respected, especially regarding assisted reproduction. Without taking into account the specific needs of donkeys, techniques originally developed for horse mares may reduce the success of artificial donkey reproduction.

The anatomy of the jennies' reproductive organs is similar to that of horse mares, but even minor differences in the formation of the reproductive organs may be important for veterinary practice. The cervix of the jennies is longer than that of the mare, very narrow and protrudes futher into the vagina (Vendramini et al. 1998). This formation complicates cervical manipulation during procedures such as uterine sampling, uterine lavage or insemination. The perineal area is usually formed perpendicular to the ground, sometimes even ventrocranially tapered. The labia of jennies are small and tightly clamped, which is a distinct advantage, limiting the possibility of bacterial contamination of the deeper structures of the genital tract.

Jennies have been shown to be susceptible to endometritis, as seen in horse mares, with the accumulation of PMN (polymorphonuclear cells) in the stratum compactum

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(Summerfield and Watson 1998; Watson 2000; Sokkar et al. 2001). In mares, acute but transient uterine inflammation is an innate immune response to sperm or bacteria (Allen and Pycock 1989; Katila 2001; LeBlanc 2010). Bacteria are also commonly found in the uterus after mating, because microorganisms from the environment, skin colonizers, enterobacteria and even microbes from the semen of the stallion are their major sources (Masarikova et al. 2014). However, inflammation can persist in up to 40% of horse mares and can cause embryonic death (Pycock and Newcombe 1996).

Smears with a cotton swab, brush or low-volume uterine lavage are the best methods for obtaining representative cytological samples (LeBlanc et al. 2007; Overbeck et al. 2011; Cocchia et al. 2012). Endometrial cells, cellular debris, and no or only isolated inflammatory cells are present physiologically in jennies in oestrus (Vilés et al. 2013).

Donkeys may be the causative agent of one of the most feared sexually transmitted infections of horses, *Taylorella equigenitalis* (Jang et al. 2001). In addition, donkeys have been reported to have a similar bacterium of the same genus, *T. asinigenitalis* (Katz et al. 2000; Jang et al. 2001) which was subsequentely discovered in horses (Båverud et al. 2006; Franco et al. 2009). The clinical relevance of *T. asinigenitalis* is currently unclear and is probably negligible (Jang et al. 2001; Meade et al. 2010).

The aim of this work was to map the form of the reproductive organs and bacterial contamination of the genital system in jennies during their oestrous cycle.

Materials and Methods

This study was carried out from May to August 2018. The experimental design was approved by the Ministry of Education, Youth and Sports (MSMT- 28509/2018-2).

The study included 10 sexually mature female donkeys (*Equus asinus*) aged 3–12 years. The jennies were housed on private farms on pastures with *ad libitum* access to water. The jennies were in the herd with other females and without the presence of a male donkey. All jennies belonged to the 'Domestic Donkey' breed registered in the Czech Republic, which are basically crossbreds of different European breeds.

A reproductive history was recorded for each jenny before entering the study. A gynaecological examination was performed and recorded. This included external assessment of the perineal area, pelvic bone palpation, and perineal taper, vaginoscopy and cervical palpation, rectal palpation of the uterus and ovaries and a rectal ultrasonografic examination. All jennies included in the study were examined at least twice to capture both phases of the oestrous cycle – oestrus and dioestrus.

After evaluating the cycle phase, the perineal area was prepared for sampling. The tail was bandaged and tied to the neck of the jenny. Perineal area disinfection was performed 3 × with iodine solution (Betadine®, EGIS Pharmaceuticals PLC, Körmend, Hungary) and 3 × with alcohol disinfectant with chlorhexidine (CITROclorex 2%, Ecolab Healthcare, Brno, Czech Republic).

For the purpose of identifying the microorganisms colonizing the jenny's reproductive system, three different sampling sites were chosen: fossa clitoridis, surroundings of the cervix uteri and endometrium. The samples were taken twice, during the dioestrus and oestrus. For the anatomical and physiological reasons, samples were not obtained from the inside of the uterus during the dioestrus phase. All material consisted of a smear taken from the area with sterile cotton swabs. Samples were also collected, whilst in the oestrus phase, from the endometrium using cytological brushes.

