
Microbiome of the mares' genital tract: from research to practice
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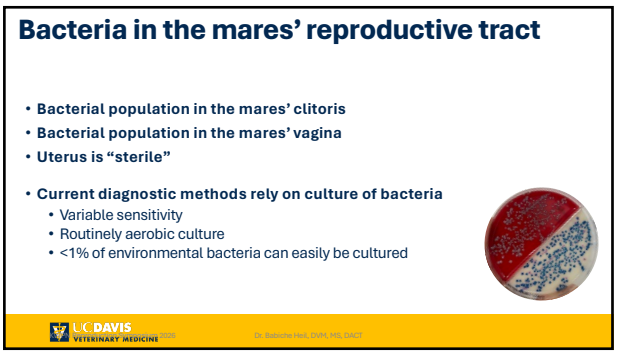
Bacteria in the mares' reproductive tract
• Bacterial population in the mares' clitoris
• Bacterial population in the mares' vagina
• Uterus is "sterile"
• Current diagnostic methods rely on culture of bacteria
• Variable sensitivity
• Routinely aerobic culture
• <1% of environmental bacteria can easily be cultured
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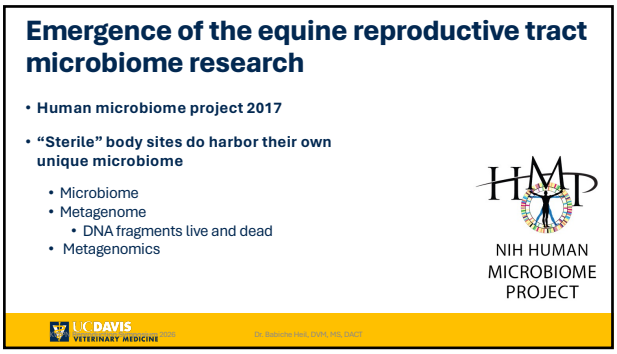
Emergence of the equine reproductive tract microbiome research
• Human microbiome project 2017
• "Sterile" body sites do harbor their own unique microbiome
• Microbiome
• Metagenome
• DNA fragments live and dead
• Metagenomics
NIH HUMAN MICROBIOME PROJECT
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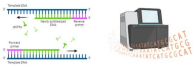
## Culture versus 16S Sequencing

**Culture**

- Bacteriology combined with cytology
- Only life bacteria identified
- Bacteria identified depend on culture conditions

**Sequencing**

- PCR amplification of 16S rRNA
- Both live and dead/fragmented DNA detected
- Many organisms identified that were previously undetected



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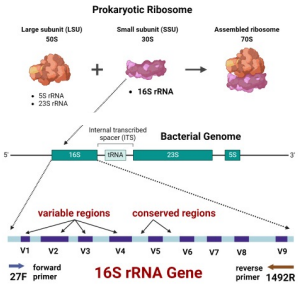
## What is 16S rRNA gene?

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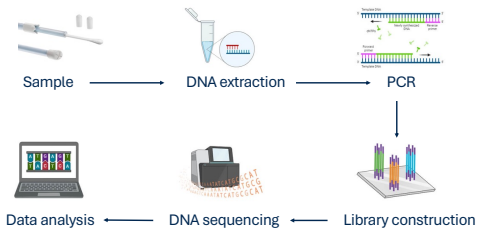
## What is 16S rRNA Gene Sequencing?

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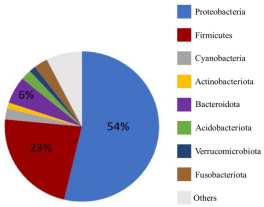
## Microbiome terminology - relative abundance

- What percentage of the microbiome is made up of a specific organism




Heil et al., *Veterinary Sciences* 2023

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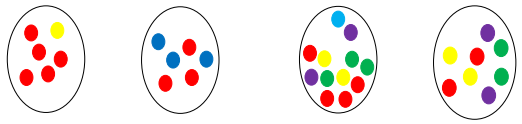
## Alpha diversity - richness and evenness of the sample

