Recent updates in the management of the problem mare

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Topics

- Basic reminders on overall health
- Uterine treatments
  - Biofilm busters
  - Settle
  - PRP
- Misoprostol

What do we need to focus on?

- Age
- Inflammation
- Weight
- Diet
- Lifestyle

Effect of Diet on Reproduction

- Continuous forage associated with increases in fertility and decreased estrus abnormalities (Benhajali 2013)
- Sugar and starch increases systemic IR when compared to forage only or diet rich in fat and fiber (increased effect) with higher BCS (Hoffman 2003)
- Regardless of diet, the endometrium of obese mares had increased expression of pro-inflammatory cytokines and affected gene transcription in the embryo (Carnevale 2018)

Effect of Diet on Reproduction

Omega-3 fatty acid supplementation

- Increases oocyte quality
  - 20 year old mares fed hay and balanced diet->23% transfer rate
  - After 8-16 weeks of DHA supplementation->51% transfer rate (Hembrooke 2018)
- Decreased inflammatory cytokines (TNFα) in the mares serum by 51% (Hembrooke 2018)
- Alters gene expression in the uterus and conceptus following supplementation (Jacobs 2018)
- Allows oocytes of older mares (13-20) to mature and metabolize energy more efficiently (Catandi 2019)
- More fertilized eggs developed into blastocysts from the mares consuming the omega-3 fatty acid supplement (58%) than from the grain and corn oil supplement group (15%) (Catandi 2019)

Reproductive Effects of Insulin Resistance (EMS)

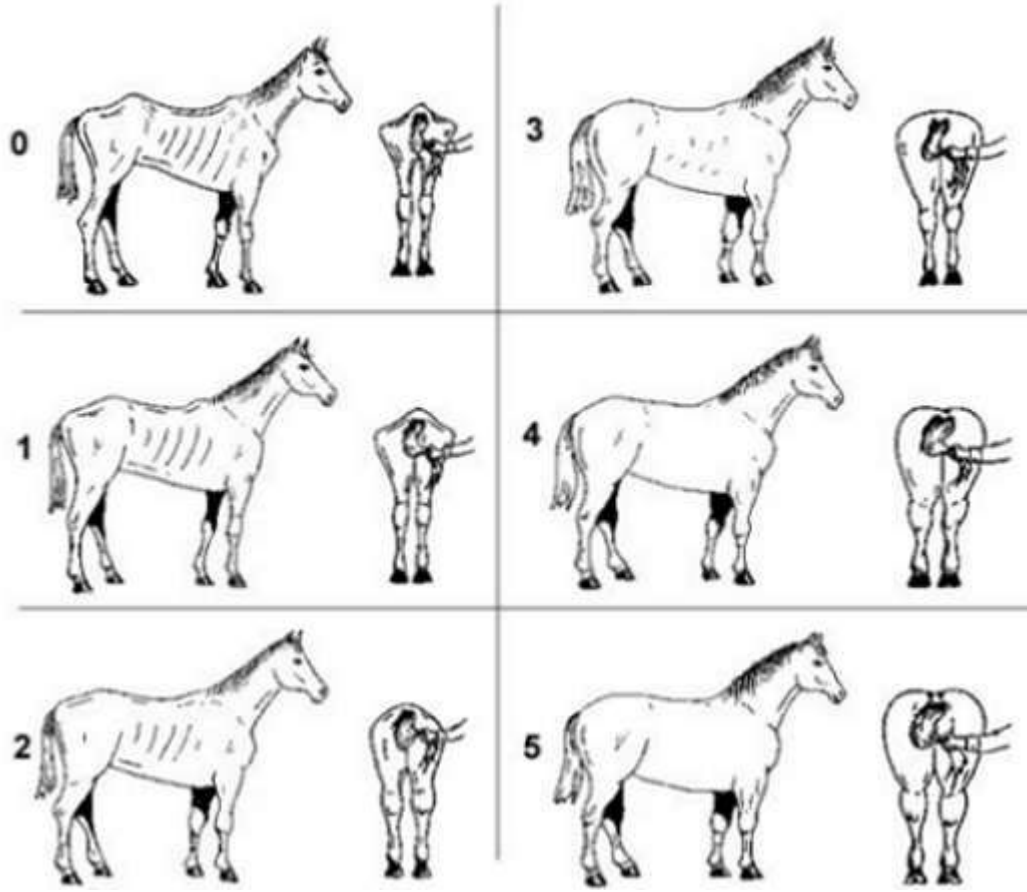
Insulin resistance(IR) increases with:

