Session 2

Laboratory Correlates of
Mare Reproductive Loss Syndrome

Chairperson: Dr. Lenn Harrison, Livestock Disease Diagnostic Center, College of Agriculture, University of Kentucky

Gross and Histopathological Correlates of Mare Reproductive Loss Syndrome

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IN THE LATE SPRING OF 2001 AND TO A LESSER EXTENT IN 2002, Central Kentucky horse farms experienced epidemic losses of fetuses and foals and other atypical equine health problems. This group of conditions has been referred to as Mare Reproductive Loss Syndrome (MRLS). During this time, increased numbers of fetuses (early and late fetal losses [EFL and LFL]), placentas, and term foals were presented to the University of Kentucky Livestock Disease Diagnosis Center (UKLDDC) for necropsy examination and diagnostic testing.

Materials and Methods

Foals, fetuses, and placentas were necropsied, and samples were taken for microbiology, serology, toxicology, and histopathology. Complete pathological reports were issued on the cases. The findings in 100 randomly selected MRLS LFL cases were reviewed and characterized for this report.

Results

During the 2001 and 2002 MRLS epidemics, approximately 550 and 250 LFL, respectively, were presented to the UKLDDC for examination. Necropsy examination and laboratory testing revealed a number of consistent findings in these fetuses.

Multiple breeds and all ages of mares were affected. Most of the fetuses were delivered at term or were aborted several weeks prior to the due date with usually no premonitory signs in the mares (Figure 1). The delivery was characterized as "red bag" in 32% of the cases, indicating that the allantochorion was presented and passed concurrently with the fetus. Lesions were seen in both the fetus and placenta. The fetuses were of normal size and weight for the gestational age and typically were in a state of good postmortem preservation. The lungs had variable inflation from case to case, ranging from no aeration to moderate aeration, indicating respiratory efforts at the time of delivery. The lungs were sometimes slightly firm, suggesting pneumonia. Hemorrhages were often present on the pleura and heart. Some allantochorions were mildly edematous; however, most were of normal size and weight. Hemorrhages were commonly seen on the chorionic and allantoic surfaces.

Figure 1. Time of LFL relative to due date in 100 randomly selected cases.
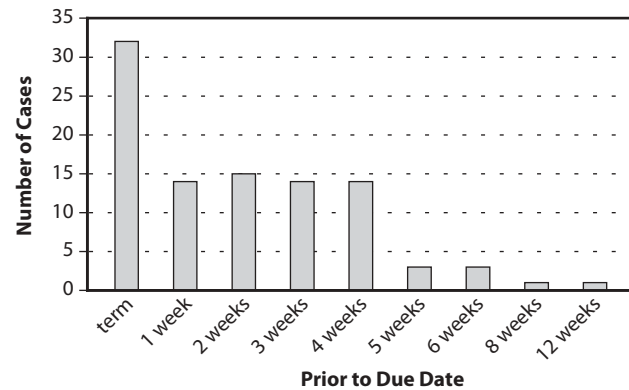
The most striking change occurred in the umbilical cord. A high percentage of the cases had roughening of the surface of the cord and enlargement due to stromal edema. There was discoloration of this area, typically a dull grayish-yellow color, and there were stromal and surface hemorrhages. The umbilical cord changes were limited to the amniotic segment with the allantoic portion having the normal smooth, glistening appearance to the surface. The amniotic membranes had variable hemorrhage and edema.

Histopathologically, the lungs often contained numerous desquamated squames of amnionic fluid origin and low numbers of neutrophils and macrophages in the alveolar spaces. Occasionally, multinucleated giant cells were present. Bacteria were often observed in the alveoli. Other fetal tissues contained only variable congestion and acute hemorrhages. Microscopically, the umbilical cords had bacteria on the surface with loss of the epithelium and light to heavy infiltrates of neutrophils and macrophages that were concentrated near the surface of the cord. Hemorrhages and edema were present in the stroma. Similar changes were sometimes present in the amniotic membrane. Allantochorions occasionally had low numbers of neutrophils in the stroma and extraembryonic coelom.

In 2002, cases consistent with MRLS recurred. The temporal, pathological, and microbiological findings were similar to the 2001 epidemic.

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Discussion and Conclusion

The cause of MRLS was not identifiable based on pathological examination; however, a number of disease conditions were eliminated as potential causes. The main pathological findings in MRLS fetuses were inflammation of the umbilical cord (funisitis), pneumonia, bacterial infection, and hemorrhages (Figure 2). The bacterial infection in aborted fetuses represented an *ante mortem* event, and most of the pathological lesions could be attributed to bacterial infection. It could not be determined if the bacteria were primary causes of abortion or merely represented secondary opportunistic infections. The epidemiological findings and bacteria isolated suggest a secondary role for the bacteria. The findings suggest that the bacteria gain access to the amniotic fluid and sites with direct contact with this fluid but show little tendency to invade. The high occurrence of "red bag" delivery, indicating possible premature placental separation, suggests placental injury or problem with placentation.

