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Case Report

Early pregnancy loss in a mare due to possible acute uterine bacterial infection

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Abstract

This case involves an 11-year Haflinger mare with breeding induced endometritis. After breeding soundness examination, mare was inseminated twice during 1 estrous cycle. On day 1 (day 0 = day of ovulation), mare was negative for endometrial cytology and culture. On day 10, an embryonic vesicle was visualized via transrectal ultrasonography; progesterone concentrations were 15.8 ng/ml. On day 11, the embryonic vesicle had increased to a diameter of 0.8 cm with clearly visible endometrial edema; progesterone concentrations were 14.4 ng/ml. On day 12, embryonic vesicle was no longer visible; progesterone concentrations were 4.7 ng/ml (possibility of premature luteolysis). Bacteriological culture of the uterus with a guarded endometrial swab had a pure culture of *Streptococcus equi subspecies zooepidemicus* (*S. zooepidemicus*). Mare underwent treatment (uterine lavage and systemic penicillin for 5 days) and became pregnant at the next estrus. This case highlighted the possibility of *S. zooepidemicus* subclinical endometrial infection, not diagnosed by routine endometrial cytology and culture, becoming dormant.

Keywords: Mare, endometritis, acute bacterial infection, early embryonic loss, treatment

Background

Early embryonic loss in mares is attributed to a range of factors of both infectious and noninfectious origins.[1,2] In horses, early embryonic loss typically refers to pregnancy loss from fertilization to day 42 of pregancy.[3] The widespread use of transrectal ultrasonography[4–6] has become a must for early pregnancy diagnosis and follow up examinations.

Endometrial infection is a major cause of impaired fertility in mares.[7] Routine endometrial culture and cytology obtained before breeding might help to predict the likelihood of conceptus growth and development. Subclinical bacterial uterine infection can result in early embryonic death.[8,9] *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*) and *Escherichia coli* (*E. coli*) are commonly isolated in uterine bacterial infections whereas *Pseudomonas spp.*, *Klebsiella*, and *Leptospira spp.*, have been less frequently identified.[7,10]

Certain reproductive strains of *S. zooepidemicus* have adapted horses as their host.[11,12] These bacteria can hide within the endometrial tissue and therefore are not easily detectable via standard bacterial culture techniques (e.g. guarded endometrial swab).[13–15] Furthermore, breeding or uterine instillation of a bacterial growth medium[13] may activate these 'dormant' streptococci resulting in acute infectious endometritis.[13] We describe a case of early embryonic death in a mare assumed to be associated with subclinical bacterial infection by *S. zooepidemicus*.

Case presentation

An 11-year Haflinger mare, weighing 535 kg with a body condition score 7/9, was enrolled (after breeding soundness examination) in a study approved by the Austrian Federal Ministry for Science and Research (experimentation license number 2020-0.547.78).[16] Mare was a maiden and has been bred multiple times with positive pregnancy outcomes while used as an embryo donor. Mare had no history of spontaneous resorption and had never carried a foal to term. Mare underwent daily transrectal ultrasonography examinations (DP-6600Vet; Mindray, Shenzhen, China). At estrus detection (preovulatory follicle of 3.9 cm in diameter accompanied by

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marked endometrial edema grade > 2/3), mare was inseminated with raw semen from a fertile stallion containing 500 x 10^6 progressively motile sperm. Artificial insemination was repeated 48 hours after the first insemination. On day 1 (day 0 = day of ovulation), an endometrial culture and cytology were collected using a double-guarded uterine culture swab (Minitube, Tiefenbach, Germany) and a cytology brush (Minitube). Blood samples were collected from the jugular vein into vacuum tubes containing EDTA (Vacuette 9ml K3EDTA, Greiner Bio One, Kremsmünster, Austria). Immediately after collection, blood samples were transferred into iced water and subsequently centrifuged at 5°C (1,200 x g, 10 minutes). Plasma was aliquoted and stored at –20°C until progesterone analysis. Plasma progesterone concentrations were analyzed by an enzyme-linked immuno-sorbent assay (Enzo Progesterone ELISA; Enzo Life Sciences.[17] The intraassay coefficient of variation was 4.9%, the interassay coefficient of variation was 7.9%, and the minimal detectable concentrations were 11.1 pg/ml.[16] Uterus was examined once daily for intrauterine free fluid via transrectal ultrasonography from insemination day until fluid was no longer detectable. The amount of intrauterine fluid was determined by measuring the area of fluid at the largest diameter in both uterine horns and the uterine body using the electronic calipers of the ultrasound machine and then calculating the total maximal area.[18]