In order to partially fulfill the objectives of the study which included both qualitative and quantitative bacteriological culture techniques, samples were taken from each of the three sites in duplicate during the oestrus phase. For qualitative assessment, the first swab was placed in Amies transport medium (sample A) after collection and the second swab was placed in an Eppendorf tube containing 1 ml of buffered saline solution (sample B) for bacterial quantification. In addition, in each jenny a fossa clitoridis smear was performed during the first examination to exclude infection by *Taylorella* spp. (sample C). Cytological brushes were used in two ways: for cytological examination of the endometrium (sample D) and also for quantitative bacteriological examination (sample B). In the dioestrus, samples were taken from fossa clitoridis and surroundings of the cervix uteri and processed in the same manner as above.

Immediately after collection, all samples were transported in a cooling box to a microbiological laboratory (Department of Infectious Diseases and Microbiology, University of Veterinary and Pharmaceutical Sciences Brno). All samples A were used for qualitative bacteriological cultivation in order to detect possible most important pathogens of the reproductive apparatus. Both universal bacteriological media and selective-diagnostic media were used for this purpose, namely Columbia Blood Agar (CBA), Rappaport-Vassiliadis semi-solid medium, XLD agar and McConkey agar (all Oxoid, Hampshire, UK). The cultivation conditions were selected according to the physiological requirements of screened bacteria and included culturing in aerobic and also

anaerobic conditions at temperatures of 37 and 42 °C for 24–48 h. The definitive identification of isolated bacteria at species level was carried out by analyzing the mass spectra of their proteins by MALDI-TOF MS method (matrix assisted laser desorption/ionization time-of-flight mass spectrometry) using a Microflex LT MALDI Biotyper (Bruker Daltonics, Bremen, Germany). Samples B stored in Eppendorf tubes for quantitative assessment of colonization of the genital tract were shaken for 10 min prior to inoculation on CBA to release the maximum amount of bacteria into saline, into which the swabs (brushes) were placed immediately after collection. One hundred microlitres of each buffered saline with B sample were applied to the surface of two CBAs and spread with a sterile glass L-shaped hockey-stick spreader. First CBA was then incubated under aerobic and second under anaerobic conditions at 37 °C. After 24 h, the total count of microorganisms was read and recalculated to Colony Forming Units (CFU) per milliliter. In addition to the quantitative assessment, one to five representatives of bacterial colonies with the same morphology were used for species identification of microorganisms from each sample B via MALDI-TOF MS.

On the day of collection, a sample C of the collected material was sent to an accredited laboratory (State Veterinary Institute, Olomouc) for identification of *Taylorella* spp. Sample D, cytological preparations of uterine swabs, were stained with Diff-Quick® staining and assessed microscopically in the laboratory of the University's Equine Clinic.

Results

The summary of information obtained from the assessment of the external perineal area of jenies describes the appropriate form of the perineal area of all the jenies included in the study. The vulva was perpendicular to the ground or even ventrocranially bevelled in most jenies. The pelvic bones were above the dorsal commissure of the vulva. By palpation of the cervix, two jennies were found to be impenetrable, so endometrium samples were not obtained from these jennies.

There were 62 different types of microorganisms belonging to 19 different bacterial genera isolated from the genital tracts of jennies, in particular from the fossa clitoridis, around the cervix and the endometrium, using qualitative bacteriological cultivation of all samples A. These bacterial genera were: *Acinetobacter, Aerococcus, Arcanobacterium, Arthrobacter, Bacillus, Bacteroides, Clostridium, Corynebacterium, Escherichia, Enterococcus, Lactobacillus, Micrococcus, Neisseria, Paenibacillus, Pantoea, Propionibacterium, Proteus, Staphylococus, Streptococcus*.

The results of the quantitative bacteriological analysis of samples B from all three levels of the donkey reproductive apparatus taken during dioestrus and oestrus are detailed for each jenny in Table 1. In 50% of jennies (5/10), the number of CFUs isolated from fossa clitoridis increased in oestrus. In one jenny the number of bacteria remained the same in both monitored periods and in four jennies (40%) the number of CFU in samples from fossa clitoridis in oestrus even decreased. A higher number of CFUs around the cervical area was found in five out of nine jennies (56%) in oestrus. In one jenny (10%), the number of CFUs decreased in estrus. The last three jennies (33%) had no change to the number of CFUs because all their samples in both dioestrus and oestrus were free from bacterial growth.

The presence of agents of mares' infectious metritis *T. equigenitalis* was not found in any of the samples; nor was the aetiological agents of salmonella abortions.

