A single post-mating uterine infusion of penicillin does not improve pregnancy rates in maiden Thoroughbred broodmares

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Abstract

Past studies have found improved pregnancy rates in mares treated with post-mating intrauterine (IU) therapies and/or oxytocin. These treatments are thus commonplace in broodmare practice. In some instances, these treatments are given regardless of a mare's history, current status and examination findings. The purpose of this study was to evaluate the effect of a single IU infusion of saline or procaine penicillin G on pregnancy and live-foal rate in maiden mares. Thirty-eight maiden Thoroughbred broodmares were evaluated over at total of 50 estrous cycles. All mares had a negative uterine culture prior to each mating. During each breeding cycle, all mares received 10 IU oxytocin (IM) 4-6 h after mating and either an infusion of physiologic saline (Control; n=24 cycles) or penicillin G (PPG; n=26 cycles) 16-24 h post-breeding. If a mare failed to become pregnant on her first cycle, another treatment was performed without consideration to the treatment given on the previous cycle. Day 0 was defined as the day of ovulation. Pregnancy status was determined by transrectal ultrasonography. Pregnancy rate per cycle was 72.00% (36/50), and Day 45 pregnancy and live-foal rates were 100% (36/36) and 90.6% (29/32), respectively. First-cycle pregnancy rates did not differ (P=0.278) between Control (83.3%; 15/18) and PPG cycles (65.0%; 13/20). No difference (P=1.0) was found in live-foal rates between Control (87.5%; 14/16) and PPG mares (93.8%; 15/16). These results indicate a single post-mating infusion does not appear to affect pregnancy and live-foal rates in maiden Thoroughbred mares.

Keywords: Broodmare, antibiotics, penicillin, ecbolics, intrauterine treatments, endometritis

Introduction

Post-mating treatment with IU infusion of antibiotics and ecbolics is common in many large broodmare practices, especially in regions that utilize live-cover breeding programs. Justifications for use are: reducing the incidence of infectious endometritis and assisting with fluid drainage.[1] In the authors' practice area (Central KY), it is not unusual for mares to receive a single intrauterine (IU) infusion of antibiotics 24-48 hours after breeding. In many cases, these treatments are performed irrespective of the mares' status, history or findings from the post-mating examination. Most commonly used agents for IU infusion are those with antibacterial activity, such as ticarcillin (+/- clavulonic acid) and ceftiofur sodium.[2] Pycock et al. reported improved pregnancy rates in mares receiving IU infusion of antibiotics within 72 hours of mating compared to untreated controls.[3] These researchers also demonstrated that a combination therapy of intravenous oxytocin with IU infusion of antibiotics provided superior pregnancy rates compared to either treatment alone and untreated controls. Unfortunately, this study did not control for age or the reproductive status (e.g., maiden, barren and foaling) of the mares. Because both are influential predictors of fertility (for a review see[4]), results may have been confounded if one or more of the treatment groups contained, for example, an increased proportion of young mares.

Despite the apparent usefulness of IU antibacterial infusions, their routine use is superfluous and potentially counterproductive by selecting for resistant organisms.[5] Frequent and unscrupulous use of antibiotics can lead to microbial resistance, which have important public health concerns.[6] In broodmare practice, IU antibiotic infusions has been associated with an increased susceptibility to fungal endometritis.[1,7] Finally, cumulative treatments can be costly and affect the breeding farms' profitability. It thus seems prudent for both clinicians and farm managers to consider a more judicious approach to the use of prophylactic antibiotics.

Breeding-induced endometritis is a normal physiologic response following coitus, and the majority of mares are capable of resolving the inflammation without any intervention.[8] Studies evaluating risk factors for bacterial infections can be useful in guiding future treatment strategies. The

use of IU antibiotic infusions is one area where antimicrobial use can potentially be stemmed, especially in certain subsets of mares where the risk of developing a clinical case of infectious endometritis is low. The purpose of this study was to evaluate the effect a single infusion of either saline or penicillin G had on pregnancy and live-foal rates in maiden Thoroughbred mares.

Materials and methods

This study involved 38 maiden Thoroughbred mares, 3-7 years of age (4.46±0.72 years) over 50 estrous cycles. It took place during the 2015 Northern Hemisphere breeding season in Bourbon County, Kentucky (38.2098° N, 84.2530° W). Mares were housed at night in two separate barns beginning December first of the previous year or upon arrival to the farm. Supplemental lighting of ~10 ft-candles per stall was provided from 4 to 11 pm each night. During the day, mares were turned out in two separate fields (>50 acres/field) unless they were under exercise restriction due to an injury. Mares were fed two quarts of 12% protein concentrate twice daily and free-choice grass hay while stalled. Fresh water was provided *ad libitum.* The first mares were bred February 16th and the last mare bred May 25th.