From day 10 onwards, the uterus was scanned daily for embryonic vesicle and if detected, its diameter was determined in 2 dimensions at a 90° angle with the electronic calipers. The mean of the 2 measurements was calculated.

On day 10, transrectal ultrasonography revealed an embryonic vesicle along with slight endometrial edema (Figure 1A). The next day, the vesicle had increased in size (Figure 1B), but on day 12, the vesicle was no longer visible in transrectal ultrasonography. Instead, a considerable amount of hyperechoic intrauterine fluid was present (Figures 1C-E). On day 14, endometrial culture (with antibiogram) and cytology (cytobrush) were collected. The endometrial cytology was negative (0% polymorphonuclear neutrophils [PMN]) but the endometrial culture resulted in moderate growth of *S. zooepidemicus* (result made available on day 16). Progesterone concentrations had dropped considerably from day 11 to day 12. Detailed description of clinical and laboratory findings is provided (Table). Based on these findings, an acute endometrial bacterial endometritis followed by premature luteolysis and early embryonic death were diagnosed.

Beginning on day 14, treatment included were uterine lavage with 0.9% NaCl solution and stimulation of mechanical uterine clearance with 20 IU intravenous oxytocin (Oxytocin, Vana, Vienna, Austria) given every 4 hours. From day 16,