We observed variations in the quantity of isolated microorganisms within specific parts of the jennies' reproductive tract (Table 1).

Cytological examination of uterine swabs, performed only in oestrus, did not reveal the presence of inflammatory cells or pathogens in any jenny. The samples showed only endometrial cells.

Discussion

The outcome of our study is an initial mapping of the microbial colonization of the endometrium and the genital tract in a group of 10 jennies. The hypothesis that the level

Table 1. Quantitative bacteriological cultivation of samples taken from individual parts of the jenny's genital tract in dioestrus and oestrus

No.	Dioestrus		Oestrus		
	Fossa clitoridis	Cervix uteri	Fossa clitoridis	Cervix uteri	Endometrium
1 Species (CFU·ml^{-1})	-	-	*Staphylococcus chromogenes* 0.3×10^{1} *Clostridium tertium* 3.7×10^{1}		
Total bacterial counts (CFU·ml^{-1})	0	0	4.0×10^{1}	Not sampled	Not sampled
2 Species (CFU·ml-1)	*Aerococcus viridans* 5.0×10^{1} *Bacillus cereus* 0.4×10^{1} *Bacillus simplex* 0.5×10^{1} *Enterococcus casseliflavus* 1.7×10^{1} *Staphylococcus chromogenes* 0.2×10^{1} *Streptococccus mitis* 1.8×10^{1}	-	*Aerococcus viridans* 2.2×10^{2} *Acinetobacter lwofii* 1.6×10^{2} *Arthrobacter nicotinovorans* 0.4×10^{1} *Bacillus cereus* 2.1×10^{1} *Corynebacterium xerosis* 1.7×10^{1} *Escherichia coli* 2.0×10^{2} *Enterococcus casseliflavus* 1.3×10^{2} *Staphylococcus chromogenes* 5.4×10^{1}	*Acinetobacter lwofii* 1.1×10^{1} *Neisseria subflava* 0.4×10^{1} *Staphylococcus equorum* 0.4×10^{1} *Staphylococcus hominis* 0.1×10^{1}	*Staphylococcus equorum* 1.0×10^{1}
Total bacterial counts (CFU·ml^{-1})	9.6×10^{1}	0	8.1×10^{2}	2.0×10^{1}	1.0×10^{1}
3 Species (CFU·ml^{-1})	*Bacteroides fragilis* 1.2×10^{1} *Corynebacterium coyleae* 2.1×10^{1} *Corynebacterium glutamicum* 1.5×10^{1} *Enterococcus faecium* 2.3×10^{2} *Staphylococcus haemolyticus* 0.4×10^{1} *Staphylococcus aureus* 0.1×10^{1} *Str. equi* ssp. *zooepid.* 0.5×10^{1} *Streptococcus equinus* 2.0×10^{1}	*Acinetobacter lwofii* 1.2×10^{2} *Enterococcus faecium* 3.5×10^{2} *Neisseria subflava* 5.0×10^{1} *Staphylococcus epidermidis* 4.2×10^{2} *Staphylococcus equorum* 2.8×10^{2} *Streptococcus mitis* 2.7×10^{2} *Str.equi* ssp. *zooepid.* 5.0×10^{1} *Streptococcus equinus* 5.9×10^{2}	*Enterococcus faecium* 1.1×10^{1} *Neisseria subflava* 0.9×10^{1} *Pantoea agglomerans* 1.0×10^{1}	*Acinetobacter lwofii* 1.1×10^{1} *Bacillus pumilus* 0.5×10^{1} *Enterococcus faecium* 4.2×10^{1} *Paenibacillus amylolyticus* 0.3×10^{1} *Staphylococcus debuckii* 0.6×10^{1} *Streptococcus equinus* 5.3×10^{1}	*Paenibacillus glucanolyticus* 0.1×10^{1} *Streptococcus gordonii* 0.7×10^{1} *Str. equi* ssp. *zooepid.* 0.1×10^{1} *Streptococcus equinus* 1.1×10^{1}
Total bacterial counts (CFU·ml^{-1})	3.1×10^{2}	2.1×10^{3}	3.0×10^{1}	1.2×10^{2}	2.0×10^{1}
4 Species (CFU·ml^{-1})	*Enterococcus casseliflavus* 1.4×10^{2} *Staphylococcus chromogenes* 4.1×10^{1} *Staphylococcus hyicus* 2.0×10^{1}	*Enterococcus casseliflavus* 2.1×10^{1} *Staphylococcus hominis* 0.9×10^{1}	*Bacteroides fragilis* 0.4×10^{1} *Staphylococcus capitis* 0.5×10^{1} *Streptococcus uberis* 0.1×10^{1}	*Staphylococcus epidermidis* 4.6×10^{1} *Staphylococcus simulans* 0.5×10^{1} *Staphylococcus warneri* 0.9×10^{1}	*Bacillus simplex* 0.5×10^{1} *Corynebacterium coylae* 3.2×10^{1} *Staphylococcus haemolyticus* 0.9×10^{1}
Total bacterial counts (CFU·ml^{-1})	2.0×10^{2}	3.0×10^{1}	1.0×10^{1}	6.0×10^{1}	5.0×10^{1}
5 Species (CFU·ml^{-1})	*Clostridium ramosum* 6.7×10^{1} *Enterococcus casseliflavus* 5.5×10^{2} *Enterococcus mundtii* 4.9×10^{2} *Escherichia coli* 2.3×10^{1} *Micrococcus luteus* 1.6×10^{1} *Staphylococcus epidermidis* 2.1×10^{2} *Staphylococcus simulans* 2.0×10^{2} *Streptococcus gallolyticus* 1.0×10^{3}	-	*Clostridium sporogenes* 8.1×10^{1} *Staphylococcus delphini* 2.1×10^{1} *Staphylococcus intermedius* 7.6×10^{1} *Streptococcus gallolyticus* 4.0×10^{2}	*Staphylococcus chromogenes* 0.3×10^{1} *Staphylococcus warneri* 0.7×10^{1}	
Total bacterial counts (CFU·ml^{-1})	2.6×10^{3}	0	5.8×10^{2}	1.0×10^{1}	