Low Richness, Low evenness

Low Richness High evenness

High richness Low evenness

High richness High evenness



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## Beta diversity - similarity between samples

Low beta diversity

High beta diversity

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## Evidence of a microbiome in the reproductive tract

- Evidence of presence of microbiome across multiple species and reproductive sites
- Variations of taxa and communities between populations

Firmicutes ● Bacteroidetes ●
Tenericutes ● Fusobacteria ●
Proteobacteria ● Actinobacteria ●

| | Vaginal | Uterine | Placental |
|---|---|---|---|
| Human | ● | ● ● ● | ● ● ● ● ● |
| Equine | ● ● ● ● | ● ● ● ● | ● ● ● ● |
| Giant panda | ● ● ● | ● ● | unknown |
| Primates | ● ● ● ● ● | unknown | unknown |
| Bovine | ● ● ● ● ● ● | ● ● ● | unknown |
| Ovine | ● ● ● | | unknown |
| Canine | ● ● ● | ● ● ● | unknown |

Heil et al., *Physiological genomics* 2019

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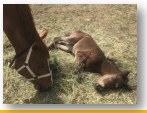
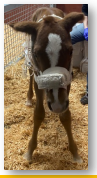
## Role of the microbiome in the equine reproductive tract?

- Contribution colonization reproductive tract to healthy immune state of the reproductive tract?
- Change in microbiome useful indicator of pregnancy outcome?



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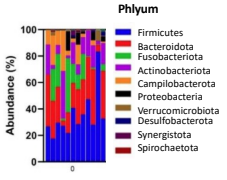
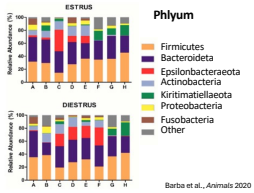
## Lower reproductive tract microbiome

### Clitoral microbiome in estrus




Herzog et al., *Veterinary Sciences* 2025

### Vaginal microbiome




Barba et al., *Animals* 2020

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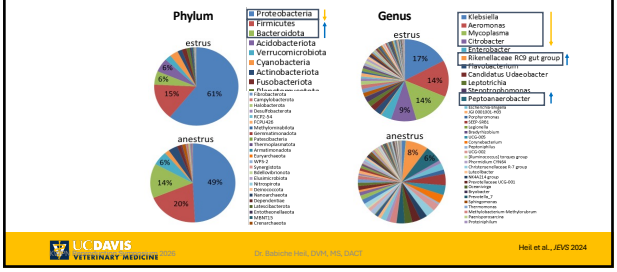
## Endometrial microbiome lower in richness and diversity in estrus




Heil et al., *JEVS* 2024

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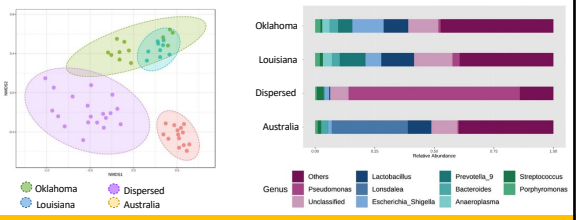
## Practical problem: uterine microbiome might differ by geographical location

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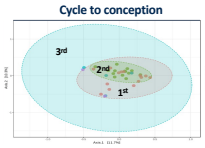
## Correlation endometrial microbiome and fertility in TB mares




| Category | # of Mares | Percent |
|---|---|---|
| First | 15 | 45.4% |
| Second | 13 | 39.4% |
| Third | 3 | 9.1% |
| Unknown | 2 | 6.1% |

- Mares sampled at the start of the breeding season that failed to become pregnant had a higher uterine microbial abundance.

Krekeler et al., *JEVS*, 2023

Sones, unpublished data

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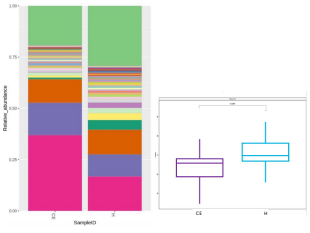
## Correlation endometrial microbiome and chronic endometritis



- Uterus microbiome of healthy mare has higher richness and evenness compared to mares with chronic endometritis.