Mares were teased daily and examined when displaying signs of estrus or a change in behavior. Reproductive evaluations consisted of transrectal palpation and ultrasonography and a vaginal speculum examination. A uterine culture was obtained on each mare prior breeding and submitted to a clinical veterinary microbiologic laboratory.* Samples were considered negative when no growth was observed after 48 hours of incubation. Mares were deemed suitable for mating when they had one or more large (≥35 mm) preovulatory follicles. The day prior to breeding, mares were administered 2500 IU human chorionic gonadotropin (hCG) intravenously to induce ovulation on the first cycle. If mares failed to become pregnant on the first cycle, then 1.8 mg deslorelin acetate (Sucromate™, Thorn BioScience, Louisville, KY) was administered intramuscularly on subsequent cycles to indue ovulation.

Mares were bred by live-cover mating to commercial Thoroughbred stallions of acceptable fertility. Four to six hours after each cover, all mares received a single dose of 10 IU oxytocin im. Sixteen to twenty-four hours after mating, a separate reproductive examination was performed to detect ovulation (defined as Day 0) and identify any unusual findings, such as the presence and amount of intraluminal fluid, excessive edema, and pneumovagina. Regardless of the findings, a single IU infusion was performed of either 35 mL physiologic saline (Control) or 5 million units procaine penicillin G (PPG) qs to 35 mL in physiologic saline. Treatments were chosen by a blinded technician by selecting from one of two infusions. If a mare failed to become pregnant on the first cycle, another treatment was performed without consideration to the treatment given on the previous cycle. Once a mare had ovulated, the only other treatment she received was a Caslick's procedure (either complete or a repair); no additional oxytocin injections or IU infusions were performed. The first pregnancy examination was performed on Day 14 and subsequent examinations were performed on Days 16, 23, 35, 45, and 60, as well as during mid-gestation. Live-foal rates were determined by evaluating produce records reported to the North American Jockey Club.

Pregnancy and live-foal rates were compared between groups by two-tailed Fishers Exact tests for small counts. Significance was defined as $p<0.05$.

Results

This study involved 38 mares, and foaling data were available for 32 of these mares. Live foals were reported from 29 of these mares; one mare died prior to foaling; one mare aborted at mid-gestation; and the third mare delivered a dead foal. Foaling data were not available for six mares: three mares were removed from the study after Day 45 of gestation due to illness, and no reports were filed for three mares. Conveniently but not intentionally, these exclusions yielded the same sample sizes (16 mares/group) between the two treatment groups when foaling data were compared between these two groups.

First-cycle, per cycle, Day 45 and live-foal rates are provided in the table. First- and per-cycle pregnancy rates are represented graphically in the figure. Thirty-eight mares were included in the

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pregnancy data. Foaling data were available for only 32 mares (32/38; 84.21%) for reasons described above.

Table. Pregnancy and live-foal data obtained from the current study. No differences (P>0.05) were observed in any of the measured parameters.

Treatment Group	No. Mares	No. Cycles	Per Cycle Pregnancy Rate (%)	First-Cycle Pregnancy Rate (%)	Day 45 Pregnancy Rate (%)	Live-Foal Rate*
Control	18	24	87.50[a]	83.33[a]	79.17[a]	87.50 (14/16)[a]
PPG	20	26	65.38[a]	65.00[a]	65.38[a]	93.75 (15/16)[a]
Total	38	50	76.00	73.68	100.00	90.63 (29/32)

*Only 32 mares were included in the live-foal data; the ratios are provided in parentheses.
[a]Values in columns with similar letters are similar (p>0.05)

Overall pregnancy rate per cycle was 76.00% (38/50), while first-cycle, Day 45 and live-foal rates were 73.68% (28/38), 100.0% (38/38) and 90.63% (29/32), respectively. No difference (P = 0.1) was observed in pregnancy rate per cycle between Control and PPG mares. Similarly, no significant interaction was seen between the two treatments groups with respect to first-cycle (P = 0.278) and Day 45 (P = 0.352) pregnancy rates, as well as live-foal (P = 1.0) rates.