There was no single diagnostic test or pathognomonic finding that permitted diagnosis of abortion due to MRLS. Diagnosis of MRLS-related abortion or stillbirth was based

Figure 2. Occurrence of pathological changes.
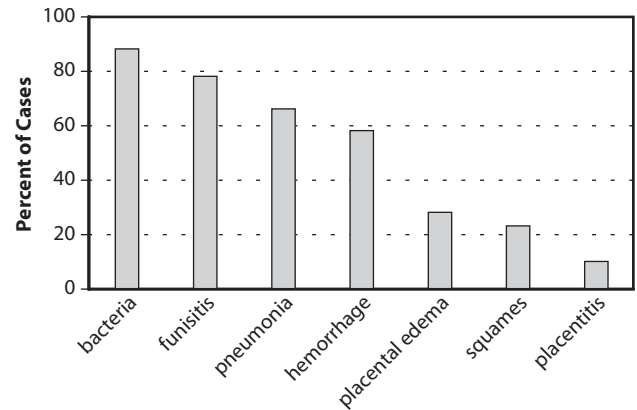
on a combination of history, time of year, bacteriologic results, and the above-described pathological findings. These pathological findings are suggestive of *in utero* fetal illness, infection, and distress but lack sufficient specificity to allow for diagnosis of a primary inciting cause.

The Pericarditis Correlate of Mare Reproductive Loss Syndrome

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AN EPIDEMIC OF PRIMARY EQUINE PERICARDITIS OCCURRED in Central Kentucky during the spring of 2001. The outbreak was temporally associated with two other syndromes in horses: early and late fetal loss (EFL and LFL) and uveitis, collectively referred to as Mare Reproductive Loss Syndrome (MRLS). All three conditions were associated with a heavy eruption of the eastern tent caterpillar (ETC), *Malacosoma americanum*, which was later identified as a risk factor in MRLS and pericarditis (3).

During the outbreak, tracheal washes, pericardial fluids, and serum samples from 13 clinical cases and 22 horses with pericarditis were submitted to the University of Kentucky Livestock Disease Diagnosis Center (UKLDDC) for diagnostic testing. Horses submitted for postmortem examination came from 22 farms and nine counties located in Central and Eastern Kentucky (Figure 1). The majority of submissions were Thoroughbreds. Quarter Horse, Rocky Mountain Spotted, and Hanoverian breeds were also represented. Two horses were of unknown or mixed breeding. The average age was 6.4 years with a range extending from three weeks to 30 years. Fifteen were female and seven male.
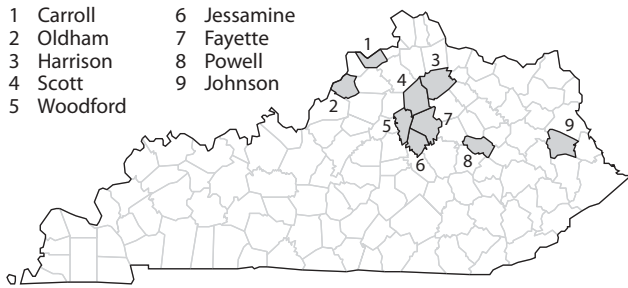
Pathologic Observations

Significant laboratory findings in the necropsy cases included lesions of effusive fibrinous pericarditis that were typically associated with varying degrees of pericardial, pleural, and peritoneal effusion. Twelve cases had received prior treatment for pericarditis. Recurrent effusions and constrictive pericarditis occurred in six individuals. Microscopically, the epicardium was covered by a thick, moth-eaten layer of fibrin within which were focally dense and diffuse accumulations of neutrophils. Bacteria were occasionally observed. The epicardium was thickened by granulation tissue of varying maturity. Myocardial lesions were generally mild and infrequent and included superficial interstitial fibroplasia and/or fibrosis, focal interstitial myocarditis, and foci of myocardial mineralization and necrosis.

Microbiologic Findings

Aerobic cultures of pericardial fluid and/or myocardium from necropsy (22) and mail-in submissions (11 of 13)

Figure 1. Geographic distribution of pericarditis cases in Kentucky.

