Journal Pre-proof

Clinical outcome of transcervical infusion of a combination of procaine penicillin and gentamicin in late-term pregnant mares

Theresa M. Beachler , Mark G. Papich , Natalie C. Andrews , Karen A. Von Dollen , Katelyn E. Ellis , Katie Withowski , C. Scott Bailey

Please cite this article as: Theresa M. Beachler , Mark G. Papich , Natalie C. Andrews , Karen A. Von Dollen , Katelyn E. Ellis , Katie Withowski , C. Scott Bailey , Clinical outcome of transcervical infusion of a combination of procaine penicillin and gentamicin in late-term pregnant mares, *Journal of Equine Veterinary Science* (2021), doi: https://doi.org/10.1016/j.jevs.2021.103727

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1

Clinical outcome of transcervical infusion of a combination of procaine penicillin and gentamicin in late-term pregnant mares

Theresa M. Beachler[ab], Mark G. Papich[a], Natalie C. Andrews[a], Karen A. Von Dollen[a], Katelyn E. Ellis[a], Katie Withowski[a], and C. Scott Bailey[ac]*

[a] Department of Clinical Sciences, North Carolina State University, Raleigh, NC

[b] Current address: Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA

[c] Current address: Claiborne Farm, Paris, KY, USA

*Corresponding author:

Claiborne Farm, 703 Winchester Road, Paris, KY 40261, USA, email address: sbailey@claibornefarm.com

Highlights

- We describe the clinical outcome of a single antimicrobial transcervical infusion.
- Fetal heart rate and CTUP remained unchanged following antimicrobial infusion.
- All mares foaled 12 to 58 days after infusion with all foals surviving to weaning.

Abstract:

Transcervical intrauterine infusion of antibiotics may more effectively treat pathogens associated with fetal and neonatal disease in pregnant mares than standard systemic routes. The objective of this study was to assess the safety of transcervical antibiotic infusion by characterizing the gestational outcome in 9 healthy pregnant pony mares following a single transcervical infusion of 2.4 million IU of procaine penicillin and 200 mg of gentamicin in a 10mL volume during late gestation. Assessment of fetal-placental health was performed through serial measurement of the combined thickness of the uterus and placenta (CTUP) and fetal heart rate and mares and foals were closely monitored in the periparturient period. Fetal heart rate and CTUP remained unchanged after infusion, with no evidence of fluid accumulation or significant increase at the time-points 24, 48, and 72 hours. All mares foaled without complication 12 to 58 days after antibiotic infusion at a mean gestational age of 322.7 ± 12.7 days. Two out of nine foals displayed signs of mild neonatal maladjustment syndrome that responded to minimal supportive care and all foals survived to weaning without further complications.

1. Introduction

Transcervical, intrauterine antimicrobial therapy is widely accepted for the

treatment of endometritis in the non-pregnant mare [1]. While transcervical

antimicrobial administration has been proposed in pregnant mares, it is not

commonly elected due to concerns of inflammation and pregnancy loss following

cervical manipulation or additional iatrogenic inoculation of the chorioallantois

and uterus [2-4]. However, a growing body of evidence suggests that this may be

the most effective means of treating important causes of pregnancy loss,

including equine ascending placentitis [5-12]. This disease has long been

associated with inconsistent or poor outcomes for the pregnancy and neonatal

health. Even in controlled research settings, up to 60% of experimentally infected

mares treated with a combination of trimethoprim sulfamethoxazole, flunixin

meglumine, and altrenogest experience stillbirth or abortion [5-8]. This may, in

part, be a result of poor antimicrobial efficacy in the clinical environment of the

infected uterus and placenta. Work at the University of Florida demonstrated that

64% of experimentally-infected and treated mares remained culture-positive for

the inoculated *Streptococcus equi* subsp. *zooepidemicus* at parturition [7,8].

Persistence of bacteria at the level of the chorioallantois may serve as a

continued nidus of infection, producing persistent upregulation in inflammatory

mediators and resulting in preterm delivery or neonatal compromise. Inactivation

of trimethoprim sulfamethoxazole and other antibiotics in purulent material has

been documented in a variety of studies and may explain bacterial persistence in the mare in the face of appropriate antibiotic treatment [9-11]. Our laboratory has confirmed these findings in purulent equine uterine fluid *in vitro* and demonstrated antimicrobial suppression of several other antibiotics using the same model [12,13]. In contrast, a combination of procaine penicillin and gentamicin at concentrations that could be achieved via intrauterine administration has been shown to completely eliminate bacterial growth for at least 48 hours in purulent equine uterine fluid *in vitro* [12]. Transcervical administration of these or similar antimicrobials may therefore complement systemic therapy and improve treatment outcomes for equine ascending placentitis by allowing direct antibiotic penetration and potentially increased antibiotic concentration in affected tissues. However, clinical concerns over cervical manipulation and proinflammatory effects of intrauterine treatment must be addressed before such an approach could be recommended. Therefore, the purpose of the current study was to assess the pregnancy outcome following a single transcervical infusion of procaine penicillin and gentamicin in healthy pregnant mares.