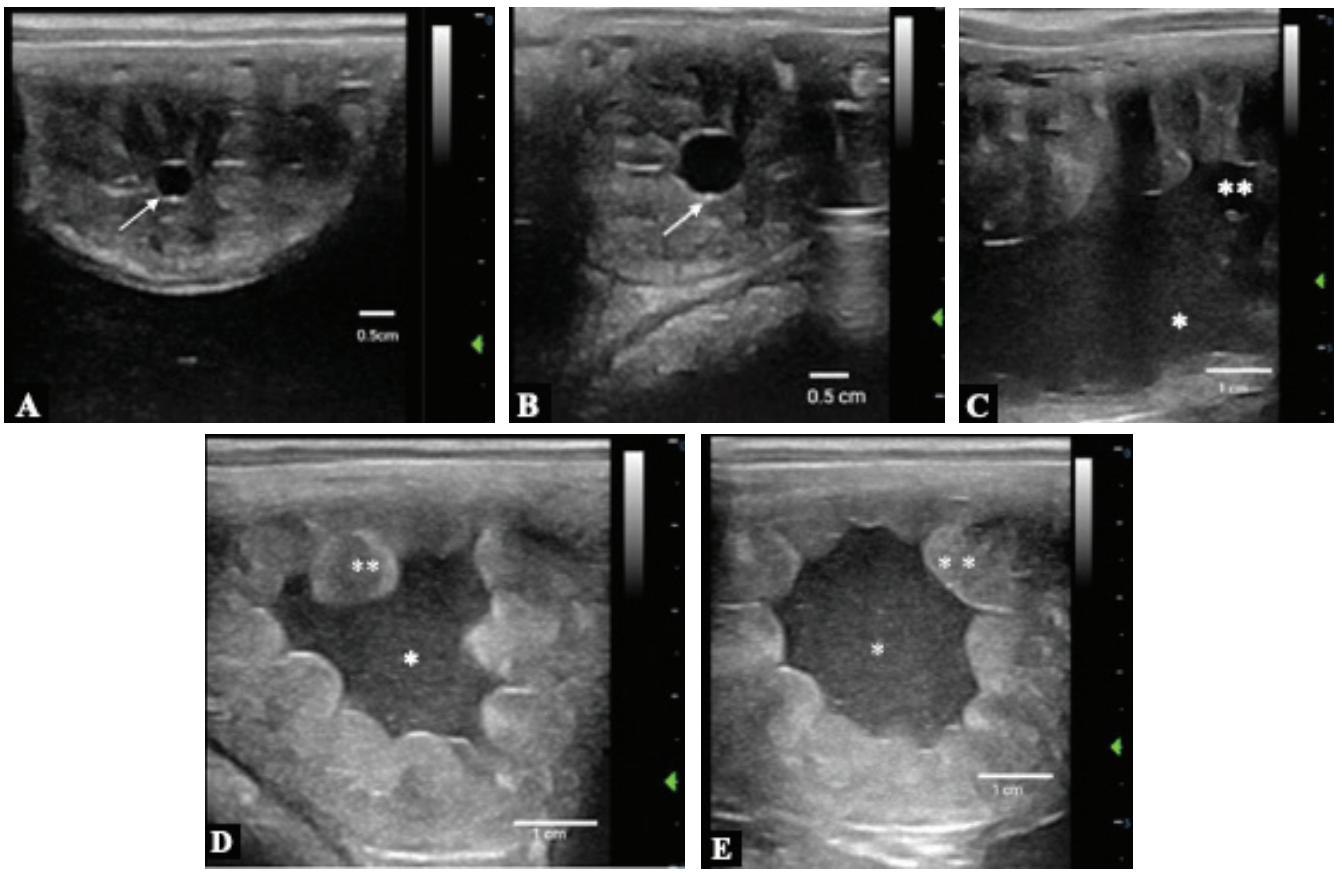

Figure 1. Transrectal ultrasonographic images of uterus: A. Day 10 embryonic vesicle (white arrow) of 0.4 cm and grade 1 endometrial edema; B. Day 11 embryonic vesicle (white arrow) of 0.8 cm and grade 2 endometrial edema; C. Day 12 embryonic vesicle; D and E. Days 13 and 14 embryonic vesicle (note hyperechoic free fluid visible in the uterine lumen* and uterine mucosa edema**)

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Table. Clinical, laboratory findings (endometrial edema: 0: none, 1: slight, 2: moderate, 3: severe), and treatment

		Transrectal ultrasonography			Insemination, endometrial samples, and laboratory results		Progesterone concentrations (ng/ml)	Treatment		
Cycle #	Day	Endometrial edema	Intra uterine fluid (cm^2)	Embryonic vesicle (cm)	Cytology (% PMN)	Culture/insemination		Uterine lavage (0.9% NaCl solution)	Subcutaneous oxytocin (20 IU)	Antibiotic treatment
	-3	3	0	not applicable		insemination				
	-2	2	8.2	not applicable						
	-1	2	4	not applicable		insemination				
	0	1	1.6	not applicable						
1	1	0	0	not applicable	2.5	culture	-	-	-	-
	3					result: negative				
	10	1	0	0.44	-		15.8	-	-	-
	11	2	0	0.8	-	-	14.4	-	-	-
	12	3	6.7	0.7	-	-	4.7	-	-	-
	13	3	7.1	not detectable	-	-	3.00	-	-	-
	14	3	4.1	not detectable	0	culture	2.8	once daily	every 4 hours	-
	15	2	4.5	not detectable	-		-	once daily	every 4 hours	-
	16	3	4.2	not detectable	-	result: moderate growth of *S. zooepidemicus*	-	once daily	every 4 hours	once daily penicillin (20,000 IU)
	17	3	5	not detectable	-	-	-	-	every 4 hours	once daily penicillin (20,000 IU)
	18	1	1.9	not detectable	0	-	-	-	every 4 hours	once daily penicillin (20,000 IU)

Table. (Continued)