No.	Dioestrus		Oestrus		
	Fossa clitoridis	Cervix uteri	Fossa clitoridis	Cervix uteri	Endometrium
6 Species (CFU·ml^{-1})	*Corynebacterium spp.* 3.0×10^{2}	-	*Corynebacterium spp.* 1.5×10^{3} *Escherichia coli* 3.2×10^{1} *Staphylococcus chromogenes* 4.6×10^{1}	-	-
Total bacterial counts (CFU·ml^{-1})	3.0×10^{2}	0	1.6×103	0	0
7 Species (CFU·ml^{-1})	*Aerococcus viridans* 2.1×10^{1} *Staphylococcus xylosus* 0.9×10^{1}	-	*Aerococcus viridans* 1.9×10^{1} *Aerococcus urinaeequi* 0.4×10^{1} *Staphylococcus xylosus* 0.7×10^{1}	-	-
Total bacterial counts (CFU·ml^{-1})	3.0×10^{1}	0	3.0×101	0	0
8 Species (CFU·ml^{-1})	*Arcanobacterium hippocoleae* 1.0×10^{3} *Bacteroides xylanisolvens* 6.0×10^{2} *Bacteroides mycoides* 1.8×10^{2} *Propionibacterium jensenii* 2.0×10^{1}	-	*Bacillus megaterium* 4.6×10^{1} *Bacillus niacini* 1.9×10^{1} *Bacillus simplex* 2.4×10^{1} *Bacteroides xylanisolvens* 2.0×10^{1} *Enterococcus casseliflavus* 1.8×10^{1} *Escherichia coli* 2.5×10^{1} *Lactobacillus equi* 1.5×10^{1} *Staphylococcus chromogenes* 4.8×10^{1} *Streptococcus dysgalactiae* 2.5×10^{1}	-	-
Total bacterial counts (CFU·ml^{-1})	1.8×10^{3}	0	2.4×10^{2}	0	0
9 Species (CFU·ml^{-1})	*Aerococcus viridans* 2.2×10^{1} *Bacillus pumilus* 1.4×10^{1} *Staphylococcus epidermidis* 0.7×10^{1} *Staphylococcus sciuri* 1.0×10^{1} *Staphylococcus xylosus* 0.7×10^{1}	*Staphylococcus sciuri* 1.4×10^{2} *Staphylococcus xylosus* 5.1×10^{1}	*Staphylococcus succinus* 1.4×10^{2} *Staphylococcus xylosus* 2.5×10^{2} *Streptococcus dysgalactiae* 7.8×10^{1} *Str. equi* ssp. *zooepid.* 2.7×10^{2}	*Enterococcus casseliflavus* 1.6×10^{2} *Staphylococcus hominis* 2.3×10^{1} *Streptococcus dysgalactiae* 9.5×10^{1} *Str. equi* ssp. *zooepid.* 1.8×10^{2} *Streptococcus equinus* 4.7×10^{1}	*Staphylococcus sciuri* 1.2×10^{1} *Staphylococcus xylosus* 1.4×10^{1} *Str. equi* ssp. *zooepid.* 1.4×10^{1}
Total bacterial counts (CFU·ml^{-1})	6.0×10^{1}	1.9×10^{2}	7.3×10^{2}	5.1×10^{2}	4.0×101
10 Species (CFU·ml^{-1})	*Bacillus pumilus* 1.9×10^{3} *Enterococcus faecalis* 1.6×10^{3} *Escherichia coli* 2.1×10^{3} *Escherichia fergusonii* 1.5×10^{1} *Staphylococcus saccharolyticus* 5.2×10^{1} *Staphylococcus sciuri* 1.4×10^{3} *Staphylococcus xylosus* 1.5×10^{2}	-	*Enterococcus casseliflavus* 5.9×10^{3} *Enterococcus faecalis* 6.8×10^{3} *Escherichia coli* 1.4×10^{3} *Proteus mirabilis* 0.1×10^{1} *Proteus vulgaris* 0.1×10^{1} *Staphylococcus sciuri* 2.1×10^{3} *Streptococcus pluranimalium* 8.4×10^{2}	*Arthrobacter sulfonivorans* 1.1×10^{1} *Staphylococcus haemolyticus* 0.9×10^{1}	-
Total bacterial counts (CFU·ml-1)	8.6×103	0	1.7×104	2.0×101	0