2. Materials and Methods

Nine healthy pregnant pony mares of unknown breed between 270 and 275 days of gestation were enrolled in this experimental study over two breeding seasons in 2018 and 2019. Mares were 7 to 15 years of age and were maintained on pasture with supplemental hay and pelleted ration on a university-owned farm in

central North Carolina. All procedures were carried out in accordance with North Carolina State University's Animal Care and Use Committee's guidelines for the humane treatment of research animals (IACUC # 18-077-O). Mares were determined to systemically healthy at the time of enrollment through a complete physical examination. Briefly, each mare was examined visually for normal eye-sight, soundness at a walk and normal skin and hair-coat. All mares had a temperature <102ºF, heartrate between 24 and 48 beats per minute and a respiratory rate between 16 and 24 breaths per minute.

Reproductive soundness and gestational health were determined through performance of transrectal and transabdominal ultrasonography for assessment of fetoplacental health. At the time of enrollment, all mares were pregnant with an active fetus of appropriate gestational size. The combined thickness of the uterus and placenta (CTUP) was measured as previously described ventro-lateral to the cervix and mean fetal heart rate was measured via transabdominal ultrasound using M-mode ultrasonography [5,7]. Three recordings were taken at the time of each examination and averaged. In addition, evidence of gas or fluid separation of the fetal membranes from the endometrium was noted at each examination.

2.1 Drug preparation and administration

A single transcervical infusion consisting of 2.4 million IU of procaine penicillin (Bactracillin G; Aspen Veterinary Resources, Ltd., Liberty, MO, USA) and 200 mg of gentamicin (GentaVed 100, VEDCO Inc., Saint Joseph, MO, USA) in a total of 10 mLs was performed between 280 and 295 days of gestation. Procaine

penicillin and gentamicin were drawn into a single 20 mL syringe immediately prior to infusion and mixed thoroughly by gently inverting the syringe several times. Mares were placed in stocks with tails tied laterally, and their perineum was washed thoroughly with water and an iodine-based soap. Using a sterile arm and clean technique, the infusion was drawn into an insemination pipette and passed through the vagina and the external cervical os under manual guidance. Antibiotic solution was deposited at the level of the internal cervical os in all mares. Placement of antibiotics at the endometrial-chorionic interface was confirmed via transrectal ultrasound examination with evidence of hyperechoic fluid internal to the cervical os.

2.2 Gestational monitoring

Mares were closely monitored following antibiotic administration. A complete physical examination and fetal health assessment via transabdominal and transrectal ultrasound as described above were performed daily for three to five days. Five mares (year one) received anti-inflammatory flunixin meglumine (1.1 mg/kg, PO, q12h) and synthetic progestin altrenogest (0.044 mg/kg, PO, q24h) for five days after antibiotic infusion, whereas four mares (year two) received no medical intervention. After the initial post-infusion period, all mares were assessed visually for vulvar discharge, changes in behavior and appetite and for signs of impending foaling twice daily. Mares that showed signs gestational compromise or imminent foaling - including signs of mammary development, vulvar discharge, lengthening of the perineum and vulva, and softening of the tail

head and gluteal musculature - were monitored continuously until foaling. At the time of foaling, all mares were allowed to foal normally unless assistance was deemed clinically necessary due to the presence of dystocia or premature separation of the fetal membranes. Each mare was additionally monitored continuously until documented complete passage of the fetal membranes after foaling. Fetal membranes were examined by laying them on a flat surface and subjectively evaluating the thickness of the amnion and chorioallantoic membrane at 3 sites. The allantoic surface was examined for discoloration and calcification that may indicate full-thickness lesions or trauma from gestational sampling. The chorionic surface was examined for regions with purulent or fibrinous exudative material, villous atrophy and edema. Any abnormal regions were noted and measured. The placenta was deemed intact if the tips of both horns were observed.