- Breed:
  - Ponies, Saddlebred, Arabian
- Increasing BCS
- Glucose dysregulation
- Age
- Mid-late Gestation

Effect of increased serum insulin

- Increased inflammation (IL-1,TNF α)
- Decreases oocyte quality
- Increased inter-ovulatory period
- Decreased fertility
- Increased uterine inflammation

How to Manage



Diagnosis:

- Resting insulin > 20µU/mL
- Resting glucose >100mg/dL
- Karo syrup test
  - Fast for 6 hours
  - Karo syrup (0.15mL/kg-75mL) oral
  - 60-90 minutes later
  - Insulin >50µU, glucose>100mg/dL

Treatment:

- Weight loss
- Decrease concentrates (<10% NSC) Hay
- Levothyroxine sodium: 0.1-0.6mg/kg
- Metformin 30mg/kg PO, SID (Durham 2012)
- Resveratrol : 750mg PO, SID (Mafredi 2018)

EQUINE ENDOCRINOLOGY GROUP

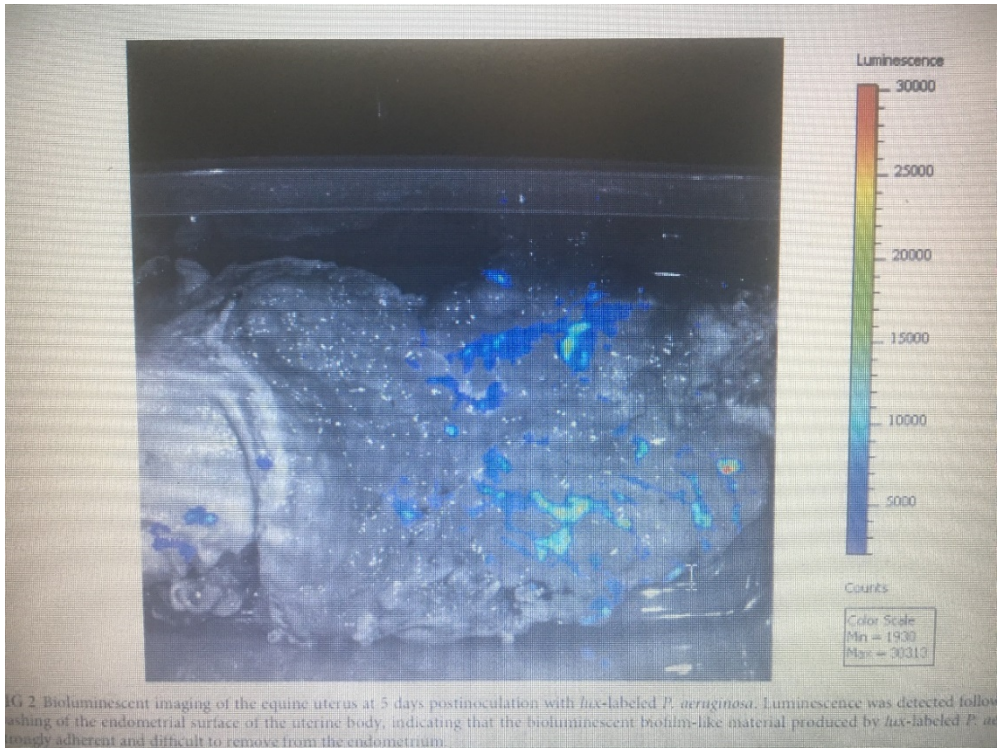
Uterine Treatment

- Efficacy of an antibiotic alone?
  - Time dependent vs. concentration dependent
- Antibiotic Resistance in Uterine Isolates
  - MDR was more frequent in Gram-negative (85.4%) than Gram-positive bacteria (23.5%)(Ferrer 2018)

In Vitro Efficacy of Nonantibiotic Treatments on Biofilm Disruption of Gram-Negative Pathogens and an In Vivo Model of Infectious Endometritis Utilizing Isolates from the Equine Uterus.