1 Carroll	6 Jessamine
2 Oldham	7 Fayette
3 Harrison	8 Powell
4 Scott	9 Johnson
5 Woodford	

yielded an *Actinobacillus* sp. (11), *Streptococcus zooepidemicus* (1), *Escherichia coli* (1), and *Enterococcus fecalis* (1). No bacteria were recovered from the remaining cases. Cultures for mycoplasma organisms were negative. No viruses were isolated in cell culture, and EHV-1 antigen was not detected in tissue by immunofluorescent methods. PCR tests for EHV-2 nucleic acid were negative except in four cases.

The inability to isolate virus from samples submitted from terminal and clinical cases of pericarditis was neither an unexpected finding nor does it exclude previous virus infection as a contributing factor in these cases. In the terminal cases, the inflammatory process in the heart and pericardium had progressed to the chronic stage. It is also likely that at the time of diagnosis most of the clinical cases were subacute to chronic in duration. Although the inability to isolate virus was not considered evidence against viral myopericarditis, epidemiologic findings and observed microscopic alterations in heart and pericardium are not supportive of a viral etiology.

Discussion

Although an etiology of the pericarditis syndrome seen in this series of horses has not been identified, epidemiologic evidence suggests a point source exposure to an environmental agent and an apparent central causative role of the ETC (3). Laboratory findings support a role for opportunistic bacterial infection in the pathogenesis of the condition but does not exclude the possibility of previous virus infection or toxic effect from an environmental or caterpillar-associated agent.

The cases were presented during a five-month period extending from May through September (Figure 2). The terminal cases submitted after June were horses that had been treated for pericarditis for two to three months and died subsequent to the development of recurrent effusions or constrictive pericarditis. The large number of cases of equine pericarditis occurring over an extremely short period of time in this report is in sharp contrast to all

previous reports that have described fewer cases collected over a period of months to years (1,2,4,5,6,7).

All of the terminal nontreated and 36% of the clinical cases were diagnosed as bacterial pericarditis on the basis of positive culture results. Bacteria isolated were similar to isolates reported in earlier studies and included *Actinobacillus* sp., *Streptococcus zooepidemicus*, and *Enterobacteri faecalis*. There is evidence to suggest the remaining cases of terminal pericarditis were also bacterial in origin. The gross and microscopic alterations in the parietal pericardium and heart of affected individuals were similar in all cases and consistent with a bacterial etiology.

Further evaluation of information for each pericarditis case is being undertaken. The additional retrospective investigation is being carried out to attempt to clarify the role of microbiologic agents in this outbreak.

Figure 2. Distribution of pericarditis cases by date of accession.
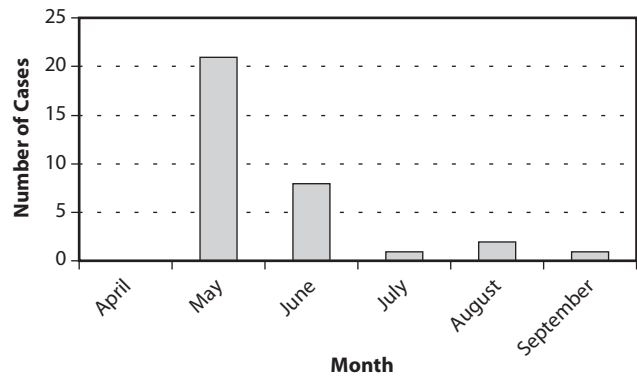
References

1. Bernard, W.; Reef, V. B.; Clark, E. S.; Vaala, W., and Ehnen, S. J. Pericarditis in horses: six cases (1982-1986). Journal of the American Veterinary Medical Association. 1990; 196(3):468-471.
2. Buergelt, C. D.; Wilson, J. H., and Lombard, C. W. Pericarditis in horses. Compendium on Continuing Education for the Practicing Veterinarian. 1990; 12(6):872-876.
3. Cohen, N.; Carey, V.; Donahue, J.; Seahorn, J.; Slovis, N., and Reimer, J. Report of pericarditis case-control study: prepared for the Governor's Task Force on the Mare Reproductive Loss Syndrome. 2001.
4. Worth, L. T. and Reef, V. B. Pericarditis in horses: 18 cases (1986-1995). Journal of the American Veterinary Medical Association. 1998; 212(2):248-253.
5. Freestone, J. F.; Thomas, W. P.; Carlson, G. P., and Brumbaugh, G. W. Idiopathic effusive pericarditis with tamponade in the horse. Equine Veterinary Journal. 1987; 19(1):38-42.

6. Robinson, J. A.; Marr, C. M.; Reef, V. B., and Sweeney, R. W. Idiopathic, aseptic, effusive, fibrinous, nonconstrictive pericarditis with tamponade in a Standardbred filly. Journal of the American Veterinary Medical Association. 1992; 201(10):1593-1598.