Guo et al., *Frontiers*, 2025

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## Correlation endometrial microbiome and fertility

**Is high richness and low abundance the key to fertility?**

- Mares sampled at the start of the breeding season that failed to become pregnant had a higher uterine microbial abundance.

Krekeler et al., *JEVS*, 2023

- Uterus microbiome of healthy mare has higher richness and evenness compared to mares with chronic endometritis.

Guo et al., *Frontiers*, 2025

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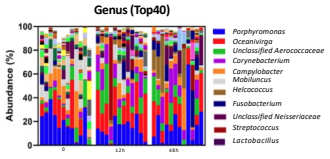
## Can we easily alter the lower reproductive tract microbiome in the healthy mare?

- Persistence of Lactobacillus at 12-hours post application, return to baseline at 48 hours. Dynamic alterations of relative abundance of top phyla.

**Clitoral microbiome in estrus before and after probiotic application**




Hertzog et al., *Veterinary Sciences* 2025

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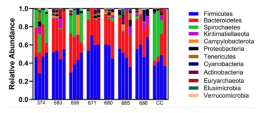
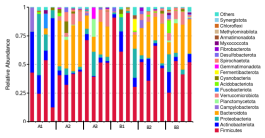
## Can we easily alter the lower reproductive tract microbiome in the healthy mare?

- Ceftiofur does not significantly alter the uterine microbiome in the healthy uterus.
- Hypochlorous acid does not significantly alter the uterine microbiome in the healthy.




Beckers et al., *Veterinary Sciences* 2025




Heil, unpublished data



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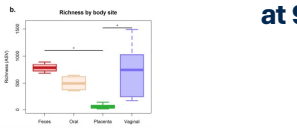
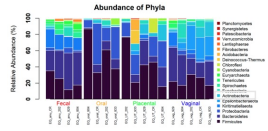
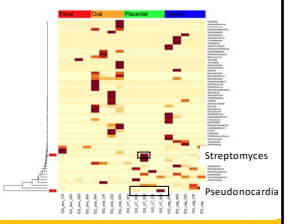
## Healthy equine placenta has low richness at 90-120 days gestation




Beckers et al., *Animals*, 2023



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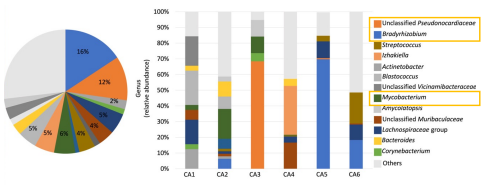
## Healthy equine placenta has low richness at 280 days gestation




van Heule et al., *Theriogenology*, 2023



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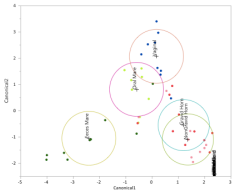
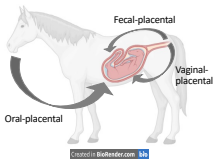
## Equine placental microbiome is closely related to oral and vaginal microbiome






Xia et al., *SFT abstract*, 2017

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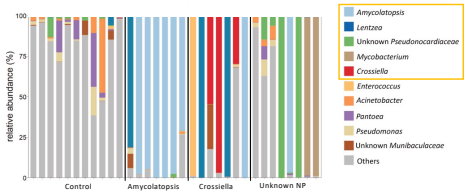
## Nocardioform placentitis is more than nocardioform bacteria




Van Heule et al., *Theriogenology*, 2024

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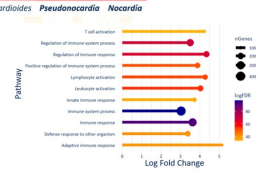
## Correlation microbial activity and placental inflammation genes

| Correlation (p value) | 0.49 (0.005) | -0.083 (0.7) | 0.24 (0.2) | 0.44 (0.01) | 0.35 (0.06) | 0.47 (0.008) | 0.43 (0.02) |
|---|---|---|---|---|---|---|---|
| | ***Amycolatopsis*** | *Crossiella* | *Streptomyces* | ***Mycobacterium*** | *Nocardioides* | ***Pseudonocardia*** | ***Nocardia*** |

- Higher activity *Amycolatopsis*, *Mycobacterium*, *Pseudonocardia* and *Nocardia* are positively correlated with genes associated with inflammation in the placenta.