Ryan A. Ferris, Patrick M McCue, Grace I Borlee, Kristen D Loncar, Margo L Hennet, Bradley R Borlee. Journal of Clinical Microbiology, (2016), Vol 54, 3

- Bioluminescence imaging of *p.aeruginosa* biofilm in-vivo
- Demonstrated variable response of disruptors between bacteria



G 2. Bioluminescent imaging of the equine uterus at 5 days postinoculation with *lux*-labeled *P. aeruginosa*. Luminescence was detected follow
ashing of the endometrial surface of the uterine body, indicating that the bioluminescent biofilm-like material produced by *lux*-labeled *P. a*
ongly adherent and difficult to remove from the endometrium.

What can we do?

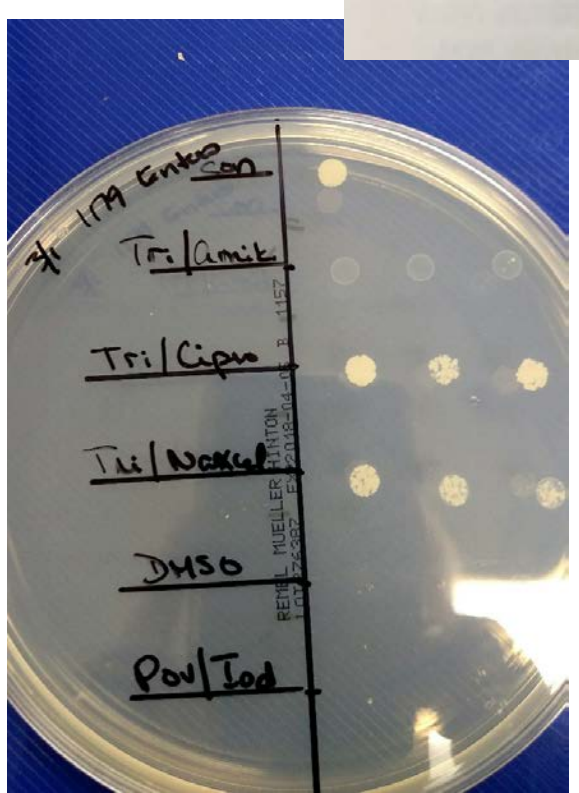
- Target treatment to disruption of biofilm
- Evidence of increased efficacy of antimicrobial treatment in conjunction with biofilm disruptors

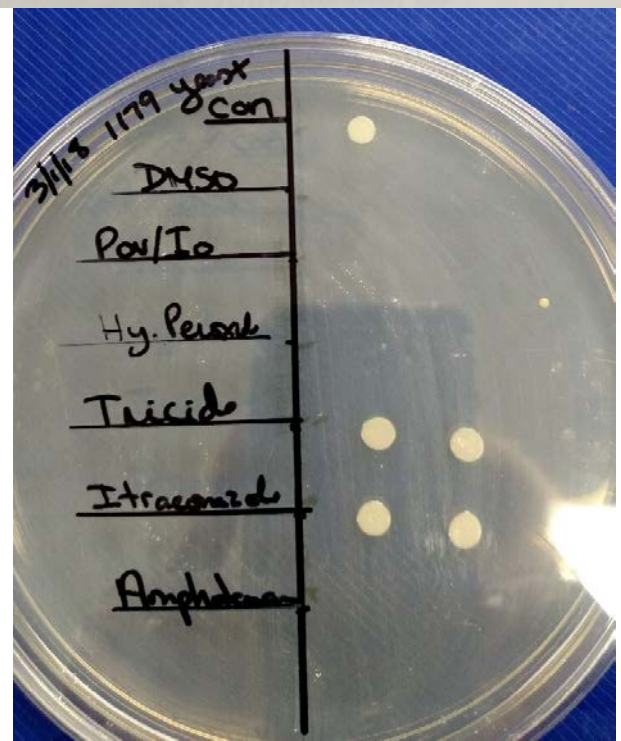
	Degradation Of Biofilm Biomass (% isolates susceptible)	Killing Of Bacteria Within A Biofilm
E coli	Tris-EDTA/Tricide-100% Acetylcysteine-100% H_2O_2-100% DMSO-100% Ceragyn®- 100%	Acetylcysteine H_2O_2
K pneumonia	Ceragyn®- 90%	H_2O_2
P aeruginosa	Tris-EDTA/Tricide- 38% H_2O_2- 50% Ceragyn®- 50%	Acetylcysteine
S equi subsp zooepidemicus	Tris-EDTA/Tricide H_2O_2 DMSO Hypochlorous Acid Ceragyn®	Tris-EDTA/Tricide H_2O_2 DMSO Hypochlorous Acid Ceragyn®