7. Dill, S. G.; Simoncini, D. C.; Bolton, G. R.; Rendano, V. T.; Crissman, J. W.; King, J. M., and Tennant, B. C. Fibrinous pericarditis in the horse. Journal of the American Veterinary Medical Association. 1982; 180(3):266-271.

Bacteria Associated with Mare Reproductive Loss Syndrome: Late Fetal Losses

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AN ABORTION OUTBREAK AFFECTING THE EQUINE POPULATION of Central Kentucky commenced in the last week of April and extended through May in 2001 and in 2002. During these time periods, there were dramatic increases in the number of early and late fetal losses (EFL and LFL) referred to as Mare Reproductive Loss Syndrome (MRLS). The losses have been associated epidemiologically with exposure of mares to the eastern tent caterpillar (ETC), *Malacosoma americanum*, but the cause of MRLS has not been identified.

After examining tissues (especially lung and umbilical cord) from the LFL received during the epidemic, pathologists at the University of Kentucky Livestock Disease Diagnosis Center (UKLDDC) observed lesions compatible with those caused by bacterial infections (1). They also observed bacteria in the fetal lungs and on the surface of the umbilical cord, and bacteria were recovered from most of the fetal tissues. The purpose of this paper is to report the microbiological findings obtained from the LFL obtained during the MRLS epidemic in 2001 and 2002.

Materials and Methods

Animals

All LFL received at the UKLDDC between April 26 and May 31 in 2001 and 2002 were examined in this study. Premature or term foals that had been treated or had been in a veterinary hospital were not included, and fetuses from mares in early gestation (less than six months) were not included.

Bacteriology

Lung, liver, and stomach content of all fetuses and placenta (if submitted) were cultured for aerobic and microaerophilic bacteria. In 2001, only the lung and stomach content from the last 275 fetuses received were cultured, and in 2002 the umbilical cord was cultured when requested by the pathologist. Each tissue was inoculated to a blood agar plate and an eosin-methylene blue agar plate, which were incubated in 8% carbon dioxide (CO_2) at 37°C for 2 to 7 days. Plates were examined daily for the presence of significant bacteria. A bacterium was considered significant if it occurred in pure or almost pure culture in moderate to numerous numbers from at least two sites. All significant bacteria were identified using conventional bacteriological media and methods (2,3).

Kidney and placenta (liver, if placenta was not received) were tested for the presence of leptospires by a fluorescent antibody test (FAT) (4,5) using a polyvalent conjugate (National Veterinary Services Laboratories [NVSL], Science and Technology, APHIS, USDA, Ames, IA). Only the first 129 fetuses received in 2001 and the first 110 fetuses received in 2002 were tested for leptospires by FAT. However, the stomach contents of all fetuses were examined by dark-field microscopy for the presence of leptospires and other bacteria.

Virology

At the UKLDDC, all fetuses were tested for EHV-1 by FAT in 2001 and 2002 and for EVA by FAT in 2002. Virus isolation was attempted on all cases from a tissue pool (lung, liver, spleen, kidney, and thymus) and, if received, from the placenta. The tissues were macerated and centrifuged. Then the supernatant was inoculated onto three cell culture lines: rabbit kidney (RK-13), monkey kidney (VERO), and equine kidney. Equine kidney was used for only 103 cases in 2001 and 93 cases in 2002.

In addition, tissues from 10 MRLS fetuses were also submitted to the NVSL for virus isolation. Those tissues were inoculated onto five tissue culture cell lines, into embryonating chicken eggs, and by the intracerebral route into 3- to 5-day-old mice.

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Results

Microbiological examinations were completed on 682 cases: 433 fetuses in 2001 and 249 in 2002. Results of bacteriological culture are reported in Table 1. Significant bacteria were recovered from 510 (74.8%) of the 682 fetuses. The most frequently isolated bacteria were non-beta-hemolytic streptococci, 353 (51.8%) of the fetuses, and actinobacilli, 98 (14.4%) of the fetuses. Eight of these fetuses yielded both bacteria. Tissues from 32 (4.7%) fetuses were overgrown by saprophytic microorganisms within 24 hours of incubation, which would mask the growth of significant bacteria. No significant bacteria were isolated from the tissues of 140 (20.5%) of the 682 fetuses.

Leptospires were not seen by FAT in any of the examined fetal tissues. Bacteria were seen by dark-field microscopy in the stomach content of many of the fetuses. The morphology of the bacteria seen correlated directly with the morphology of bacteria that were isolated from the fetuses.

Four (4 in 2001 and 0 in 2002) of the 682 fetuses were positive by FAT for EHV-1. In 2002, all 249 fetuses were negative by FAT for EVA; none were tested in 2001. Except for the four FAT-positive EHV-1 cases, no evidence of viral infection was observed in the cell cultures set up from the fetuses at the UKLDDC. From the 10 cases sent to the NVSL, there was no evidence of viral infection in cell cultures, eggs, or mice.