2.3 Gestational sampling

Plasma, cervical fluid, and allantoic fluid were collected from mares to quantify local drug concentrations and identify metabolomic profiles of equine fetal fluids. Data are reported elsewhere or under investigation [15]. We have included the timing and methodology of sample collection here due to the fact that both cervical manipulation and allantoic fluid sampling may represent risk factors for abortion [6,15]. Sample collection schedules were as follows: In five mares (year one), plasma, cervical fluid and allantoic fluid were collected upon enrollment into the study (270-275 days of gestation), immediately prior to antibiotic infusion

(280-290 days of gestation) and 5 days after infusion. In four mares (year two), cervical fluid was collected at 2 hours, 4 hours, 8 hours, 24 hours, 32 hours, 48 hours, and 72 hours after infusion and allantoic fluid was collected at 8 hours, 24 hours and 48 hours after infusion. In all mares, plasma was collected at the same time-points as other procedures.

Briefly, plasma was obtained by collecting whole blood via jugular venipuncture into sodium heparin tubes. Cervical fluid was collected with a sterile arm and clean technique widely used for routine uterine culture by manually passing a sterile cotton swab or umbilical tape through a double-guarded culturette into the external cervical os for a total of 30 seconds to allow for the collection of secretions. Fetal fluids were collected via transabdominal ultrasound-guided fetal fluid puncture with an 18-gauge, 20 cm echogenic tip needle as described previously [6,15].

2.4 Neonatal care

All live-born foals received a complete physical examination consisting of temperature, pulse, respiration, ocular exam, oral exam, palpation of ribs for evidence of fracture, palpation of joints for evidence of sepsis, palpation of umbilicus for evidence of herniation or omphalophlebitis and observation of normal activity and nursing behavior. Foals were initially deemed viable if they were able to breathe without mechanical assistance, right themselves after birth, and respond to stimuli. These foals were assessed for the presence of adequate transfer of immunoglobulins (>800mg/dL) within 8 to 24 hours of birth (Snap

Equine Immunoglobulin test kit, Idexx Pharmaceutical Inc., Greensboro, NC,

USA). Foals showing signs of systemic compromise and/or neonatal

maladjustment syndrome with difficulty nursing were treated with squeeze-

induced somnolence and administered supportive care consisting of either

administration of thawed equine colostrum via orogastric intubation or 1 L of

intravenous equine plasma, the systemic antimicrobials ceftiofur sodium (5 mg/kg

q12h IM; Naxcel, Zoetis, Parsippany, NJ) and/or amikacin (25 mg/kg q24h IV;

Amikacin Sulfate Injection, USP, Heritage Pharmaceuticals Inc, Eaton, NJ, USA)

and intravenous lactated ringer fluid therapy (500 mLs q12h for 24 to 48 hours)

[14]. All viable foals were monitored regularly and received daily physical

examinations as described above for the first five to seven days of life.

2.5 Statistics

Measurements of CTUP and fetal heart rate were compared at the time-points

baseline (T0), 24 hours, 48 hours, and 72 hours with a one-way ANOVA.

Gestational age at foaling was compared via paired t-test with the most recent

natural gestation of the same mare and between years (year one vs. year two).

Analysis was performed using Statistix software with significance for all tests set

at $p<0.05$ (Statistix 10.0, Analytical Software Inc., Tallahassee, FL). All data are

presented as mean ± standard deviation.

3. Results

Physical exam parameters remained within normal limits in all mares for the duration of the study until foaling. Immediately following infusion, evidence of hyperechoic material and/or air was noted at the level of the chorioallantoic and uterine interface and/or cervical lumen in all mares (Figure 1). CTUP remained within normal limits for gestational age at all time points (5.2mm; range 3.9-5.9), with no evidence of fluid accumulation between the endometrium and chorioallantois or significant increase (p>0.05) at the time-points 24, 48, or 72 hours compared to baseline values (Figure 2) [5,7]. Fetal heart rate was within normal limits (mean 78, range 67-85) and remained unchanged at each time-point (p>0.05) [5,7].

All mares (n=9) foaled without complications and produced viable foals. Mares foaled between 12 to 58 days after antibiotic infusion (mean 35.9 ± 13.0 days). The mean gestational age at foaling was 322.7 ± 12.7 days. Mares foaling in year one foaled significantly earlier than in their most recent pregnancy (314.4 ± 10.7 vs. 333.4 ± 8.82 days; n=5; p=0.0097) while no difference in gestation length was seen during year two compared to the most recent untreated pregnancy (333.0 ± 9.41 vs. 330.0 ± 8.48 days; n=4; p>0.05) (Table 1).