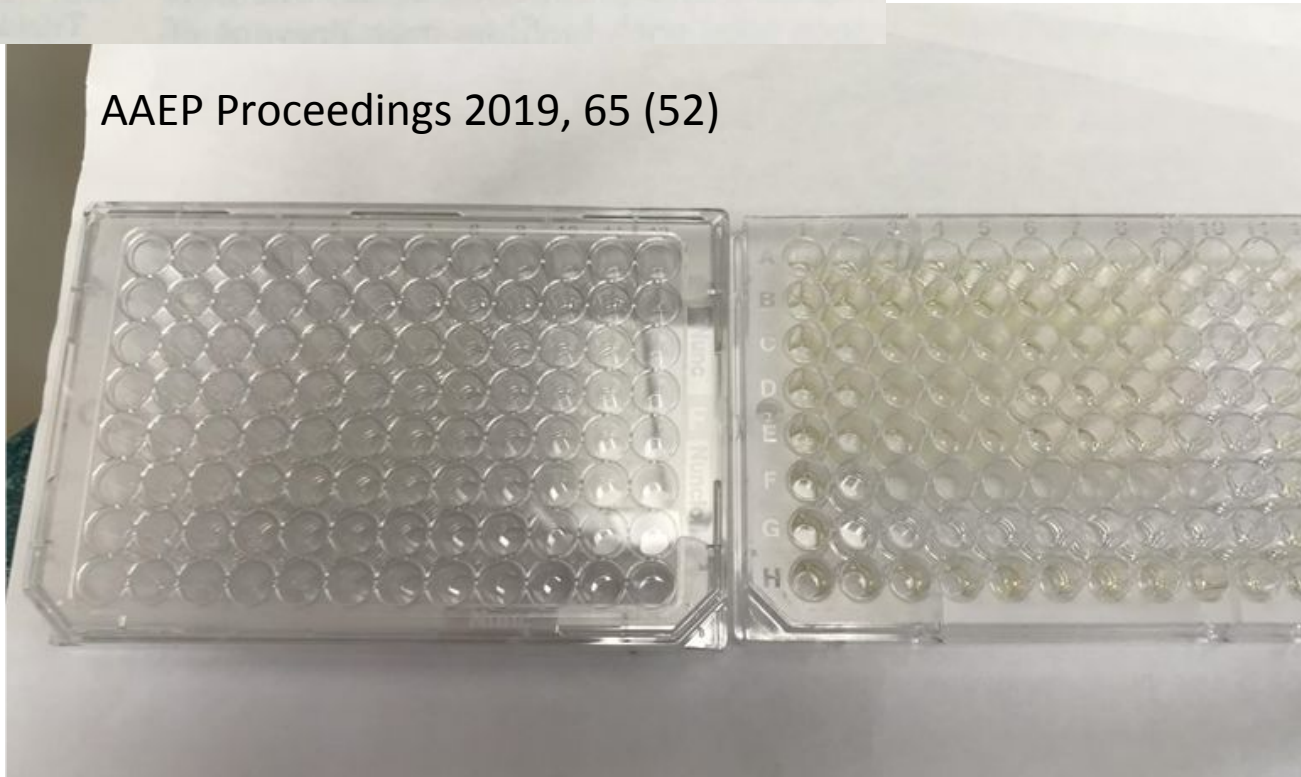
Table 1. Suggestions of compounds to use to degrade a preformed bacteria biofilm and kill bacteria residing within a preformed biofilm.

How to Use a Modified Biofilm Assay in Clinical Practice

Cassandra Cromer, DVM*; Ryan A. Ferris, DVM, MS, DACT;
Etta Bradecamp, DVM, DACT, DABVP; and Maria Schnobrich, VMD, DACT







AAEP Proceedings 2019, 65 (52)

Table 1. Antimicrobials (Non-Antibiotic and Antibiotics) at Their Final Stock Concentrations

