

## Experimentally Induced Mare Reproductive Loss Syndrome Late Fetal Losses with Eastern Tent Caterpillars

M. Sebastian, D. Williams, L. Harrison, J. Donahue, T. Seaborn, N. Slovis,  
D. Richter, T. Fuller, C. Trail, R. Douglas, and T. Tobin

DURING 2001, CENTRAL KENTUCKY HAD AN EPIDEMIC OF early and late fetal losses (EFL/LFL), which was together called Mare Reproductive Loss Syndrome (MRLS). The LFL began in the last week of April, peaked on May 5, and declined rapidly. EFL was identified on April 26 and had a similar course and ultimately totaled about 1,500 cases. A total of 450 LFL cases were submitted to the University of Kentucky Livestock Disease Diagnostic Center (UKLDDC) for detailed pathological examination during a period of two months (1). Concurrent with the epidemic was a local population explosion of eastern tent caterpillar (ETC), *Malacosoma americanum*.

Experiments conducted by Webb et al. (2) and Bernard et al. (3) have established a causal role for ETC in EFL. The necropsy examination of LFL demonstrated lesions not observed in EFL and bacteriological findings that can be compared to EFL. If reproduced experimentally, these observations will help in defining the specific pathogenesis, etiological agent, and clinical picture. Hence, a study was undertaken to evaluate the ability of ETC to reproduce abortion in late and midterm pregnancies.

### Materials and Methods

Eleven pregnant mares obtained from a commercial nurse mare operation in late stage of pregnancy (9 to 11 months of pregnancy) were selected for the experiment. Mares were divided into two groups, six in the treatment group and five in the control group. Mares in the treatment group were administered 50 g of ETC collected from the Upper Peninsula of Michigan fed on wild cherry tree leaves mixed in 70 ml of normal saline for 9 days. The control group mares received 120 ml of normal saline for 9 days. All of the mares were confined to the stall during the entire period of experiment. They were fed hay and had constant access to clean drinking water. Mares were walked once daily to provide exercise. Blood was collected from the jugular vein 5 days prior to experiment and all 9 days of the experiment and every other day after the experiment for 10 days. Complete blood count and biochemical assays were performed within 2 to 3 hours of collection with an auto analyzer. Blood was collected aseptically from all the mares prior to experiment and also during the entire period of 9 days of experiment for bacterial culture. The serum concentration of progesterone and estrogen were estimated on the day before the experiment and on the day of abortion.

All the mares were monitored prior to the experiment by rectal ultrasonography and on days 3, 5, 7, and 9 by transabdominal ultrasonography for fetal heart rate, placental thickness, and appearance of fetal fluid.

### Results

Five of the treatment mares aborted during the experiment, and the sixth mare aborted on day 15 from the first day of exposure. A complete necropsy was done on all aborted fetuses and placentas. A uterine biopsy was taken from all the aborted mares on the day of abortion. FATs were done on pooled tissue samples of all fetuses for *Leptospira* species and EHV. The first abortion occurred between 56 to 69 hours, and the last abortion at 357 hours on the fifteenth day. All the placentas had intact cervical stars, and two had tears of the allantochorion. The weight of the placentas ranged from 5 to 10 pounds. The length of the umbilical cords ranged from 55 to 82 cm. All the placentas' chorionic surface had a pale brown color compared to the dark red color seen on the chorionic surface of fresh placentas. The weight of the fetus ranged from 35 pounds to 82 pounds.