Discussion

Since there was no single or combination of tests that definitively diagnosed all cases of MRLS-related losses, the data in this study were from all the fetuses received during the two MRLS outbreaks. The common infectious causes of equine abortions (6,7), such as EHV-1, *S. zooepidemicus*, *E. coli*, and *Leptospira* spp., were recovered from only a few (<9.0%) of the fetuses received. The fetuses with these infections probably represented the normal fetal loss that would occur without the presence of MRLS. Most of the fetuses were infected with non-beta-hemolytic streptococci or actinobacilli. These bacteria have not often been associated with equine abortions. During a six-year study of 3,527 fetuses submitted to the UKLDDC, non-beta-hemolytic streptococci were isolated from only 26 fetuses and actinobacilli from 14 fetuses (6). A review of the records at the UKLDDC for the equine fetuses received between April 26 and May 31 in 1999 and 2000 revealed that only three were infected with actinobacilli and 11 with non-beta-hemolytic streptococci (Table 2). However, these bacteria were isolated from 433 (66%) of the 682 fetuses cultured during the MRLS epidemics. For the time period involved, the fetal loss was about 75 fetuses per year in 1999 and 2000 (Table 2), the two years preceding MRLS. If this number (75 per year) is used as normal fetal loss,

Table 1. Bacteriological findings on fetuses received in 2001 (433 fetuses) and 2002 (249 fetuses).

Bacterium Isolated	Number (%) of Fetuses		
	2001	**2002**	**Total**
Non-beta-hemolytic streptococci	223 (51.5)	112 (49.0)	245 (50.6)
Actinobacilli	74 (17.1)	16 (6.4)	90 (13.2)
Actinobacilli & non-beta-hemolytic streptococci	8 (1.8)	0	8 (1.2)
Escherichia coli	7 (1.6)	8 (3.2)	15 (2.2)
Pantoea agglomerans	4 (0.9)	6 (2.4)	10 (1.5)
Serratia marcescens	2 (0.5)	4 (1.6)	6 (0.9)
Aeromonas species	4 (0.9)	2 (0.8)	6 (0.9)
Enterobacter species	0	5 (2.0)	5 (0.7)
Acinetobacter species	4 (0.9)	0	4 (0.6)
Beta-hemolytic streptococco	2 (0.5)	2 (0.8)	4 (0.6)
Staphylococcus species	1 (0.2)	3 (1.2)	4 (0.6)
Other coliforms	4 (0.9)	2 (0.8)	6 (0.9)
Other bacteria	4 (0.9)	3 (1.2)	7 (1.0)
No significant bacteria	70 (16.6)	70 (28.1)	140 (20.5)
Overgrown by saprophytes	26 (6.2)	6 (2.4)	32 (4.7)
TOTALS:	433 (100)	249 (100)	682 (100)

then the actual number of fetuses that died due to MRLS for the two years was 532 (682 minus 150). Therefore, about 83% (443 of 532) of the fetuses from MRLS-related losses were infected with non-beta-hemolytic streptococci or actinobacilli. Also, bacteria with the morphology of streptococci or actinobacilli were seen by histopathological examination in the tissues of more than 80% of the LFL associated with MRLS (1).

Presently, only streptococci from 12 of the fetuses have been speciated, and all were *Streptococcus bovis*. Most (277) of the other streptococcal isolates were biochemically very reactive and similar, but not identical, to *Streptococcus mutans* (8). Studies are under way to identify these isolates using phenotypic and genotypic characteristics.

The species of *Actinobacillus* isolated from the fetuses remains to be determined. Recently, the classification of actinobacilli from horses was examined using phenotypic characterization and DNA-DNA hybridization (9,10). The species reported from horses were *A. equuli* subsp. *equuli* subsp. nov., *A. equuli* subsp. *haemolyticus* subsp. nov., *A. arthritidis*, Bisgaard taxon, and *Actinobacillus* genomospecies 1 and 2 (10,11). Studies are in progress to determine if the actinobacilli recovered from the fetuses associated with MRLS are identical to the species of *Actinobacillus* listed previously.

Conclusions

Based on the results obtained from the microbiological analysis of LFL submitted to the UKLDDC during the MRLS epidemics, the following conclusions were made. Viral infections, such as EHV-1 and EVA, that can be detected

Table 2. Isolation of non-beta-hemolytic streptococci and actinobacilli from fetuses: April 26-May 31, 1999-2002.