Antimicrobial	Concentration
HP	3%
HP + ciprofloxacin	3% + 0.7 mg/mL
HP + amikacin	3% + 4 mg/mL
HP + ceftiofur	3% + 0.4 mg/mL
Tricide	8 mM disodium EDTA dehydrate and 20 mM 2-amino-2-hydroxymethyl-1,2-propanediol
Tricide + ciprofloxacin	8 mM disodium EDTA dehydrate and 20 mM 2-amino-2-hydroxymethyl-1,2-propanediol + 0.7mg/mL
Tricide + amikacin	8 mM disodium EDTA dehydrate and 20 mM 2-amino-2-hydroxymethyl-1,2-propanediol + 4mg/mL
Tricide + ceftiofur	8 mM disodium EDTA dehydrate and 20 mM 2-amino-2-hydroxymethyl-1,2-propanediol + 0.4mg/mL
DMSO	30%
Povidone/Iodine	0.1%
Acetylcysteine	3.3%
Vinegar	1.25%

DMSO, dimethylsulfoxide; EDTA, ethylenediaminetetraacetic acid; HP, hydrogen peroxide.

Results

Antimicrobial	Killing of bacteria in biofilm phenotype
Povidone/iodine	100%, (23/23)
HP	95.6%, (22/23)
DMSO	95.6%, (22/23)
Vinegar	95%, (19/20)
Tricide	39.1%, (9/23)
Acetylcysteine	10.5%, (2/19)

Antimicrobial	Killing of bacteria in biofilm phenotype
HP + Ciprofloxacin	100%, (19/19)
HP + Ceftiofur	100%, (18/18)
HP + Amikacin	94.4%, (17/18)
Tricide + Ciprofloxacin	94.4%, (17/18)
Tricide + Amikacin	88.8%, (16/18)
Tricide + Ceftiofur	88.8%, (16/18)

Compounds with 100% efficacy

	HP	HP+ Cipro	HP+ Ceftiofur	HP+ Amikacin	Tricide+ Ciproro	Tricide + Ceftiofur	Tricide+ Amikacin	PI	DMSO
Strep	✔	✔	✔	✔	✔	✔	✔	✔	✔
E. coli		✔	✔	✔	✔	✔	✔	✔	
Klebsiella		✔			✔		✔	✔	✔
Pseudo-monas	✔							✔	
Yeast	✔							✔	✔

How to use in Practice?

- Povidone Iodine
  - In Cromer study 0.1% Povidone-iodine solution effective in 100% of cases
  - Antimicrobial activity at 0.01% solution (Brinsko 1991)
  - Range of published reports with minimal inflammation 0.05 (Brinsko 1991) 1% - solution (Kalpokas 201(

  Recommendation:

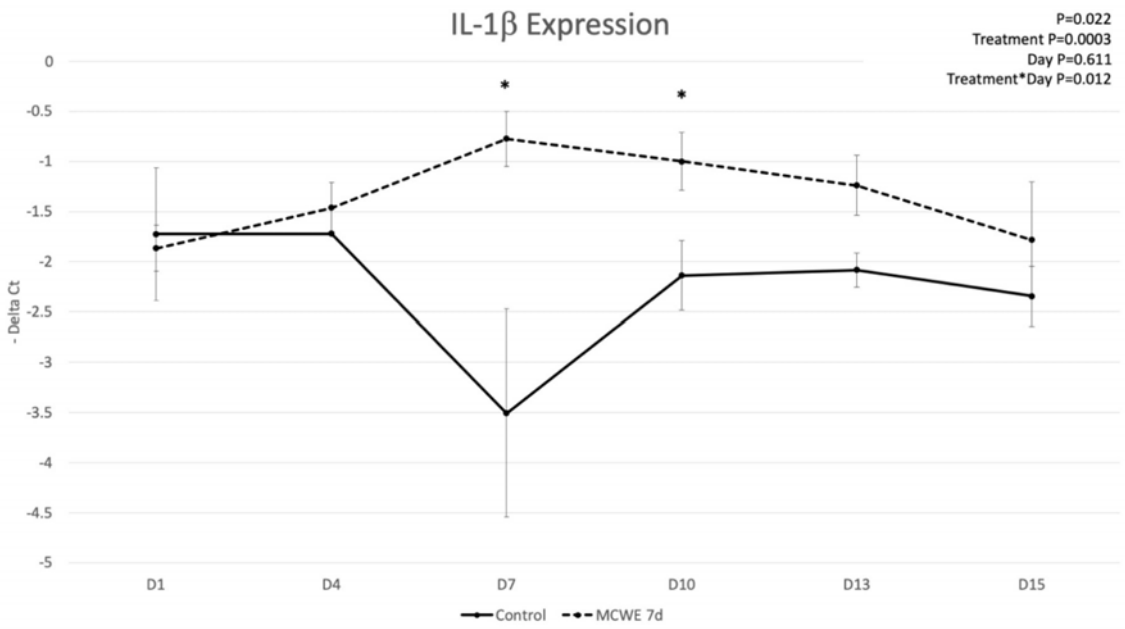
    - Large volume .1% solution Povidone-Iodine in LRS
      - 20mL Betadine solution/ 1 L LRS from 5% povidone iodine stock solution

