RELATIONSHIP BETWEEN BACTERIOLOGICAL AND CYTOLOGICAL EXAMINATION OF THE MARES’ UTERUS DURING FOAL HEAT AND FERTILITY RATE

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In the study, 75 mares were examined during foal heat by rectal palpation and ultrasound scanning. Samples obtained from the uterus were investigated bacteriologically and cytologically. Bacteriological examinations were positive in 49 (65.3%) and β-haemolytic Streptococcus (25.3%) and Escherichia coli (24%) were the most frequently isolated bacteria. Fungi were found in 6 mares. Negative result of cytological examination was obtained from 25 (33.3%) mares. Pregnancy was diagnosed in 54.5% of mares with normal uterus while in groups with uterine disorders pregnancy was found in 18-23%. Our results showed that uterine disorders in foal heat decrease the fertility rate.

Key words: mares, uterus, bacteriology, cytology, fertility.

The ovarian cycle appears very early after parturition in a mare and the foal heat is often used for mating. Some authors noticed that fertility rate in foal heat is lower compared to following heats (15, 17). The main reasons for this situation might be existing subclinical endometritis or delayed involution of the uterus after parturition. Rectal palpation supported by ultrasound examination is not often sufficient for detection of this problem. Therefore, for proper diagnosis additional examinations such as bacteriology and cytology of uterine smears must be used (4, 13, 22). Due to the differences in bacterial strains isolated from the vagina and uterus, only collection of samples from the uterus was adequate for diagnosis and treatment of this organ (2, 4, 8). Examinations of the uterine smears allow for identification and evaluation of endometrial cells, white blood cells (leukocytes, eosinophils, macrophages, lymphocytes), erythrocytes, and bacteria (6, 13, 22, 25).

The aim of this study was to evaluate, using bacteriological and cytological examinations, status of health of the mares’ uterus during foal heat as well as the fertility rate of the examined mares in the north-eastern part of Poland.
Material and Methods

As experimental animals served 75 mares aged from 3 to 20 years. This group included full-blooded, standardbreed and cold-blooded horses. Animals belonged to two National Horse Studs in Liski and Rzeczna as well as to private owners whose mares were treated at the Clinic of Obstetrics and Pathology of Reproduction.

All the animals were controlled for appearance of foal heat during early postpartum period. Mares with signs of heat were examined by rectal palpation and ultrasound scanning and the status of the uterus and ovaries was determined. Samples for bacteriological and cytological examination were collected from the uterus using a disposable catheter (Equi Vet). Detailed description of samples collection is included in other articles (1, 2). All mares in heat were mated.

The bacteriological examinations were carried out using: 5% blood agar, MacConkey medium and Müller-Hinton medium. Samples were incubated for 24-48 h at 37°C. Presence of fungi was checked at Sabouraud medium. Plates were incubated for 5 d at 37°C. For cytological examination the cotton swab was smeared onto the slide, the preparation was air dried, and Papanicolau staining was done. Smears were observed with light microscope under 125x and 250-500x magnification. For diagnostic purpose ten high powered fields were chosen and polymorphonuclear leukocytes (PMnL), macrophages, and erythrocytes were controlled.

Results

First signs of foal heat appeared between 6th and 12th d p. p. (43% on day 8th and 9th) and average time of the heat was 5.04 ±1.71d. All the examined mares had enlarged and asymmetric uterine horns, but only 9 of them had uterine fluid during the foal heat.

Bacteriological examinations of the smears were positive in 49 (65.3%) mares. The most frequently isolated bacteria were: β-haemolytic Streptococcus - 19 (25.3%), Escherichia coli - 18 (24%), α-haemolytic Streptococcus and coagulase-negative Staphylococcus - 14 (18.6%). Staphylococcus aureus and Pseudomonas aeruginosa were found in 1 (1.3%) mare each (Fig. 1). Mycological examination was positive in 6 mares and Candida (3 mares), Aspergillus fumigatus (3 mares) and Mucor (1 mare) were isolated. Cytological examination was positive in 50 mares (66.7%). Large number of erythrocytes testify to incomplete regeneration of endometrium was found in 34 mares (45.3%). Ten or more leukocytes, what indicates endometritis, was seen in 21.4% of mares. Negative result of cytological examination were obtained from 25 (33.3%) mares.