		Transrectal ultrasonography			Insemination, endometrial samples, and laboratory results		Progesterone concentrations (ng/ml)	Treatment		
Cycle #	Day	Endometrial edema	Intra uterine fluid (cm^2)	Embryonic vesicle (cm)	Cytology (% PMN)	Culture/insemination		Uterine lavage (0.9% NaCl solution)	Subcutaneous oxytocin (20 IU)	Antibiotic treatment
2	0	0	1.6	not applicable	-	-	-	-	every 4 hours	once daily penicillin (20,000 IU)
	1	0	1.1	not applicable	-	-	-	-	every 4 hours	once daily penicillin (20,000 IU)
	2	0	0	not applicable	-	-	-	-	every 4 hours	-
	11	0	0	not applicable	0	culture	-	-	-	-
	13	0	0	not applicable	-	result: negative	-	-	-	-
	17	2	0	not applicable	-	insemination			-	
	18	2	6.3	not applicable	-				every 6 hours	
	19	2	7.9	not applicable	-	insemination			every 6 hours	
	20	1	6.8	not applicable						
3	0	1	0.8	not applicable	-	-	-	-	every 6 hours	-
	1	0	1.82	not applicable	0	culture	-	-	every 6 hours	-
	2	0	1.14	not applicable	-	result: negative	-	-	every 6 hours	-
	3	0	0	not applicable	-		-	-	-	-
	10	0	0	0.28	-	-	23.9	-	-	-
	11	0	0	0.47	-	-	30.6	-	-	-
	12	0	0	0.83	-	-	27.1	-	-	-
	13	0	0	1.2	-	-	30.1	-	-	-
	14	0	0	1.71	-	-	24.6	-	-	-

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intramuscular penicillin (Vanapen, Vana, Vienna, Austria) was given (20,000 IU/kg bodyweight once daily) and continued for 5 days.

Outcome

Mare ovulated on day 19 of cycle 1 (corresponding to day 1 of cycle 2), underwent diagnostic assessments, including endometrial culture and cytology on day 11 of cycle 2; results were negative. Mare was inseminated on days 17 and 19 of cycle 2 with raw semen collected from the same stallion as in cycle 1. On days 18-20, a considerable amount of intrauterine fluid accumulation was detected. Mare ovulated on day 21 of cycle 2 (i.e. day 1 of cycle 3). An embryonic vesicle was detected via transrectal ultrasonography on day 10. The vesicle continued to develop until day 14; no signs of endometritis or pregnancy loss were detected. Mare was treated with intramuscular 250 µg prostaglandin $F_{2\alpha}$ (Estrumate, MSD, Vienna, Austria) for termination of pregnancy and induction of estrus according to the experimental protocol of the research study. Further examinations during 3 consecutive cycles did not reveal any signs of acute endometritis.

Discussion

This case emphasizes some critical points regarding the diagnosis of activated dormant bacteria, management, and treatment of endometritis in mares, particularly acute bacterial endometritis. Endometritis in horses occur either clinically or subclinically. In either case, laboratory tests (e.g. endometrial cytology and culture) are crucial for diagnosis and for identifying the pathogens involved and their susceptibility to antibiotics.[4,19] In this case, breeding soundness examination of the mare did not reveal any clinical signs of endometritis, and the endometrial culture performed with a double-guarded swab was negative. Endometrial cytology, however, revealed 2.5% PMN on day 1 after ovulation and the mare had not been bred during the previous estrous cycle. This finding might have been considered suspicious because subclinical endometritis in mares without clinical signs was assumed when > 2% of PMN were in an endometrial cytology sample.[20] Other researchers, however, suggested that a PMN count < 5% is nonindicative for endometrial inflammation.[21,22] In this context, the stage of the estrous cycle should be considered because it may influence endometrial cytology. Although healthy mares outside estrus have an inflammatory cell count close to zero because of reduced PMN migration into the endometrium during progesterone dominance,[20] a small resident population of neutrophils (< 5%) may exist in the endometrium of mares from 24-96 hours after ovulation (i.e. when progesterone concentrations gradually increase).[21] Based on these suggestions, we interpreted the initial endometrial cytology (2.5% PMN) together with a negative endometrial culture collected on day 1 after ovulation as physiological. Progression of the case supports the suggestion to include endometrial histology in breeding soundness examination of mares. This approach considerably improved the likelihood of detecting increased numbers of PMN in the endometrium.[23]

