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Occurrence of bacteria and polymorphonuclear leukocytes in fetal compartments at parturition; relationships with foal and mare health in the peripartum period

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ABSTRACT

This study investigated the relationship of the health of the newborn foal and (1) number of polymorphonuclear leukocytes (PMNLs) in the amniotic fluid, (2) bacteria present in the amniotic fluid and the venous umbilical blood, and (3) bacteria present in the uterus of the newly foaled mare. A further aim was to investigate relationships between the bacteriologic findings in the amniotic fluid, umbilical blood, and uterus postpartum. Samples were taken from 50 Standardbred trotter foaling mares from a well-managed stud in Sweden. Parturition was spontaneous in all cases. Length of pregnancy, parturition and postpartum complications, health status of the foal, the time between foaling and the expulsion of the placenta, and the number of postfoaling mares becoming pregnant after insemination were recorded. Amniotic fluid was collected when the amniotic vesicle was clearly visible; it was analyzed for bacteriology and occurrence of PMNLs. Umbilical blood was analyzed for the presence of bacteria and the concentration of serum amyloid A. The uterus of the mare was swabbed for bacteriology 6 to 17 hours postpartum. A blood sample was taken from the foal before administering plasma. The foals were divided into two groups: group 1 required up to 2 hours to rise after birth ($\leq$2 hours; 31 foals) and group 2 required more than two hours (>2 hours; 19 foals). The length of gestation varied between 332 and 356 days; there was no significant difference in gestation length between the two foal groups. Partus and postpartum complications occurred in a significantly higher proportion of mares giving birth to group 2 foals than group 1 foals ($P = 0.02$), although uterine culture postpartum and the subsequent pregnancy rate per season were not different between the groups. Compromised health status was significantly higher among foals belonging to group 2 than group 1 ($P = 0.001$). Most of the amniotic samples contained 5% or less PMNLs. Only three samples contained more than 30% PMNLs; group 2 foals had the highest percentage of PMNLs. Bacterial growth was found in both amniotic fluid (57%) and umbilical blood (35%) in mares irrespective of whether their foals were healthy or compromised. Coagulase-negative staphylococci were the most frequent bacteria. There were no differences in bacterial occurrence in amniotic fluid or in umbilical blood between the two foal groups.

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1. Introduction

A previous study showed the occurrence of a low number of polymorphonuclear leukocytes (PMNLs) in the amniotic fluid of foaling mares giving birth to healthy foals; these PMNLs were found to originate from the fetus [1]. Similar findings have been reported previously in human amniotic fluid, where the number of PMNLs is used as an indicator of human fetal well-being [2].

The presence of bacteria in both amniotic fluid and venous umbilical blood has been reported in healthy human babies, delivered by cesarean section [3–5]. These bacteria are thought to have an impact on the fetal immune system, preparing the newborn baby for the environment outside the uterus [6]. In veterinary medicine, the occurrence of bacteria in fetal compartments is not expected in healthy animals at normal parturition and the effect of the presence of bacteria during the peripartum period is documented [7]. To our knowledge, no investigation of the presence of bacteria in the amniotic fluid and umbilical blood in mares giving birth to healthy or compromised foals has been published. In the previously mentioned study [1], only the presence and the number of PMNLs in the amniotic fluid were investigated.

The objectives of the present study, therefore, were to investigate the relationship of the health of the newborn foal and (1) number of PMNLs in the amniotic fluid, (2) bacteria present in the amniotic fluid and the venous umbilical blood, and (3) bacteria present in the uterus of the newly foaled mare. A further aim was to investigate if there are any relationships between the PMNLs in the amniotic fluid and the bacteriologic findings in the amniotic fluid, umbilical blood, and uterus postpartum.

2. Materials and methods

2.1. Animals

Samples from 50 foaling mares from a well-managed stud in Sweden were collected during the foaling season in 2013. The stud has approximately 120 mares foaling each year, supervised by a resident veterinarian and well-trained personnel, with well-established routines for foaling and insemination. The mares, aged 4 to 15 years old, were Standardbred trotters. All reproductive groups of mare were represented, that is maiden (10 mares), older maiden (five mares), foaling (24 mares), and barren mares (11 mares). Parturition was spontaneous in all cases and was supervised by the trained staff at the stud. Length of pregnancy, parturition and postpartum complications, health status of the foal, the time between foaling and the expulsion of the placenta, and the number of postfoaling mares becoming pregnant after insemination were recorded. All foals were given colostrum from a baby bottle within 1.5 hours postpartum, when they were still lying down, by different members of the trained staff. The foals were divided into two groups according to the time required to stand, less than 2 hours and more than 2 hours, respectively.

2.2. Sampling

The following samples were collected:

2.2.1. Colostrum

Colostrum was obtained by milking 1 to 2 mL from the mare's udder for measurement of immunoglobulin G (IgG). All foals received their dam's colostrum from a baby bottle within 1.5 hours postpartum and were given 1 liter of hyperimmune plasma intravenously within 24 hours postpartum.

2.2.2. Fluid from the amniotic vesicle

The amniotic fluid was collected when the amniotic vesicle was clearly visible; it was swabbed with alcohol three times at its lowest point. A 20-mL syringe was filled using a sterile 18-ga needle. Aliquots of amniotic fluid (5 mL each) were placed in two plastic tubes for cytologic examination and a further 2.5 mL for bacteriologic culture.

2.2.3. Blood from the umbilical vein

After the birth of the foal, the umbilical vein was identified by palpation of the pulsations from the uterine contractions. The umbilicus was washed three times with alcohol more than 10 cm from the natural breaking point, and 20-mL blood was aspirated using a sterile syringe and 18-ga needle. For aerobic and anaerobic blood culture, two glass bottles containing a blood culture broth were prepared. After changing the needle on the syringe containing the umbilical blood, 2.5-mL blood was injected into each bottle. A small hematoma was seen within the umbilical tissue after the puncture. It was not possible to take the blood from the umbilical artery because of negative pressure.

2.2.4. Uterine fluid

The uterus of the mare was swabbed for bacteriology 6 to 17 hours (average 11 hours) postpartum using a double-guarded endometrial swab (Equi-Vet; Kruuse, Marslev, Denmark).

2.2.5. Jugular blood from the foal

All blood samples were taken from the foal's jugular vein within 24 hours of parturition using one serum and one EDTA tube, after they had received colostrum but before they were given plasma, i.e., 8 to 22 hours old (mean 15 hours old).

2.3. Analyses

2.3.1. IgG level in colostrum

The IgG level was measured using the colostrometer Brix chart. An IgG level above 50 g/L was regarded as good quality colostrum [8]. The level was calculated as the sum of the beta and gamma globulin fractions after electrophoresis.

2.3.2. Polymorphonuclear leukocytes in the amniotic fluid

The number of PMNLs was determined semi-quantitatively as a proportion of the total number of cells by an experienced cytologist in cytospin preparations after staining with May-Grünwald-Giemsa. The number of PMNLs was classified in the following categories: "none to moderate (0%–30%) per field of vision (magnification: × 100)" and "high (>30% per field of vision; magnification: × 100)".