CFU·ml^{-1} - colony forming units per millilitre

of bacterial contamination is associated with perineal formation and oestrus cycle phases was only partially confirmed, as all randomly selected jennies in the study had appropriate perineal formation which significantly reduced the risk of endometritis. In most jennies, the vulva was perpendicular to the ground or even ventrocranially bevelled, and the pelvic bones were above the dorsal commissure of the vulva. Therefore, it was not possible to assess the level of bacterial contamination of the urogenital tract in jennies with poor perineal formation. On the other hand, this natural physiological and for jennies typical formation of the perineal region, which creates a natural barrier against the entry of microorganisms from the external environment into the genital tract, has not been suppressed in this animal species by inappropriate genetic development, as is encountered e.g. in mares (Allen and Pycock 1989).

There were 62 different types of microorganisms belonging to 19 different bacterial genera isolated from the genital apparatus of the jennies, in particular from the fossa clitoridis, around the cervix and the endometrium. The vast majority of isolated bacteria are classified as commensal microorganisms that occur in soil, dust, water, skin and mucosal surfaces of domestic and wild animals, on the surface of plants, seeds, fruit and animal or human faeces and are of no clinical importance. From this group, we detected *Acinetobacter, Aerococcus, Arcanobacterium, Arthrobacter, Bacillus, Bacteroides, Clostridium, Corynebacterium, Lactobacillus, Micrococcus, Neisseria, Paenibacillus, Pantoea, Propionibacterium, Proteus,* and *Staphylococcus.* These microorganisms have no major influence on the development of diseases of the genital apparatus, and their presence and localization are only of interest in terms of the dynamics during the oestrous cycle. From the group of microorganisms belonging to potential urogenital pathogens, *Escherichia coli, Streptococcus equi* ssp. *zooepidemicus, Enterococcus faecalis* and *Enterococcus faecium* were detected. These are microorganisms which, under specific conditions, can cause the development of diseases of the genital tract. In horse mares, one of the main reasons for decreased fertility is bacterial edometritis, which is most often caused by the pathogens *S. equi* ssp. *zooepidemicus* or *E. coli*, and is diagnosed in 25–60% of breeding mares (Riddle et al. 2007; Overbeck et al. 2011). However, jennies that tested positive for potential pathogens (*E. coli, S. equi* ssp. *zooepidemicus, E. faecali*s and *E. faecium*) did not show any clinical or cytological signs of endometritis and, after the completion of the trial, all jennies became pregnant without any problems. This finding is all the more important when confronted with the work of Sokkar et al. (2001). Their study reported the incidence of bacterial pathogens classified as endometritis-inducing microorganisms (*S. equi* ssp. *zooepidemicus, Staphylococcus aureus, Corynebacterium*, and coagulase-negative staphylococci) in the *post mortem* group of jennies, and described these symptoms of endometritis with a worsening fertility prognosis. But there is a lack of reproductive history of the jennies that were examined *post mortem*; there is no record of consequences regarding the influence in pregnancies in their study. So our work casts doubt on their conclusions regarding the same pathogenicity of bacteria between donkey and horse species.