Combined results of these additional examinations are presented in the Fig. 2. The doubly negative results were obtained on only in 18 (24%) mares. About 60% of mares showed positive results of bacteriological examination and 20% of animals were cytologically positive.

Fertility rate of mares without subclinical disorders of the uterus was much higher than that of mares with pathological changes (Fig. 3). Pregnancy was diagnosed in 54.5% of mares with healthy uterus while in the groups with uterine disorders, the result was 18-23%. The worst result showed mares with positive bacteriological and cytological examinations and in this case the pregnancy was diagnosed only in 18.2% of the mares.
**Fig. 1.** Bacteria isolated from the uterus during foal heat.

**Fig. 2.** Combined results of bacteriological and cytological examinations of mares during foal heat.
Discussion

It has been suggested that healthy uterus during physiological anoestrus does not contain any bacteria (24). During parturition and heat the uterine cervix is opened and tissues are swollen. These changes increase the possibility of uterine infection with both pathogen and non-pathogen bacteria (9, 19). In this study, bacteriological examination during foal heat was positive in 66.2% of mares. In most cases haemolytic *Streptococcus* and *E. coli* were isolated, what is in agreement with observations of other authors (12, 20, 28). However, in the cited studies bacteria were found only in 36% of cases. Studies dealing with colonisation of the uterus with bacteria in postpartum period and foal heat (10, 12, 23, 28) demonstrated that type of bacteria and intensity of the infection after parturition changed in the uterine environment. The noted in this study of infected animals is very high percentage (66.2) in contrast to the results of other authors who found only 20-30% of infected mares (18, 20, 28). The reason for such numerous uterine infections in our experimental animals might have been poor environmental conditions, lower local and systemic resistance of the animals, as well as possibility of infections from bedding and hay.

Cytological examination of the smears showed in many cases presence of a high number of red blood cells without signs of inflammation. According to other researchers (23, 27) this phenomenon is physiological only during a short period after normal parturition (5-7 d p.p.). In later phases of puerperium, presence of high number of erythrocytes points at damage of blood vessels or their increased permeability probably due to an incomplete regeneration of endometrium. Saltiel (23) found that
number of erythrocytes decrease during postpartum period and slightly increase in the heat. This author noticed that mares which did not conceive had much more red blood cells than those which did. Prolonged regeneration of endometrium during puerperium was also observed in mares susceptible to endometritis during a longer period after parturition (10, 21). Its usefulness for the anticipation of fertility was also stressed by these authors. The results obtained in our study verify this suggestion because only 35.7% of mares with prolonged endometrium regeneration conceived after mating in first heat.

Endometritis recognized by cytological examination was found in 23% of mares in this study, while other authors report higher frequency of this disorder (5, 18). White blood cells, because of their ability of migration, are involved in resistance against factors which cause inflammation both in interior structures of endometrium and in the uterus lumen (5, 11, 16, 22, 29). It is assumed that these cells are associated with involution of the uterus and presence of the infectious or inflammatory factors (5, 16).

Relationship between the results of bacteriological and cytological examination and fertility of the mares was analyzed in our study. The best outcomes (54.5% of pregnancies) were observed in the group of animals with both negative examinations, while the worst fertility (18.2%) was show in mares with positive cytological and bacteriological examinations. These results pointed at the influence of a healthy status of the uterus diagnosed by laboratory examinations for fertility after the mating in foal heat what is in agreement with other authors’ opinion (3, 10, 27). Total 32% fertility rate after the mating in foal heat was relatively low in this study. Low pregnancy rate in the examined animals could be caused by pathological uterine environment or post mating endometritis. Troedson (26) and Watson (29) suggest this phenomenon to be very important and can be one of the reason for low fertility rate. Unfortunately, this aspect was not investigated in this study. Results obtained from other studies are discrepant. Lieux (14) found 39% and Lenz (15) 42% of pregnant mares after the mating in foal heat, while in contrast Camillo et al. (7) achieved 70% of the fertility rate.

In our study, the fertility rate after the second heat increased significantly, especially in the group with both positive results of the laboratory investigations. The explanation of this phenomenon is progress in endometrial regeneration as well as elimination of bacteria from uterus during the period from foal heat to mating in the following heat (12).

Our results showed that uterine disorders in foal heat decrease fertility rate. To get more information for mating strategy in foal heat, the bacteriological and cytological investigations are necessary. These laboratory tests should be used as an important tool for improvement of fertility in mare.

References