All mares passed the fetal membranes within 2 hours of foaling and gross examination did not reveal any abnormalities. Seven of nine foals displayed normal expected neonatal behaviors after birth and through weaning. Two foals displayed signs of mild neonatal maladjustment syndrome with inconsistent and/or difficulty nursing (n=1 year one; n=1 year two). Each was treated acutely in accordance with established protocols in our laboratory as described above.

No foal required oxygen, continuous intravenous fluids or continuous monitoring. Following therapy, both foals were able to maintain hydration and to nurse independently within three days of birth and maintained a normal health status through weaning. Immunoglobulins were greater than 800 mg/dL in all other foals within 24 hours of birth.

4. Discussion

Paired with data from our laboratory that demonstrate complete suppression of bacterial growth in purulent material in the presence of procaine penicillin and gentamicin *in vitro*, the results of the study described here suggested that a local, transcervical treatment approach may safely reduce or eliminate growth in late pregnant mares with ascending placentitis [12,13]. Additional treatment trials are needed to establish the clinical efficacy of a transcervical antibiotic infusion as primary or adjunctive therapy for infectious gestational conditions.

Mares in both study-years received a total of three allantocenteses for the sampling of fetal fluids prior to foaling, as well as repeated cervical stimulation for the purpose of antibiotic infusion and collecting intracervical samples. Previous studies have documented an increase in abortions related to allantocentesis and cervical manipulation has previously been shown to affect progesterone concentration during the peri-ovulatory period [4,6,16]. However, in our study, viable foals were produced from all mares that received the transcervical infusion despite necessary cervical dilation and manipulation involved in drug delivery and sampling protocols of the study. Further, infusion of a 10 mL volume of

antibiotic between the endometrium and fetal membranes was not associated with grossly detectable changes of the cervical star or chorionic villi at the time of foaling.

Two foals exhibited signs of neonatal maladjustment syndrome following delivery. Clinical signs in each remained mild following birth and resolved quickly with supportive care, including thoracic compression to induce squeeze induced somnolence in the field in addition to supportive care consisting of fluid therapy, colostrum and/or plasma administration, and broad-spectrum antimicrobials [17]. Neonatal maladjustment syndrome, which is also commonly referred to as neonatal encephalopathy or dummy foal syndrome has been reported to occur in 1 to 2% of all live births [18]. Pathogenesis has been suggested to be related to the presence of a combination of perinatal ischemia and hypoxia, reperfusion injury, electrolyte disturbances, and neurosteroid imbalances [19,20]. These mechanisms may occur during periods of maternal illness in the period prior to or during parturition, placental disease such as placentitis or premature separation of the fetal membranes, periods of hypoxia and/or ischemia during or immediately surrounding parturition or in cases where foaling was uneventful with no known maternal comorbidity present [20]. In the mares affected in this study, one mare foaled unobserved at 299 days of gestation while another foaled at 345 days of gestation. Samples of these animals were not collected for histologic or microbial diagnostics and a cause for neonatal maladjustement syndrome was not apparent in either case. Thus, either a treatment effect or an effect of allantoic and cervical sampling cannot be ruled out. However, these concerns

must be weighed against the well-established frequent treatment failure of traditionally applied therapeutic regimens in the face of clinical or experimental placentitis. Clinical and experimental treatment success for both ascending placentitis and nocardioform placentitis is poor, with up to 60% of animals aborting and a substantial percentage of foals born small and dysmature [5,6,21].

We have proposed that this treatment failure is, in part, a result of inactivation of the most commonly-used antibiotic (TMS) in the face of the environment of the inflamed chorioallantois and endometrium. This is supported by work from the University of Florida, which documented a high prevalence of *Streptococcus equi* subsp. zooepidemicus in infected, treated pony mares at the time of foaling irrespective of outcome, but failed to demonstrate those bacteria in normal foaling mares in the same premises [7,8]. A review of foaling data from similar trials in our laboratory conducted in 2011 and 2017 confirmed the presence of *Streptococcus equi* subsp. *zooepidemicus* on periparturient uterine cultures in 9/10 inoculated, treated mares after an average of 23 days of treatment and despite the fact that 4/10 of those mares delivered apparently healthy foals (Supplementary Table 1). Sensitivity testing of bacterial isolates failed to demonstrate any evidence of microbial resistance to the antibiotic used for treatment (trimethoprim sulfamethoxazole). Local treatment with procaine penicillin and gentamicin, already well-established to enhance pregnancy rates in mares at the time of breeding, may sufficiently suppress intrauterine bacterial growth to enhance fetal survival and development and improve neonatal well-being of foals [22-24].