	Year			
Number of Fetuses	**1999**	**2000**	**2001**	**2002**
With streptococci	2	9	231*	112
With actinobacilli	0	3	82*	16
Without streptococci or actinobacilli	71	78	128	111

* Eight fetuses were infected with both non-beta-hemolytic streptococci and actinobacilli.

using common animal virological procedures (FAT, tissue culture, egg inoculation, and suckling mouse inoculation) were not involved in the MRLS epidemics. Bacteria such as *S. zooepidemicus*, *E. coli*, and *Leptospira* spp. that are common agents of equine abortions were not responsible for the MRLS epidemics. Non-beta-hemolytic streptococci or actinobacilli, two groups of bacteria not normally considered to be important causes of equine abortions, were recovered from most of the fetuses associated with MRLS. Their role in MRLS is unknown, but most of the pathological lesions observed could be attributed to infection by these bacteria.

References

1. Williams, N. M.; Bolin, D. C.; Donahue, J. M.; Giles, R. C.; Harrison, L. R.; Hong, C. B.; Poonacha, K. B.; Roberts, J. F.; Sebastian, M. M.; Swerczek, T. W., and Tramontin, R. R. Gross and histopathological correlates of MRLS. In: Powell, D. G.; Tobin, T., and Troppmann, A., editors. Proceedings: First Workshop on Mare Reproductive Loss Syndrome (MRLS); Lexington. Lexington: University of Kentucky, College of Agriculture; 2003.

2. Carter, G. R. and Cole, J. R. Diagnostic procedures in veterinary bacteriology and mycology. 5th ed. San Diego: Academic Press; 1990.

3. Murray, P. R. Manual of clinical microbiology. 7th ed. Washington, D.C.: ASM Press; 1999.

4. Donahue, J. M.; Smith, B. J.; Donahoe, J. K.; Rigsby, C. L.; Tramontin, R. R.; Poonacha, K. B., and Wilson, M. A. Prevalence and serovars of *Leptospira* involved in equine abortions in central Kentucky during the 1990 foaling season. Journal of Veterinary Diagnostic Investigation. 1992; 4(3):279-284.

5. Miller, D. A.; Wilson, M. A., and Kirkbride, C. A. Evaluation of multivalent leptospira fluorescent antibody conjugates for general diagnostic use. Journal of Veterinary Diagnostic Investigation. 1989; 1(2):146-149.

6. Giles, R. C.; Donahue, J. M.; Hong, C. B.; Tuttle, P. A.; Petrities-Murphy, M. B.; Poonacha, K. B.; Roberts, A. W.; Tramontin, R. R.; Smith, B., and Swerczek, T. W. Causes of abortion, stillbirth, and perinatal death in horses: 3527 cases (1986-1991). Journal of the American Veterinary Medical Association. 1993; 203(8):1170-1175.

7. Hong, C. B.; Donahue, J. M.; Giles, R. C.; Petrites-Murphy, M. B.; Poonacha, K. B.; Roberts, A. W.; Smith, B. J.; Tramontin, R. R.; Tuttle, P. A., and Swerczek, T. W. Equine abortion and stillbirth in central Kentucky during 1988 and 1989 foaling seasons. Journal of Veterinary Diagnostic Investigation. 1993; 5(4):560-566.

8. Ruoff, K. L.; Whiley, R. A., and Beighton, D. Streptococcus. In: Murray, P. R., editor. Manual of clinical microbiology. 7th ed. Washington, D.C.: ASM Press; 1999; pp. 283-296.

9. Christensen, H.; Bisgaard, M.; Angen, O., and Olsen, J. E. Final classification of Bisgaard taxon 9 as *Actinobacillus arthritidis* sp. nov. and recognition of a novel genomospecies for equine strains of *Actinobacillus lignieresii*. International Journal of Systematic and Evolutionary Microbiology. 2002; 52(4):1239-1246.

10. Christensen, H.; Bisgaard, M., and Olsen, J. E. Reclassification of equine isolates previously reported as *Actinobacillus equuli*, variants of *A. equuli*, *Actinobacillus suis* or Bisgaard taxon 11 and proposal of A. equuli subsp. equuli subsp. nov. and A. equuli subsp. *haemolyticus* subsp. nov. International Journal of Systematic and Evolutionary Microbiology. 2002; 52(5):1569-1576.

11. Bisgaard, M. Ecology and significance of *Pasteurellaceae* in animals. International Journal of Medical Microbiology. 1993; 279(1):7-26.