Discussion

Results from this study indicate that a single post-mating IU infusion of penicillin the day after live-cover mating did not significantly affect pregnancy or live-foal rates in maiden Thoroughbred mares. No significant interactions were observed between mares receiving a single IU infusion of saline or penicillin when evaluating first-cycle and seasonal pregnancy rates, as well as live-foal rates. Furthermore, data from this study suggest that uterine infusions did not negatively impact fertility, but that cannot be stated with absolute certainty due to a lack of negative controls (e.g., mares receiving no IU treatments) in this study. Antibiotic residues been found in the milk of dairy cows receiving IU infusions of procaine penicillin G,[9] and there is conjecture that a prevailing residue within the endometrium may adversely affect embryonic or fetal development. No data were collected to determine if such a residue occurs; however, if a residue does form after a single infusion of procaine penicillin G, results from this study indicate that pregnancy and live-foal rates were not adversely affected. Finally, data contained herein provide further evidence of what has been known for some time in clinical practice: young maiden mares appear to be at very low-risk of clinical infectious endometritis and thus require minimal post-mating management.

The researchers chose to study this topic because uterine infusions are a fairly common practice in areas of high equine breeding activity, particular live-cover operations. In some instances, it is used as blanket or stopgap treatment on large stud farms because of pressure to achieve high pregnancy rates. However, commercial operations are also businesses and must be treated as such to remain profitable. Because there does not appear to be a clinical benefit for a single IU infusion in young reproductively normal mares, this procedure could be abandoned in this subset of mares without sacrificing fertility yet improving profitability. In the USA, a single uterine infusion can range in cost from tens to hundreds of dollars, depending on the product. Assuming a median cost of $50 per infusion and using the mare numbers from this study, reduction in farm expenses would be ~$2,500 (e.g., 50 cycles x $50 = $2,500).

Findings from this study may also have pertinence with respect to public health. Antimicrobial resistance is of immediate concern to health professions and the global community, and strategies to curb emergence are in high demand.[10] Overuse is one of the main reasons attributed to the development of

resistance, and livestock programs are considered purveyors of resistance due to the shear quantities of antimicrobials used in agricultural production systems.[11,12] While the amount of antimicrobials used for IU infusions in mares is relatively low compared to volumes used in food-producing species, antimicrobial stewardship should apply to all clinicians. Veterinarians should assume some of the onus in promoting judicious use of antimicrobials, regardless of how much or little they use.

Pregnancy and live-foal rates reported in this study were consistent with—if not slightly higher—than previously published reports.[13-15] This is most likely due to the present study including only young and healthy maiden mares. Past studies have evaluated the efficacy of IU antibiotic infusion[3] or uterine irrigation in conjunction with a single IU antibiotic infusion,[16] but they either did not control for age or involved only barren mares. Because age and status can affect mare fertility, direct comparison of fertility data in the present study with that of the past should be interpreted with caution. It should also be mentioned that in these previous studies oxytocin was administered six hours after breeding. Oxytocin was used in a similar manner in the current study, and its clinical benefit should not be overlooked. It is a potent ecbolic that is highly useful in assisting both normal and subfertile mares with uterine fluid clearance.[17] Additionally, treatment with oxytocin alone has been shown improve pregnancy rates in mares receiving 25 IU (IV) within 48 hours of mating compared to untreated controls.[3]

The use of a single IU infusion was similar to what was performed in previously cited studies. While a clinical benefit of a single IU infusion has been noted in previously cited studies, a direct association with improved pregnancy rates and its effect in combating infectious endometritis remains in question. For example, published systemic doses for procaine penicillin in adult horses are 22,000-44,0000 unit/kg twice daily or 12 million units per dosage. The dosage used in the present study (5 million units) was previously reported in the literature as adequate for treating susceptible infections.[18] However, it is less than half that and given only as a sole infusion. Whether or not therapeutic concentrations were created and maintained with this treatment is unknown but warrants further investigation.

Along these same lines, the choice of penicillin G, and specifically procaine penicillin G, could have influenced pregnancy rates. The drug used in this study was compounded from the commercially available intramuscular injection by dissolving 5 million units of penicillin G (17 mL of a 300,000 units/mL) in approximately 18 mL of physiologic saline. Even though this formulation is relatively uncommon from a clinical standpoint,[2] it should have good activity against *Streptococcus equine* ssp *zooepidemicus* (beta-*Strep*),[19] which is the most common bacteria isolated from mares with infectious endometritis.[20] Furthermore, the author has used it with good clinical success in confirmed cases of beta-*Strep* endometritis, and procaine penicillin G was a component of an antibiotic used in a past study evaluating the efficacy of IU infusions.[3]