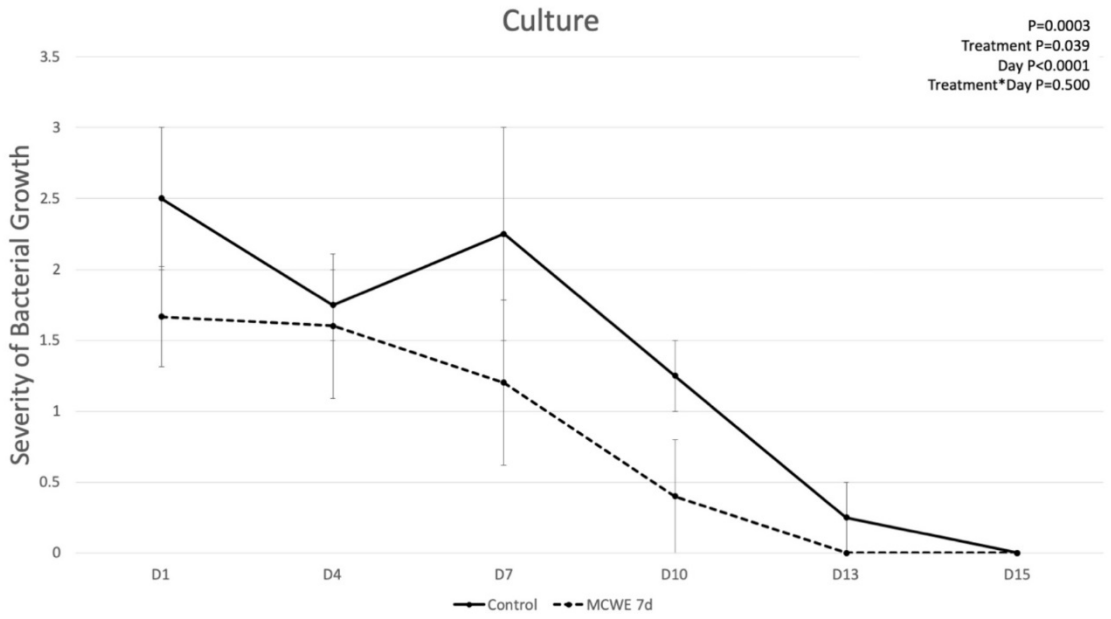
Mycobacterium Cell Wall Extract

- Immunomodulator made from Mycobacterium cell wall extract (MCWE)
- Treatment of susceptible mares with MCWE served to downregulate IL-1b and TNFα levels at estrus and IL-6and TNFα levels during diestrus, resulting in cytokine levels similar to those of resistant mares (Fumoso 2003)
- IU and IV routes equally as effective (Fumoso 2004)
- Single dose of Settle caused significant reduction of infection with *S. zooepidemicus.* Elimination of endometritis in 35% of the mares by the time of ovulation, and 70% of the mares by 7 days post-ovulation (Rogan 2007)

MCWE in post-partum mares (Fedorka 2019)

- Administration of a single IV dose led to:
  - increase in pro-inflammatory cytokines
  - Decrease of bacterial growth severity
  - Decreased time to clean culture