4.1. Conclusions

In summary, this preliminary study is the first to demonstrate that procaine penicillin and gentamicin can be administered as a transcervical infusion in late-pregnant mares without affecting neonatal viability. Additional work is needed to demonstrate treatment efficacy of such an approach in mares with placentitis.

Conflict of interest statement: The authors declare no conflict of interest.

6. References

[1] LeBlanc MM. The current status of antibiotic use in equine reproduction. Equine Vet Educ 2009;21:156-67.

[2] Zent WW, Williams NM, Donahue JM. Placentitis in central Kentucky broodmares. Pferdeheilkunde 1999;15:630-2.

[3] Hinrichs K, Cummings MR, Sertich PL, Kenney RM. Clinical significance of aerobic bacterial flora of the uterus, vagina, vestibule and clitoral fossa of clinically normal mares. J Am Vet Med Ass 1988;193:72-5.

[4] Handler J, Konigshofer M, Kindahl H, Schams D, Aurich C. Secretion patterns of oxytocin and PGF2 alpha-metabolite in response to cervical dilation in cyclic mares. Theriogenology 2003;59:1381-91.

[5] Bailey CS, Heitzman JM, Buchanan CN, Bare CA, Sper RB, Borst LB, et al.. B-mode and doppler ultrasonography in pony mares with experimentally induced ascending placentitis. Equine Vet J 2012;44:88-94.

[6] Beachler T, Gracz H, Long NM, Borst L, Morgan D, Nebel A, et al. Allantoic metabolites, progesterone, and estradiol-17β remain unchanged after infection in an experimental model of equine ascending placentitis. J Equine Vet Sci 2019; 73:95-105.

[7] Bailey CS, Macpherson ML, Pozor MA, Troedsson MHT, Benson S, Giguere S., et al. Treatment efficacy of trimethoprim sulfamethoxazole, pentoxifylline, and altrenogest in experimentally induced equine placentitis. *Theriogenology* 2010;74:402-12.

[8] Diaw M, Bailey CS, Schlafer D, Pozor M, Troedsson M, Benson S, et al. Characteristics of endometrial culture and biopsy samples taken immediately postpartum from normal mares compared with those from mares with induced placentitis. Anim Reprod Sci 2010;121S:S36970.

[9] Wagner C, Sauermann R, Joukhadar C. Principles of antibiotic penetration into abscess fluid. Pharmacology 2006;78:1-10.

[10] Zeitlinger MA, Derendorf H, Mouton JW, Cars O, Craig WA, Andes D, et al. Protein binding: do we ever learn? Antimicrobial Agents Chemother 2011;55:3067-74

[11] Thomas J, Linton S, Corum L, Slone W, Okel T, Percival SL. The affect of pH and bacterial phenotypic state on antibiotic efficacy. Int Wound J 2012;9:428-35.

[12] McKelvey K, Jacob M, Papich M, Beachler T, Bailey CS.. A comparative efficacy trial for trimethoprim-sulfamethoxazole, gentamicin and penicillin using an ex vivo model of gestational disease. Clin Theriogenology 2014;6:403.

[13] Von Dollen KA, Jones M, Beachler T, Harris TL, Papich MG, Lyle SK, et al.Antimicrobial activity of ceftiofur and penicillin with gentamicin against escherchia coli and streptococcus equi subspecies zooepidemicus in an ex vivo model of equine postpartum uterine disease. J Equine Vet Sci 2019;79:121-6.

[14] Toth BA, Aleman M, Brosnan RJ, Dickinson PJ, Conley AJ, Stanley SD, et al. Evaluation of squeeze-induced somnolence in neonatal foals. Am J Vet Res 2012; 73:1881-9.

[15] Beachler TM, Bailey CS, Gracz HS, Morgan DR, Von Dollen KA, Ellis KE, et al. Metabolomic profile of allantoic and amniotic fluid in late-term gestational mares characterized by [1]H-nuclear magnetic resonance spectroscopy. J Eq Vet Sci 2020;94:Article103235 IN PRESS.

[16] Schmidt AR, Williams MA, Carleton CL, Darien BJ, Derken FJ. Evaluation of transabdominal ultrasound-guided amniocentesis in the late gestational mare. Equine Vet J 1991;23:261-5.

