Detecting fetal microchimerism in the mare

Veterinary Research Scholars Program
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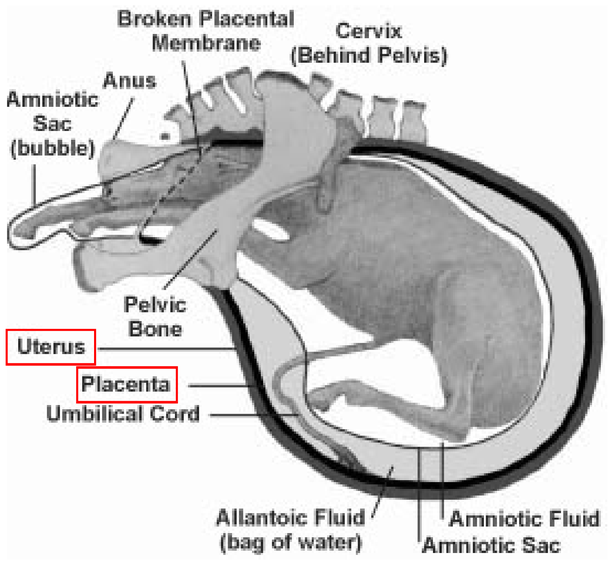
What is fetal microchimerism (FMC)?

- Trophoblasts are the cells that make up the outer layer of the chorion (placenta)
- As the fetus grows, more of the chorion comes into contact with the uterine lining of the mare
- At this contact, some trophoblasts will detach and migrate into the maternal bloodstream
- It is hypothesized that the trophoblasts can then de-differentiate into progenitor-like stem cells
- These cells can be harbored in maternal organs or circulate in the bloodstream



Background

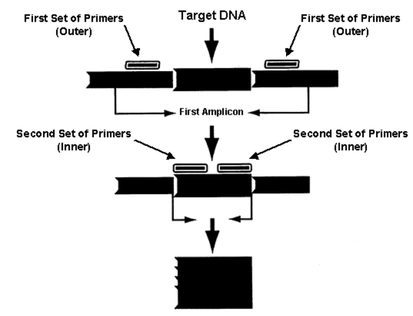
- Fetal microchimerism has been found in humans, dogs, and cows
- Previously, our lab identified a 36% microchimerism rate in dogs with FMC persisting in the mother for up to 96 months post-partum (1,2)
- Can trace fetal cells with male DNA by designing primers for a section of the male Y-chromosome
- Goals of the project:
  - optimize a detection protocol
  - test sensitivity of assay to detect fetal microchimerism during and after pregnancy
- May be useful in the breeding industry with early fetal sexing

Hypothesis

We hypothesize that a nested polymerase chain reaction (PCR) assay can be used to identify the presence of male fetal DNA circulating in mare maternal blood both prior to and post-partum.

Methods

- Banked samples from 2014 used for initial study
- Fresh samples collected to broaden data set
- DNeasy Blood and Tissue kit (Qiagen CA) was used to extract DNA from buffy coat of collected blood
- Two sets of previously generated primers were used to complete a nested PCR reaction
- Nested PCR, as demonstrated in the diagram below, is used to increase sensitivity and decrease false positive results from contamination



Specific Amplification of the Target DNA

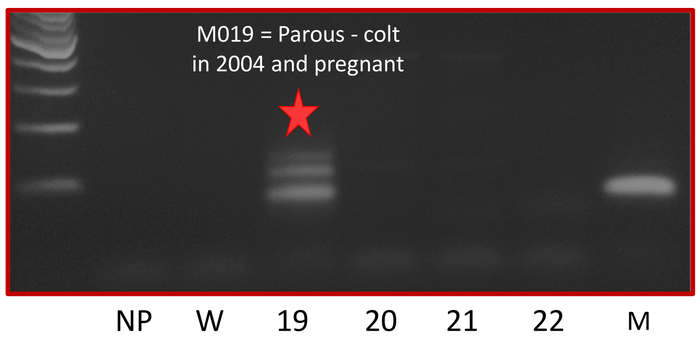
- DNA from virgin mares and water was used as negative controls
- DNA from male horses was used as positive control
- Strict room isolation to prevent male positive control contamination of test samples
- PCR master mix was prepared and aliquoted in separate room – negative controls and test samples added and PCR tubes were sealed
- Male DNA added and PCR was run in main lab

Results

NP = Nulliparous (-) Control, W = Water, Gel A-E = Banked Sample, Gel F = Fresh Sample, ★ = Positive Result

A.