Platelet Rich Plasma

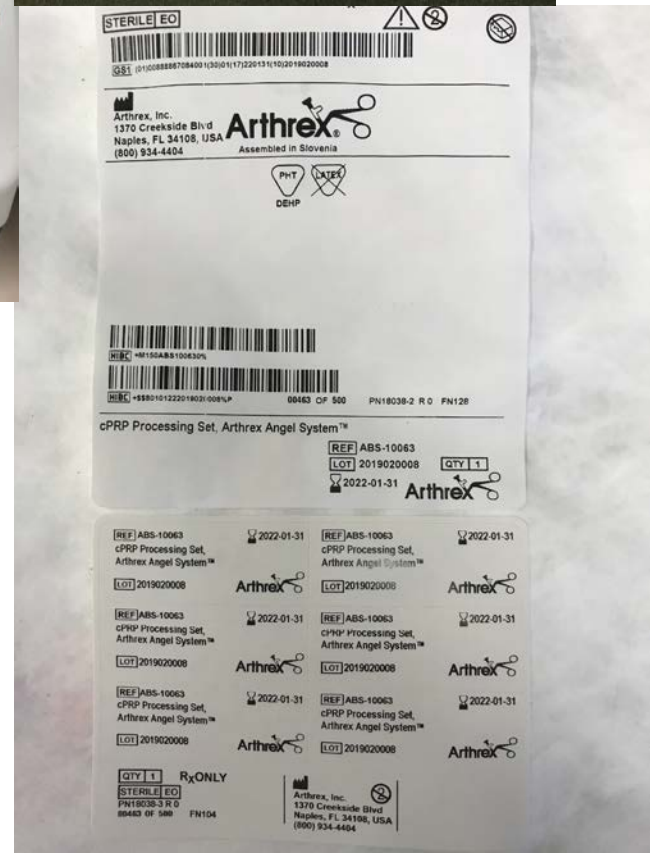
What is it and why is it good?

- Platelet-rich Plasma (PRP) or Autologous conditioned plasma is made when whole blood is centrifuged and red cells are removed. Contains variable platelets and their associated plasma proteins.
- Contains growth factors important in tissue repair due to mitogenic, chemotactic, neovascular, and anti-inflammatory effects
- The chemokines alter the chemotactic gradient to inhibit migration of leukocytes from the circulation into the tissue(Alam 1992)
- Another study found that platelets cause an initial suppression of IL-1 released by activated macrophages(Woodall 2008)

Breeding Applications or PRP

- PRP was effective in modulating the exacerbated uterine inflammatory response to semen in mares with Chronic Degenerative Endometritis(CDE) but did not reduce NO concentrations in intrauterine fluid (Reghini 2016)
- PRP infused (20mL) 24hour prior to or 4 hours after insemination beneficially reduces inflammatory response in PMIE mares (Segabinazzi 2017)
- Conception rates were significantly higher in the mares treated with PRP pre- and post-breeding (Segabinazzi 2017)

How to prepare PRP



(Reghini 2016)

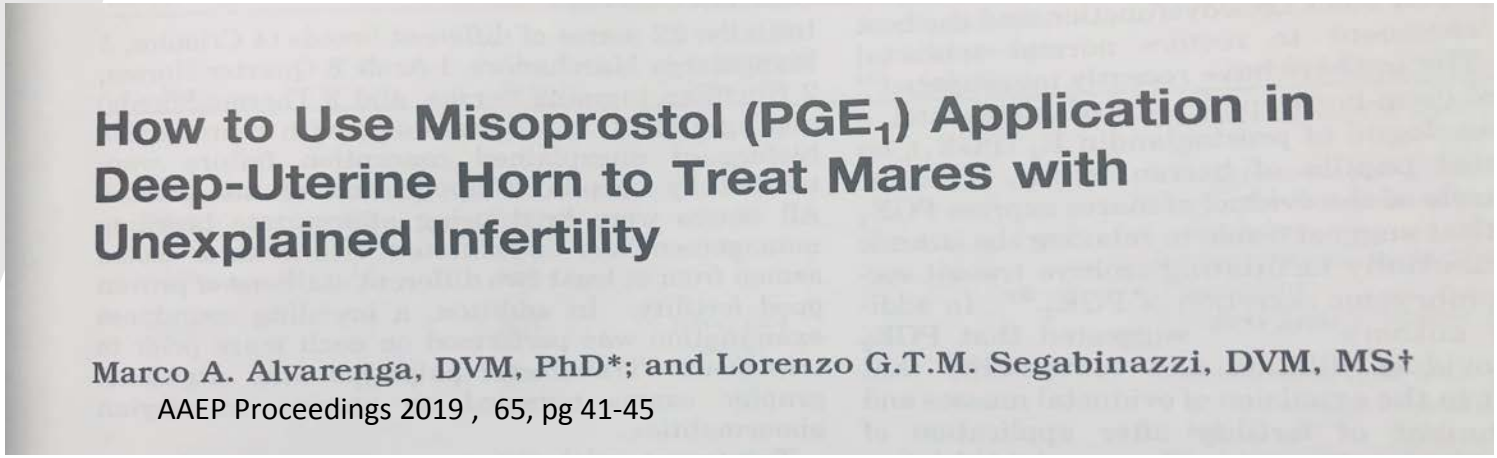
- 100mL in 3.2% Sodium citrate tube
- Centrifuge #1 : 120g for 10min-discard upper 50%
- Centrifuge #2 : 240g for 10 min-discard upper 50%
- PRP maintained for 1 hour at 20- ° 25°C
- Calcium chloride solution at 0.068 mEq calcium / mL of PRP
- The minimal platelet concentration used as a treatment was 250.000 platelets/mL