An Overview of Reproductive System Changes during and after Mare Reproductive Loss Syndrome

W. W. Zent

MARE REPRODUCTIVE LOSS SYNDROME (MRLS) CAUSED abortion in early pregnancy, and even though the abortion happened with two months remaining in the breeding season, only rarely were these mares able to get back in foal. Mares that aborted with MRLS often were contaminated with bacteria immediately after they aborted. The most common organisms isolated were non-beta hemolytic streptococcus and actinobacilli; however, in almost all instances, these organisms were quickly cleared from the reproductive tract, and the mare was free of the bacteria. The primary reason for these mares not becoming pregnant was their refusal to cycle normally, due to the presence of eCG (equine chorionic gonadotrophin) in their circulation. The eCG levels are maintained due to the presence of endometrial cups produced by the placenta before the pregnancy was lost.

Endometrial cups form between 34 and 38 days of gestation from specialized trophoblastic cells called the chorionic girdle. The chorionic girdle forms between the allantois and the yoke sac and begins to invade the uterine wall at about 30 days of gestation. Once invasion of the uterus is complete, the girdle cells lose their attachment to the trophoblast and become part of the uterine wall. They mature at about day 60 and slough between days 100 and 110. The endometrial cups derive their nourishment from the uterus, not the placenta, so that they will continue to function and produce eCG even in the absence of the placenta and fetus.

The secretion of eCG is first detectable at about day 40 and peaks at day 60. It is detectable in the circulation until days 120 to 150. The presence of eCG in the mare's circulation even after the sloughing of the endometrial cups is due to its long half-life (6 days) and its high levels in mare's plasma. It has luteinizing and follicle-stimulating activity and in non-equine species can be used as a follicle-stimulating drug. In the equine, its role is as a luteinizing agent. Secondary follicular development during early pregnancy is stimulated by follicle-stimulating hormone (FSH), which is secreted from the pituitary gland. There is evidence that this occurs in 10- to 11-day cycles and continues until mid-gestation. This FSH stimulation causes secondary follicular development, and these follicles are driven to luteinize by the circulating eCG. The corpora lutea formed by these follicles provide a secondary source of progesterone that lasts until mid-gestation when the placenta takes over progestin production.

The above process works well unless the fetus dies and an attempt is made to encourage the mare to return to estrus so that she can be rebred. Because of the presence of corpora lutea in varying stages of maturity and follicles that are also of different ages, the administration of a single injection of prostaglandin rarely will destroy all of the luteal tissue and allow the mare to return to estrus.

Attempts to rebred mares that have lost fetuses from any reason after the formation of endometrial cups have in general not been very successful. The occasional mare may become pregnant, but this is usually, in the author's opinion, due more to luck than skill. It is also important to be sure that the mare had functioning endometrial cups, with positive eCG blood levels. Some fetuses, due to an accident in development, will not form cups or at least the mare will not have circulating eCG, and these mares will usually return to cycling with very little intervention.

Several therapy regimens have been tried to encourage mares with endometrial cups to return to estrus. Multiple doses of prostaglandin will eventually destroy all of the luteal tissue, and if the mare has a functional follicle, they will return to estrus. Due to the presence of eCG, these mares have a tendency to very quickly luteinize these follicles and go out of heat without ovulating. Mares have been treated with progesterone and estradiol in an attempt to inhibit the pituitary from producing FSH and allow the ovaries to become more synchronized. This method allows all the corpora lutea to become prostaglandin sensitive at the same time and reduce the need for multiple doses of prostaglandin. This has met with some success, but the problem of eCG causing follicles to luteinize before ovulation still remains. Mares that lose their fetuses at a later stage in gestation, 80 days or more, are likely to have ovaries that become quiescent, and even though the corpora lutea are destroyed, these mares tend not to produce follicles. Their ovaries remain inactive even following attempts at stimulation. Products such as natural gonadotropin-releasing hormone (GnRH), deslorelin, sulpiride, and domperidone have all been tried with very little documented success.

Most mares with functional endometrial cups that have lost fetuses will remain in this cyclic state until about 150

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days post-conception, at which time many will return to normal cyclic behavior. Several of the mares that suffered from MRLS during the 2001 northern hemisphere breeding season were bred to the southern hemisphere season and readily became pregnant. The mares that lost pregnancies to MRLS in 2001 behaved normally at the start of the 2002 breeding season.

Suggested Reading

Gunther, O. J. Reproductive biology of the mare: basic and applied aspects. 2nd ed. Cross Plains: Equiservices; 1992.

McKinnon, A. O. and Voss, J. L., editors. Equine reproduction. Philadelphia: Lea and Febiger; 1993.

Summary

K. Benirschke

THANK YOU FIRST OF ALL FOR INVITING ME TO COME TO Kentucky again. I was here many years ago, and I enjoyed it then and am enjoying it now very much.