[17] Toth B, Aleman M, Brosnan RJ, Dickinson PJ, Conley AJ, Stanley, SD, Nograndi, N, Williams, DC, Madigan, JE. Evaluation of squeeze-induced somnolence in neonatal foals. Am J Vet Res 2012;73:1881-9.

[18] Bernard WV, Reimer JM, Cudd T. Histological factors, clinicopathologic findings, clinical features, and outcome of equine neonates presenting with or

developing signs of central nervous system disease. Proceedings of the

American Association of Equine Practitioners 1995;41:221-4.

[19] Toribio RE. Equine neonatal encephalopathy: facts, evidence, and opinions.

Vet Clin North Am Equine Pract 2019;35:363-78.

[20] Aleman M, Weich KM, Madigan JE. Survey of veterinarians using a novel

physical compression squeeze procedure in the management of neonatal

maladjustment syndrome in foals. Animals 2017;7:69.

[21] Barr BS. The outcome of foals born to mares treated for placentitis. Uterine

Infection in Mares and Women: a comparative study II. In Havemeyer

Foundation Monograph Series. 2005;19:49-50.

[22] Pycock JF, Newcombe JR. Assessment of the effect of three treatments to

remove intrauterine fluid on pregnancy rate in the mare. Vet Rec 1996;138:320-

3.

[23] Pascoe DR. Effect of postbreeding infusion on conception and foaling rates.

Proceedings of the twelfth Bain-fallon memorial lectures. Equestrian

Veterinarians Australia, Townsville, QLD:187-92.

[24] Noseir, WMB. Effect of clearance of uterine fluid on pregnancy rates in

mares susceptible to endometritis. Vet Med J Giz 2002;50:281-7.

18

7. Tables

Mare	Year of Study	Infusion Date	Foaling Date	Days to foaling post Infusion	Viable	Complication
Mare 1	Year one	287	299	12	Viable	Mild NMAS
Mare 2	Year one	296	329	33	Viable	None
Mare 3	Year one	285	312	27	Viable	None
Mare 4	Year one	285	316	31	Viable	None
Mare 5	Year two	280	316	36	Viable	None
Mare 6	Year two	287	322	35	Viable	None
Mare 7	Year two	287	333	46	Viable	None
Mare 8	Year two	287	332	45	Viable	None
Mare 9	Year two	287	345	58	Viable	Mild NMAS

Table 1: Gestational outcome following transcervical infusion of a procaine penicillin and gentamicin. Infusion date and foaling date depicted as days post ovulation. NMAS, Neonatal maladjustment syndrome.

19

Figure 1: Representative image of the appearance of the chorioallantoic-uterine interface immediately after infusion of 10mL of compounded procaine penicillin and gentamicin.
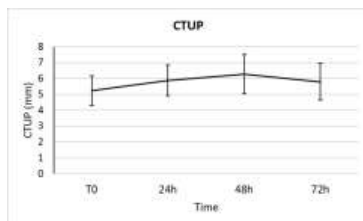
20

Figure 2: The combined thickness of the uterus and placenta (CTUP) were not significantly different at different time points before or after infusion of 10 mL of procaine penicillin and gentamicin. All values were within published values for healthy pregnant pony mares between 280 and 300 days of gestation.

Year	Mare	Gestational age	Outcome	Uterine Isolates at Foaling
2011	MARE 1	326	LIVE	*Streptococcus equi* subsp. *zooepidemicus*
	MARE 2	300	ABORT	*Streptococcus equi* subsp. *zooepidemicus*
	MARE 3	302	ABORT	*Streptococcus equi* subsp. *zooepidemicus*
	MARE 4	315	LIVE	No growth

21

	MARE 5	303	ABORT	*Streptococcus equi* subsp. *zooepidemicus*	*Pseudomonas aeruginosa*
2017	MARE 1	291	ABORT	*Streptococcus equi* subsp. *zooepidemicus*	
	MARE 2	298	ABORT	*Streptococcus equi* subsp. *zooepidemicus*	Eschericia coli
	MARE 3	300	LIVE	*Streptococcus equi* subsp. *zooepidemicus*	Eschericia coli
	MARE 4	305	ABORT	*Streptococcus equi* subsp. *zooepidemicus*	
	MARE 5	325	LIVE	*Streptococcus equi* subsp. *zooepidemicus*	

Supplementary Table 1: Summary of gestational outcome in ten mares experimentally inoculated with *Streptococcus equi* subsp. *zooepidemicus* [5,6].