Application of Misoprostol as a Treatment of Unexplained Infertility in Mares

Alvarenga MAA, Segabinazzi LG.

Journal of Equine Veterinary Science 2018;71:46-50

- One Cytotec tablet (200 mcg of Misoprostol, Pfizer, USA) diluted in 3 mL of sterile water, in each horn, aiming the application as close as possible to the papilla of uterine tubes.
- These procedures were performed during diestrus
- Mares were bred in the next cycle.
- Embryos were produced, or a pregnancy was obtained from 15 of the 22 mares within the first two cycles following Cytotec treatment.



How to Use Misoprostol (PGE_1) Application in Deep-Uterine Horn to Treat Mares with Unexplained Infertility

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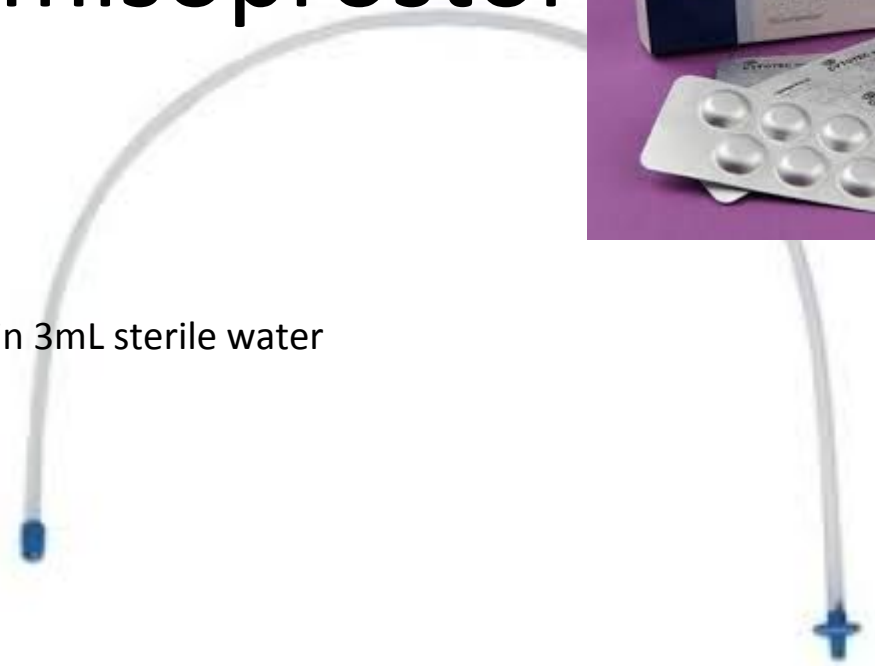
AAEP Proceedings 2019 , 65, pg 41-45

- 42 mares, failure to conceive multiple cycles,≥ two stallions
- Deep-horn application of 200ug misoprostol in 3mL sterile water in estrus or diestrus
- 69%(29/42) conceived following treatment
  - 18/28 on first cycle
  - 9/28 on second cycle
  - 2/28 on third cycle

When and how to use misoprostol



- Case selection:
  - No evidence of uterine pathology (culture, biopsy, hysteroscopy)
  - Failure to conceive when bred well to multiple stallion:
- Treatment:

  Deep-horn application of 200ug misoprostol (1 Cytotec tab) in 3mL sterile water in estrus:
  - Lavage daily (LRS) until efflux is clear
  - Oxytocin 10 iU, IM, BID through last day of lavage

  Diestrus:
  - Administer luteolytic agent 6 hours after treatment
  - Lavage the next day, and daily until efflux clear

  Allow mare to ovulate and breed on next estrus.


Questions?