I'm supposed to make some sensible remarks, and they may not be sensible, but they're out of the box. What this problem reminds me of very much is that in the 1960s we had the same hand-wringing in human pathology—I'm a human pathologist—about the occurrence of amelia, or absence of arms, fingers, and feet in humans. It was during the thalidomide epidemic, if you all recall, that started in Germany from a drug that was thought to be absolutely harmless, Contergan. In tests in animals, the drug was harmless. This is the reason I have totally different ideas about this process. Lenz, a German pediatrician and the son of a geneticist, visited patients with flipper arms in Hamburg, Hannover, and Cologne. Such cases had never been reported previously, but he concluded that the cause was of environmental origin.

How does all this fit together? This epidemic is just like the flipper arms, which in one significant experiment in a pregnant rhesus monkey was shown to be due to a single exposure on a specific day. One pill caused flipper arms in a rhesus monkey. You could poison rabbits and rats with Contergan, and it caused no problems, except in one rhesus monkey. So can I—with an interest in the human placenta—add anything to your discussion of the fetal loss syndrome that you experience in mares?

Placentation is very different in the mare from that of primates. Ascending infection of the placenta is the most important disease that obstetricians in human biology have to contend with. It is the principal cause of cerebral palsy and of premature birth. We have eliminated hyaline membrane disease and many other diseases by prenatal or neonatal care. Many babies born prematurely at 20 to 25 weeks that develop brain hemorrhages are the result of chorioamnionitis because of infection *in utero*. They aspirate *in utero* and swallow infected amnionic fluid containing organisms including ureaplasma and Chlamydia and other agents that enter via the cervix into the amnionic cavity. One exception as a result of maternal septicemia is *Listeria monocytogenes,* which causes abscesses in the placenta. I cannot believe the bacteria you find in the foals can get there any other way than by ascending infection. You've got to find out how that comes about.

I have had the privilege of seeing sections that Dr. Sebastian sent me of cases, and there are some significant differences from the pathology we see in human placental chorioamnionitis. One is that you see leukocytes on the umbilical cord surface, but you hardly ever see any in the umbilical vessels themselves. In humans, we see a large number of vessels packed with leukocytes. In the equine fetuses, aspirated bacteria are present in the stomach and the lungs, but that is secondary. They entered, I assume, through the cervical star, and I couldn't agree with you more that you have to examine the cervical star much more carefully for bacteria.

Even though it is often hypothesized by obstetricians that it is a prolonged rupture of the membranes in humans that causes the chorioamnionitis, in very many cases there is no rupture of the membranes, and bacteria just penetrate the membranes and invade the amnionic sac.

Human obstetricians refer to abruptio placentae cases in prematurely delivered babies with amnionitis and chorioamnionitis. Abruptio is detachment of the placenta from the uterine wall. In these cases, it is a separation at the insertion site of the membranes at the lower pole of the uterus. It is not truly a retro-placental hemorrhage. From what I've seen of the placentas that Dr. Sebastian has sent me, I do not think that the "red bag" syndrome is the cause of fetal death, nor of the problem. I think it's the end result of a very expulsive phenomenon.

We see a lot of intrauterine chorioamnionitis in humans, for many different organism reasons, including streptococci infection from which babies rarely die *in utero*. It is uncommon early in pregnancy; it's usually halfway

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through pregnancy. The babies are born prematurely, they may have sepsis and are taken care of by neonatalogists, but they don't die *in utero*. It seems to me that many of the foals have died *in utero* sometime earlier, and we need to find out if it's a toxin and what kind of toxin it is.

Why do babies die *in utero*? It reminds me of an old experience. Babies die *in utero* often when their mothers are diabetic. When the sick foals are born, they're hypoglycemic, in shock, and cold. This is also true of diabetic mothers' newborns—they are immediately, severely hypoglycemic. In order to prevent neonatal death of diabetic mothers in pregnancy and delivery, doctors in Boston years ago performed Caesarean sections so the babies wouldn't die in the last few weeks of pregnancy. Most of the placentas of diabetic mothers that I now see are induced or Caesarean sections a week and a half or so prior

to their expected date of delivery because of the fear of intrauterine death. I believe there is some good correlative evidence for this in the foals—I'm thinking out of the box—that this is due to hypoglycemia *in utero*. The foal doesn't have enough to eat, so to speak, because changes in the pancreas reduce the blood sugar.

A toxin may cause the demise of glucagon-producing cells in the pancreas resulting in death of the foal or fetus. There may be subtle toxins as in the thalidomide cases. The children were perfectly normal; they just had significant reductions in the growth of the extremities. The effects may be on one organ system like the islets of Langerhans. I say this because we see a lot of newborns with islets that show beta-cell hyperplasia because of the tremendous increase in obesity.