B.



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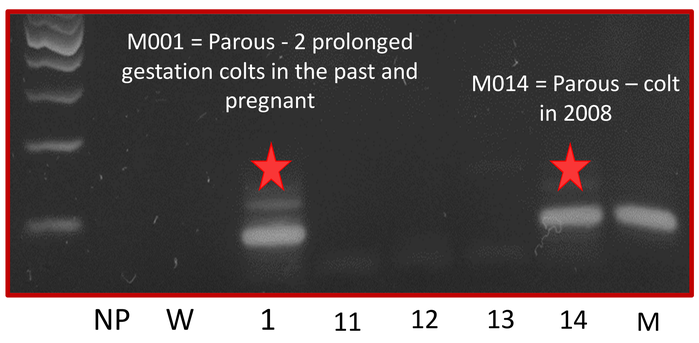

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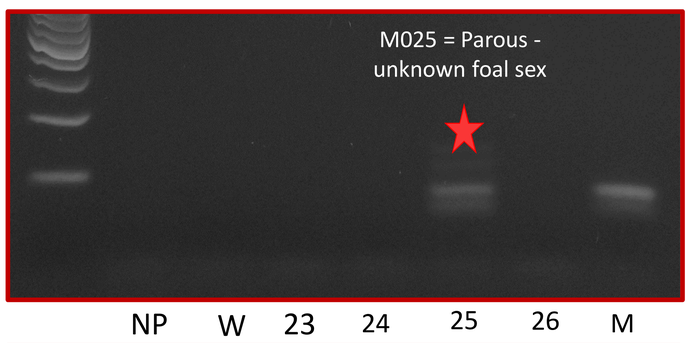

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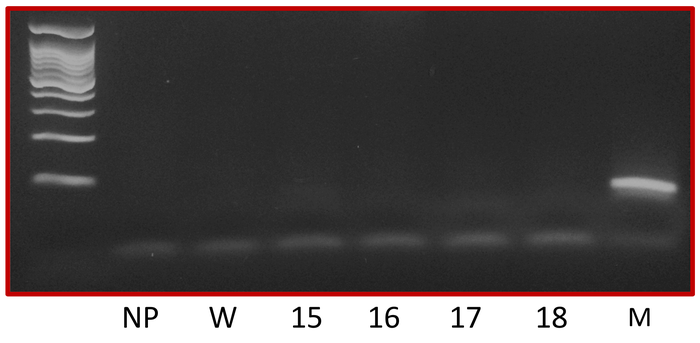

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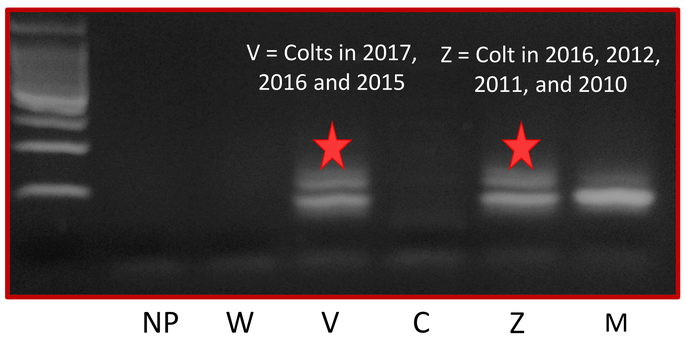

Conclusions

- Banked Samples: 5 out of 22 (22.7%) samples yielded bands for Y chromosomal DNA
  - 4 out of 8 (50%) mares with previous colts show FMC – other samples were abortions or fillies
- Fresh Samples: more detailed foaling records allowed better analysis
  - FMC found in 2/3 (66%) of parous mares with colts in the past
  - Mare that did not show FMC was embryo transfer recipient for colt
- Future Directions: More pregnancy samples of varying gestation lengths & more detailed foaling histories

Special thanks to the MU Equine Teaching Facility for sample acquisition

References

1. Bryan JN. Fetal Microchimerism in Cancer Protection and Promotion: Current Understanding in Dogs and the Implications for Human Health. *AAPS Journal* 2015;17(3):506-512.
2. SM Axiak-Bechtel et al. Y-chromosome DNA is present in the blood of female dogs suggesting the presence of fetal microchimerism. *PLOS One* 2013;8(7):e68114.