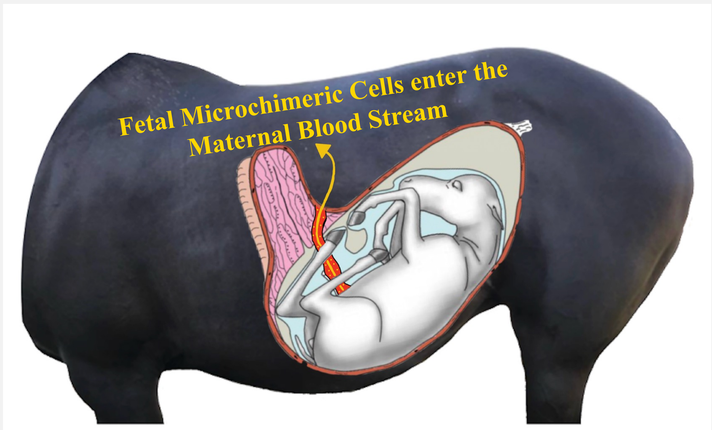
Fetal microchimerism and its presence in the equine hoof lamellar interface in health and laminitis

Veterinary Research Scholars Program
University of Missouri

Jessica R. Warwick, Angelynn P. Simenson, Julia M. Baldrighi (co-sponsor), Jeffrey N. Bryan (co-sponsor), Philip J. Johnson (co-sponsor)
Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO

Background



Fetal microchimerism is the presence of a small number of genetically distinct cells in an individual that originated from another individual.

Fetal microchimerism has been demonstrated in the human, canine, bovine and ovine species.

Fetal cells with male DNA can be traced using primers designed for the equine SRY gene.

Objectives

- **Nested polymerase chain reaction (PCR) analysis of equine whole blood to affirm equine fetal microchimerism**
- **Fluorescence *in situ* hybridization (FISH) analysis of archived paraffin-embedded formalin fixed equine hoof lamellar tissue (healthy and laminitic) to identify Y-chromosome positive microchimeric cells.**

Hypotheses

Nested PCR assays will identify the presence of male fetal DNA circulating in parous/multiparous mares who have given birth to male offspring.

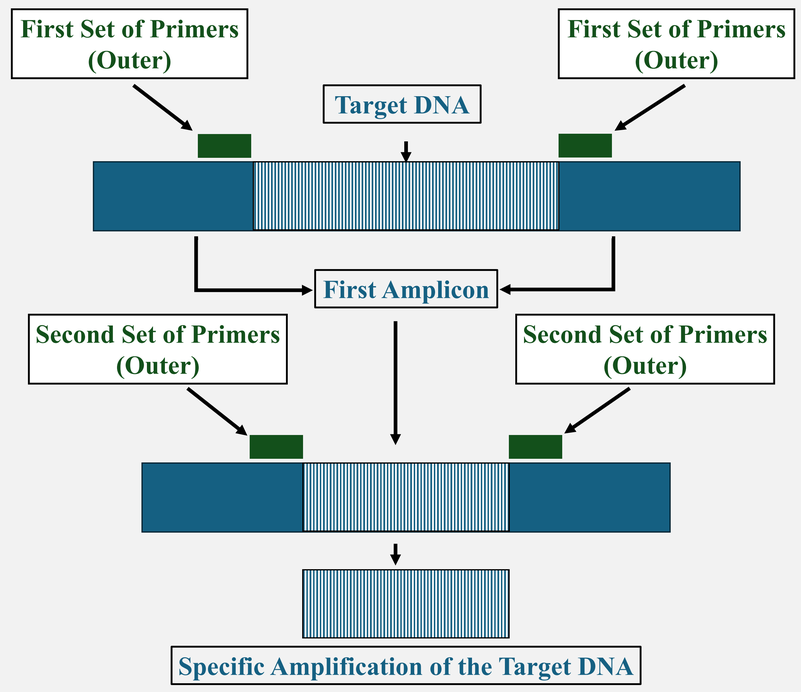
FISH analysis will identify Y chromosome-positive microchimeric cells in the hoof lamellar interface in normal and laminitic conditions.

Methods

Fresh whole blood samples were collected from males (geldings), nulliparous mares, and parous/multiparous mares.

DNA was extracted from blood samples via DNeasy Blood and Tissue kit (Qiagen CA).

Nested PCR Analysis



DNA from males (geldings) was used as positive controls.

DNA from nulliparous mares and water were used as negative controls.

Strict room isolation was performed to prevent contamination of test samples by male positive controls.

Polymerase Chain Reactions

Figure 1.