Van Heule et al., *Equine Veterinary Journal*, 2025

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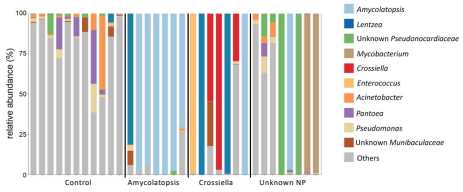
## Mucoid placentitis is more than nocardioform bacteria




Van Heule et al., *Theriogenology*, 2024

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## Is risk for developing ascending placentitis related to vaginal microbiome?

- 60 TB broodmares on one farm
- Vaginal samples at 45- and 120-days gestation
- Ultrasonographic and post foaling placental evaluations
  - Normal placenta group
  - Abnormal placenta group
  - Placentitis group

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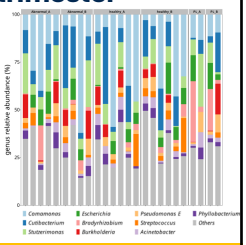
## Similar microbial diversity healthy vs abnormal placenta in first trimester

- **Abnormal placenta 2nd trimester:**
  - *Pseudomonas sihuiensis* higher
- **Abnormal placenta 1st and 2nd trimester:**
  - *Actinomyces naeslundii*
  - *Actinobacter heamolyticus*
  - *Haemophilus parainfluenzae*
  - *Ralstonia insidiosa*

  } lower




Van Heule et al., *Under review*

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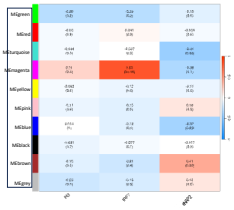
## Correlation vaginal gene expression and vaginal microbiome

- Presence of *Pseudomonas* and *Streptococcus* associated with vaginal genes *PI3, IRF7* and *eNAP2*
  - ***PI3:*** anti-viral, -fungal and immunomodulatory function, also upregulated in ascending and nocardioform placentitis
  - ***IRF7:*** critical for innate immune response to bacterial infection
  - ***eNAP2:*** role in neutrophil-mediated antimicrobial defence



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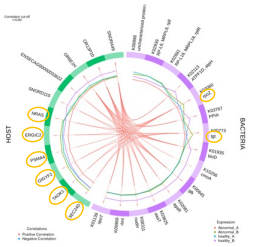
## Correlated vaginal and bacterial gene expression

- **Abnormal placenta 2nd trimester**
  - **6 host genes** (inflammation and apoptosis markers)
  - **2 bacterial genes** (associated with virulence)

elevated

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## Is risk for developing ascending placentitis related to vaginal microbiome?

- **Bacterial gene expression *rpoZ* and *tgt* influences vaginal gene expression altering vaginal environment**
  - Creation of condition allowing ascension of bacteria through vagina and cervix into placenta?
  - Microbe-host interactions in vagina rather than broad community shifts predispose to ascending placentitis?

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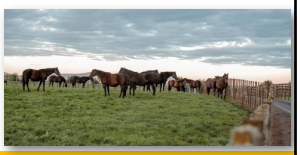
## Conclusions

- There are good bacteria in the mares' reproductive tract
- Presence of live and dead bacteria impact uterine environment
- Prediction of risk of developing disease is ongoing research
- Rapid DNA test are not yet available but will come



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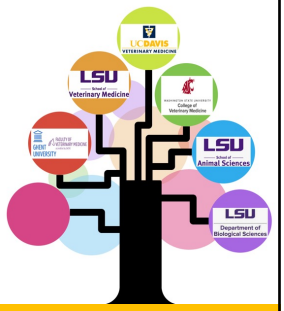
## Acknowledgements

- Dr. Jenny Sones
- Dr. Pouya Dini
- Dr. Machteld van Heule
- Dr. Kalie Beckers
- Dr. Fiona Herzog
- Dr. Erin Oberhaus

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## Questions?

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