Interestingly, more recent investigations demonstrated that specific bacteria influence endometrial cytology; not only *E. coli* infections, but similarly dormant *Streptococci spp*. may reduce PMN in endometrial cytology.[11,13,24,25] This may be considered as an explanation for the small number of PMN detected in the initial endometrial cytology of the present case. *S. zooepidemicus* was only determined by endometrial culture once the mare developed acute endometritis during early pregnancy. This might suggest that dormant bacteria were activated during estrus or postovulatory phase. Dormant *S. zooepidemicus* are likely undetectable in a routine endometrial culture obtained by double guarded swab but require activation. For diagnostic purposes this is possible by intrauterine instillation of the bacterial growth medium bActivate.[13] In addition, intrauterine misoprostol infusion provoked clinically significant bacterial endometritis in 63% of mares that had previously tested negative for endometritis, highlighting its potential utility in uncovering undiagnosed infections.[26] Furthermore, culture of an endometrial biopsy was often successful.[27] In the present case, repeated intrauterine manipulation associated with insemination and endometrial sample collection or even more intrauterine fluid accumulation indicating delayed uterine clearance after insemination of the mare may have resulted in activation of dormant *Streptococci*. Under experimental conditions, intrauterine instillation of bActivate in early estrus resulted in positive endometrial cytology and culture already after 48 hours.[13] In the present study, mild signs of endometritis can be suspected by the detection of a mild endometrial edema together with the conceptus on day 10. This is considerably later but unfortunately, no information on transrectal ultrasonographic findings after treatment is provided.[13]

Alternative to activation of dormant *Streptococci*, infection with *S. zooepidemicus* due to contamination at insemination or culture collection must be considered in the present case. This scenario is, however, relatively unlikely because the stallion used for semen collection was restricted to breeding by artificial insemination limiting transfer of bacteria from another mare. Also, any intrauterine manipulation was performed under strict hygienic conditions to minimize the likelihood of bacterial contamination in an experiment where uterine health was a key requirement.[16] This approach was highly successful as none of the 7 mares kept together with this mare discussed had any positive culture or any clinical signs of endometritis during the entire experimental period which included a total of 7 closely monitored estrous cycles per mare.

Although embryonic vesicle size was within the physiological range indicating normal conceptus development on days 10 and 11,[28] growth had ceased on day 12 demonstrating a switch to abnormal embryo development often indicative of early pregnancy loss.[29] At this period, also progesterone concentrations had considerably dropped which paralleled by increasing endometrial edema indicating return of the mare to estrus.[30] In addition, the sudden appearance of free intrauterine fluid suggested an endometrial inflammatory condition.

In agreement with good practice of antimicrobial stewardship, antibiotic treatment of the mare was not initiated before the infectious agent, *S. zooepidemicus* was isolated via endometrial culture.[7] Nonantimicrobial treatment, however, was already started as soon as embryonic loss and return to estrus were diagnosed to take advantage of the beneficial effects of removal of debris and inflammatory material from the uterus.[4,9,28,30] Two day delay in initiating antibiotic treatment had no negative impact.

Identification of bacteria in mares with mild subclinical endometritis may be difficult. *Streptococci* are among the most frequent bacteria causing endometritis in horses, and because some strains may enter a dormant state, their detection is diagnostically challenging. Observation of PMN in endometrial cytology samples must therefore be considered cautiously.

Additional diagnostic approaches may help to identify bacteria that have developed strategies to escape the host immune response.[31]

Learning points

- *S. zooepidemicus* endometrial infections may be dormant in horses
- *Routine endometrial cytology and culture may not detect S. zooepidemicus*
- Advanced diagnostic methods are necessary
- Endometrial infections are treatable
- Proper treatment can help mares conceive successfully

Conflict of interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content.

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