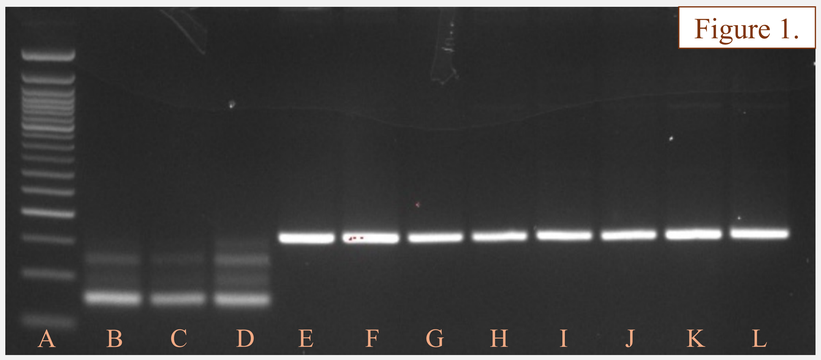


Figure 1. Lane A, 50 bp ladder. Lanes B-D, nested PCR with DNA template concentrations of 200 ng, 250 ng and 300 ng, respectively. Lanes E-L, male/female blood dilution of PCR 1.

Figure 2.

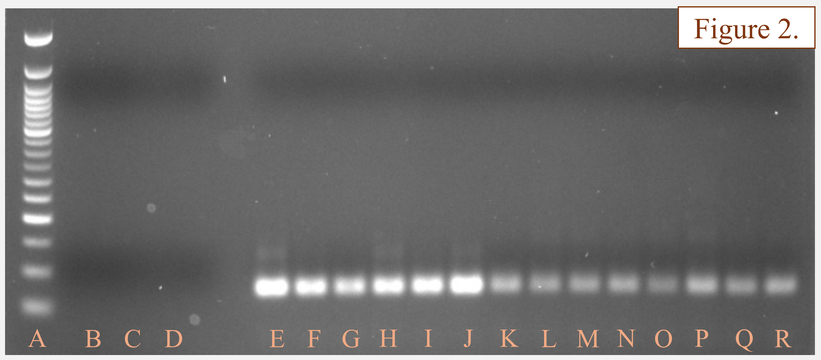


Figure 2. Lane A, 50 bp ladder. Lanes B-D, failed nested PCR using only 1 µl of DNA template. Lanes E-J, cleaned dilution PCR product ran via nested PCR. Lanes K-R, cleaned dilution gel bands ran via nested PCR.

Figure 3.

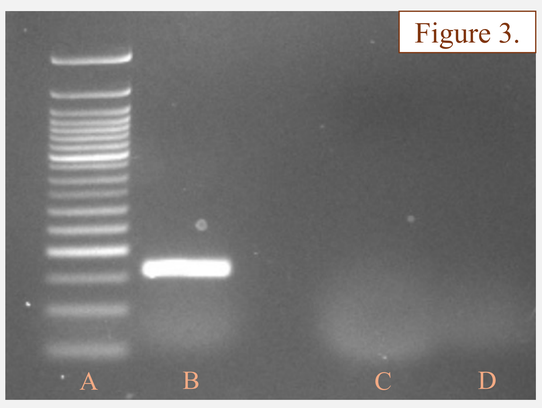


Figure 3. Lane A, 50 bp ladder. Lane B, positive control male sample. Lanes C and D, negative control female samples.

Figure 4.

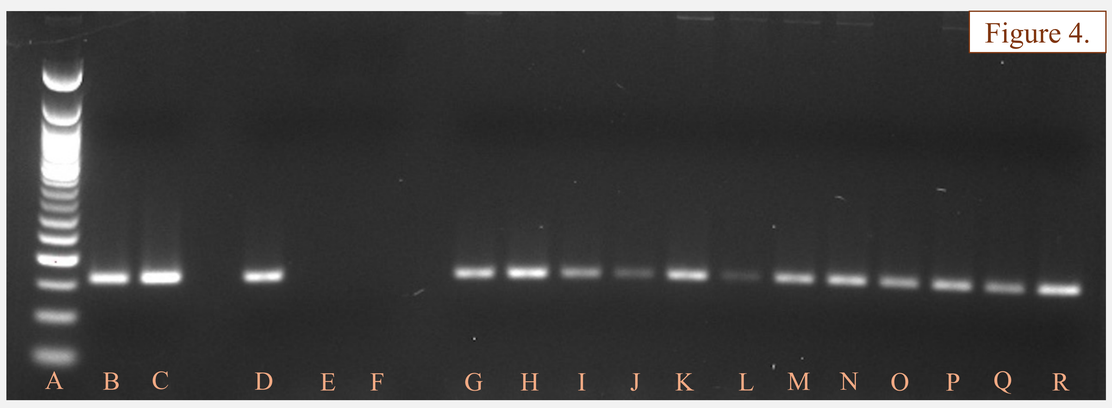


Figure 4. Lane A, 50 bp ladder. Lanes B and C, positive male controls. Lane D, negative water control. Lane D tested positive which indicates contamination of the water or other PCR products. Lanes E and F, negative female controls. Lanes G-R, test parous/multiparous female samples.

Future Goals

- **Ongoing troubleshooting to eliminate contamination in water lane.**
- **FISH analysis on archived paraffin-embedded formalin hoof lamellar tissue from healthy and laminitic horses.**

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