*Enterobacter sakazakii* (two fetuses), *Serratia marcescens*, and *Enterococcus* species (two fetuses) and *Enterobacter cloacae* (two fetuses) were isolated from multiple organs of the aborted fetuses. There was no significant difference in the complete blood count, serum biochemistry, coagulation factors, and ammonia levels between the treatment group and the control group. Detailed histopathological examination of the fetus and placenta showed hemorrhage and congestion of adrenals (6 of 6 fetuses), congestion of liver (5 of 6 fetuses), congestion of kidney (5 of 6 fetuses), hemorrhage in epicardium (3 of 6 fetuses), congestion of thymus (6 of 6 fetuses), congestion of spleen (5 of 6 fetuses), endometritis (6 of 6 mares), bronchopneumonia (1 of 6 fetuses), hepatic necrosis (1 of 6 fetuses), placentitis (1 of 6 fetuses), amnionitis (1 of 6 fetuses), and

Sebastian, Williams, Harrison, Donahue, Trail: Livestock Disease Diagnostic Center, College of Agriculture, University of Kentucky, Lexington, Kentucky. Seaborn, Slovis: Hagyard-Davidson-McGee Associates, Lexington, Kentucky. Richter: School of Forestry and Wood Products, Michigan Technological University, Houghton, Michigan. Fuller, Tobin: Maxwell H. Gluck Equine Research Center, College of Agriculture, University of Kentucky, Lexington, Kentucky. Douglas: Bluegrass Embryo Transplants, Lexington, Kentucky.

funisitis (1 of 6 fetuses). The fetal heart rate was increased in all fetuses on days prior to the day of abortion. FATs done on the pooled tissue samples of all aborted fetuses were negative for EHV and *Leptospira* species. Blood culture for bacteria yielded no bacteria during the entire period of experiment. There were no significant observations in the fetal fluid or placental thickness. All the fetuses showed significant increase in fetal heart rate before they aborted or were diagnosed dead *in utero*. The serum concentration of progesterone from the day before exposure of caterpillars, when compared to the day of abortion, had a drastic drop in concentration. The serum concentration of estrogen on the day of abortion was significantly lower than the day before the exposure of caterpillars.

### Discussion

One consistent finding for all abortions was an intact cervical star similar to the gross finding in naturally occurring cases of LFL. Also, the chorionic surface of 4 of 6 allantochorions had a light brown yellow appearance, indicating placental detachment that remained *in utero* for sufficient time to cause fetal death. The ultrasound examination of the fetal heart rates showed an increase in heart rate indicating the fetuses may have been under stress due to hypoxia. Placenta detachment may have occurred leading to physiological effects consistent with fetal hypoxia. The fetus of the mare that aborted on day 15 had fetal diarrhea, which likewise underscores a possible connection to fetal hypoxia. Congestion and hemorrhage noted in fetal tissues may also be seen as indication that fetal hypoxia is a factor in the pathogenesis of MRLS. The fetus of the mare aborted on day 15 had fetal diarrhea and funisitis similar to the fetuses in naturally occurring cases of LFL (4). Enterobacter species, *Serratia marcescens*, and Enterococcus species bacteria isolated in these cases were also isolated in naturally occurring cases of LFL (5).

The dose of ETC used for this experiment was based on the dose used in the EFL experiments (2,3). Abortion occurred earlier in this experiment as compared to the EFL experiment by Bernard et al. (3) in which the first abortion occurred on day 8. The first abortion in this study was observed at 69 hours post administration of ETC. The apparent difference in the early onset of abortion may be due to ETC in this experiment being fed fresh cherry tree leaves until the day of preparation, while in the EFL study by Bernard et al. (3), the ETC had been kept away from cherry tree leaves several days prior to preparation and administration. This difference in the onset of abortion/fetal death suggests that the caterpillars fed cherry tree leaves have more abortifacient potential when compared to starved caterpillars. Another fact to consider is that metabolically active ETC may be producing an abortifacient agent in much higher quantities compared to starved ETC.

### Conclusion

This experiment indicates that dosing late-term pregnant mares with a preparation of ETC that had consumed wild cherry trees leaves causes abortion consistent with MRLS. Caterpillars were collected from the Upper Peninsula of Michigan and were shipped to Lexington for the experiment, indicating that ETC from other states have the potential to induce LFL in horses.

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