Recorded amounts of isolated microorganisms varied in different phases of the oestrous cycle (oestrus and diestrus). However, the study has not confirmed the hypothesis that CFU will be higher in oestrus than in dioestrus due to a change in tonus and passage of the genital tract. To compare the differences in contamination associated with the penetrability of the reproductive tract in different phases of the oestrous cycle, a comparison was made with the number of CFUs trapped around the cervical area.

The changes that were detected on the external parts of the genitalia also brought interesting findings. The question remains whether it is a natural cleansing and defensive ability of the genital tract, which intensifies during the oestrous period, or whether the results obtained are burdened by a diagnostic error. A higher number of samples would

certainly help to clarify this uncertainty. Samples for microbiological examination collected by swabing and their subsequent examination in the laboratory can show a high rate of false negative findings. In order to refine the diagnosis of the presence of pathogens, it is advisable to confirm the presence of the inflammatory condition that usually accompanies the infection. For these reasons, microbiological examinations should be combined with cytological examinations and evidence of inflammatory cells. Cytological smears are twice as sensitive in terms of confirming inflammation (Nielsen 2005).

In our study, endometrial samples were only taken in oestrus (increased risk of uterine contamination in dioestrus, cervical penetrability). They were sterile in four jennies (50%), and contained between 10 and 50 CFUs in the other four (50%), indicating a very low endometrial contamination rate overall. *Streptococcus equi* ssp. *zooepidemicus* was cultivated from the endometrium as the only potentially pathogenic microorganism that may cause subfertility in horse mares (Riddle et al. 2007; Overbeck et al. 2011), but its clinical importance has not yet been described. In two jennies we were unable to take samples from the endometrium. One of them had a very narrow birth canal and it was not possible to insert a hand into the vagina for cervical palpation, and then insert a catheter into the uterus. In the other jenny, it was not possible to pass the sampling catheter through the cervix.

Oestrus swab cytology did not reveal the presence of inflammatory cells or pathogens in any of the jennies. The samples showed only endometrial cells, cell debris, and none or rare inflammatory cells, which is considered physiological (Vilés et al. 2013). This cytological examination can be used together with a microbiological examination as a double control and diagnosis of inflammatory conditions of the endometrium. And if both examinations are negative, it can be concluded that these are not false negative results (Nielsen 2005) or a subclinical form of endometritis (Nielsen 2005; Riddle et al. 2007). All jennies became pregnant at the end of the study without difficulty.

Taylorella equigenitalis (causative agent of infectious endometritis in mares) was not detected by culture and subsequent microscopic examination. Bacteria of the same genus *T. asinigenitalis* that had been described in donkeys (Katz et al. 2000; Jang et al. 2001) were not isolated in our study. Both bacteria have similar requirements for growth, morphology and their biochemical properties are similar. To differentiate between them, it is appropriate to extend laboratory diagnostics to molecular methods with species-specific primers, preferably the quantitative polymerase chain reaction (Wakeley et al. 2006; May et al. 2016). Štritof et al. (2017) showed low sensitivity when using a culture alone, and recommended always using other molecular methods to identify positive cases of *Taylorella* spp. The clinical significance of this bacterium in donkeys is not very clear yet (Jang et al. 2001; Meade et al. 2010), but we can conclude that the symptoms may be the same as in equine diseases caused by *T. equigenitalis*. It is possible that donkeys only develop a latent disease without external clinical manifestations. In the case of cross-species mating, even through artificial insemination, disease transmission could occur. Katz et al. (2000) describes a case of two mares exposed to intrauterine isolates of *T. asinigenitalis* that developed detectable vaginal and cervical discharge. The main reason for this study was the lack of previously published information on bacterial contamination of the donkey reproductive system, which can cause subfertility. Obtaining detailed information is important for improving assisted reproduction, which will lead to increased fertility in jennies.

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