There were a few drawbacks to the current study design. First, as previously mentioned, there were no negative controls. Second, this study involved only a small number of test subjects (N=38) and reproductive cycles (N=50) and certain mares had to be excluded after Day 45 due to extenuating circumstances. Thus, the statistical power of this study was relatively low. Also, endometrial cytology data were not available for these mares; all mares had only a "clean" uterine culture. The combination of a culture and cytology increases the sensitivity of diagnosing clinical endometritis,[21] so there is a possibility that a culture alone failed to identify mares with low-grade endometritis, which could adversely affect pregnancy rates. Finally, there were three foaling reports that had yet to be filed at the time of this writing, and all three mares were part of the Control group. If all three report a dead foal, then the live-foal percentage would drop to ~74% and approach statistical significance.

In conclusion, results from this study indicate that a single post-mating IU infusion of penicillin has no effect on pregnancy or live-foal rates in young maiden mares. Per cycle pregnancy rates for Control mares verged on a trend (P=0.1) of being higher than PPG mares. Unfortunately, the limitations of this study do not allow for the claim that IU infusions are contraindicated, but data presented herein could suggest that such treatment is unnecessary to achieve adequate fertility in young maiden mares. Other factors, such as past reproductive history, proper breeding management, and stallion selection appear to be much more important factors dictating pregnancy and live-foal rates in horses.

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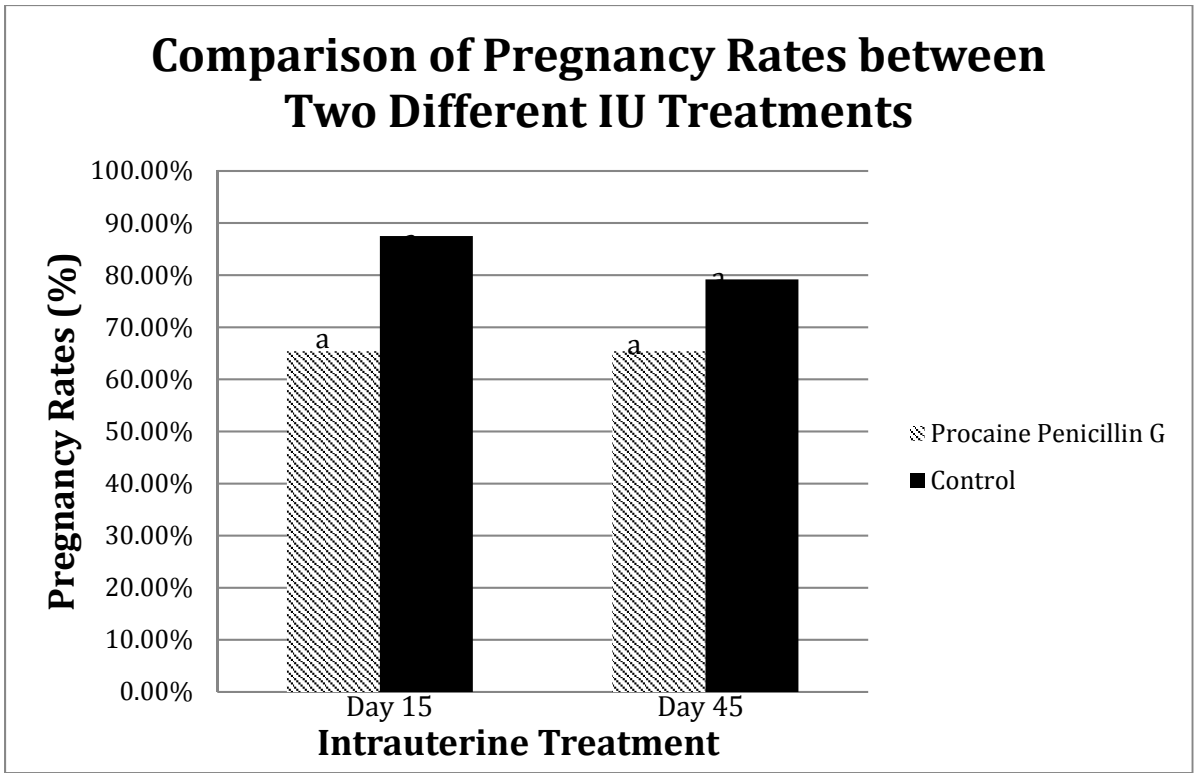

Figure. Day 15 and 45 pregnancy rates of Control and PPG mares