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Antimicrobial prophylaxis post-amniocentesis procedures in cattle: A randomized controlled equivalence study

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ABSTRACT

Amniocentesis is a routine procedure utilized on several species including human, equine, and bovine patients. Early assessment and discovery of new genetic traits in the cattle industry are highly desirable in order to accelerate genetic gain by shortening generational intervals. One of the main concerns from this procedure is the introduction of pathogenic bacterial contamination into the amniotic cavity thereby increasing the risks of spontaneous pregnancy losses post procedure. In this randomized controlled equivalence study, we have tested the effect of antimicrobial prophylaxis on the incidence of spontaneous abortions and contrasted it to untreated individuals post amniocentesis. On the treated group ($n = 67$) all heifers remained pregnant whereas 1 of the untreated group ($n = 65$) resulted in a spontaneous abortion during the study period. The latter represents 1.54% of pregnancy losses attributed to the risk associated to the amniocentesis procedure. However, the probability of inducing spontaneous abortion from the technique itself is not different to that of the contemporaneous population ($n = 694$) not undergoing amniocentesis viz., 1.59%. Following a two-tailed distribution, statistical analyses showed no significant differences across treatments (Fisher's exact test $P = 0.49$). The current prospective study indicates that performing amniocenteses on cattle have resulted in similar spontaneous pregnancy losses comparable to those of pregnant heifers without undergoing amniocentesis and regardless of antimicrobial use. In conclusion, prophylactic antimicrobials may not be applicable within the cattle amniocentesis framework.

Introduction

The amniocentesis technique was developed in the human medical field during the mid-1950′s to determine fetal gender by karyotypical assessment of the sexual chromosomes (Fuchs & Riis, 1956). Prenatal sex-determination became a topic of interest in the cattle industry coincidental with the advent of in vivo embryo production technologies being developed and improving rapidly; hence, information about the fetal gender was valuable. The first reports on amniocentesis were generated without the aid of ultrasonography (Bongoso & Basrur, 1975; Leibo & Rall, 1990) which limited the full potential benefits of the technique. It was not until better ultrasound technology became available, coupled with improved laboratory techniques, where such benefits were realized (Garcia & Salaheddine, 1997; Makondo et al., 1997). Improved ultrasonographic imaging permitted a more accurate identification of the genital tuberculum; thus, amniocentesis procedure for fetal gender determination was no longer justifiable. However, in the genomic era we live in today the amniocentesis technique has regained value in terms of assessing genetic conditions in the human field (Daum et al., 2019) as well as determining relevant metabolites for the livestock industry (Wood, Ball, Scoggin, Troedsson & Squires, 2018). Additionally, early identification of desirable traits could aid in the selection process as well as multiplication of individuals with superior genetics while still in utero (Gonzales da Silva et al., 2016).

Inherent pregnancy loss risks have been associated to amniocentesis since its early establishment in both human and cattle species. Additionally, the effects of antimicrobials as prophylactics post-procedure have remained controversial. Early amniocentesis attempts in bovine species reported no use of antimicrobials and spontaneous pregnancy losses as high as 13% (Bongoso & Basrur, 1975; Leibo & Rall, 1990). Moreover, a later study using an ultrasound-guided transvaginal amniocentesis approach without providing prophylactic antimicrobials, reported a similar 14% spontaneous pregnancy loss (Kamimura, Nishiyama, Ookutsu, Goto & Hamana, 1997). On the other hand, a study by Garcia and Salaheddine (1997) reported no spontaneous losses post amniocentesis procedures using antimicrobials. However, in a

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contemporaneous study Makondo et al. (1997) reported a high incidence of spontaneous losses (>60%) due to intrauterine infections occurring within a week post amniocentesis even when antimicrobial prophylaxis was used. Reports on bacterial infection showed an incidence ranging from 1% to 18% in human patients being subjected to amniocentesis and such infections were predominantly due to species belonging to the Mycoplasma family (reviewed in Gramellini, Fieni, Casilla, Raboni & Nardelli, 2007).

Human studies with substantially larger databases (≥ 4000 cases) have reported a lower incidence of spontaneous pregnancy losses ranging from 0.13% to 2.2% when compared to those on cattle (reviewed in Nizard, 2010). A retrospective study by Gramellini et al. (2007) reported that the average spontaneous pregnancy loss amongst 1744 amniocenteses cases was 1.26%. Additionally, the difference between prophylactically treated vs. non-treated groups was 0.1% which was not statistically significant and, more importantly, the risk of spontaneous abortion was not different in patients with a procedure-related risk factor. However, in a prospective randomized controlled trial, Giorlandino et al. (2009)) provided with prophylactic antimicrobials to a subpopulation of individuals undergoing amniocentesis ($n = 21,219$) while the other group remained antimicrobial-free ($n = 12,529$). In the study, spontaneous pregnancy loss rates for the treated and untreated groups were 0.03% vs., 0.28%, respectively. Although, statistical significance was found across treatments the magnitude of the main effect remained less than 1% and authors recommended the use of antimicrobial prophylaxis for amniocentesis. It is for the above reasons that the overarching objective of this study was to measure the effects of antimicrobial prophylaxis on spontaneous pregnancy losses post amniocentesis in cattle using a randomized complete equivalence trial.

Materials and methods

Experimental design

A randomized controlled equivalence trial was conducted at STgenetics® Ohio Heifer Center facilities located in South Charleston Ohio USA, from November 2019 to May 2020 with a total of 151 animals enrolled in the study. Standard estrus synchronization protocols were used either for artificial insemination or embryo transfers (Sala et al., 2020; Fig. 1). Detailed demographical information is presented in Table 1. Nineteen heifers were removed from statistical analyses because they were punctured 2 times during amniotic fluid collection, and they received prophylactic antimicrobial. Therefore, final statistical analyses accounted for a total of 132 heifers with a single puncture during collection. Further, all animals were under close observation for at least 4 weeks post procedure and pregnancy diagnosis was performed at ~100 days of gestational age which also corresponds when these animals were moved to a different facility for subsequent management.

Table 1
Demographic distribution of prophylactic antimicrobial versus control groups during the amniocentesis procedures.

Variables	Prophylactic treatment $n = 67$	Control $n = 65$
Average age in months (±SEM)[a]	20.13 (±0.56)	20.62 (±0.57)
Enrolled heifers (n)[b]	–	–
Holstein (%)	94.03	95.38
Jersey (%)	4.48	4.62
Crossbred (%)	1.49	0.00
Average times bred (±SEM)[c]	2.49 (±0.16)	2.46 (±0.17)
Average BCS (±SEM)[d]	3.33 (± 0.04)	3.37 (±0.05)
Average CL tissue area in cm^2 (±SEM)[e]	21.09 (±1.42)	18.54 (±1.49)
Average gestational age in days (±SEM)[f]	68.34 (±0.23)	68.58 (±0.23)
Embryo breed (n)[g]	–	–
Holstein (%)	83.58	78.46
Jersey (%)	10.45	13.85
Brown Swiss (%)	5.97	7.69
Average embryo stage (±SEM)[h]	5.86 (±0.12)	6.05 (±0.13)
Average embryo quality (±SEM)[h]	1.20 (±0.05)	1.22 (±0.06)
Derived pregnancies by (n)	–	–
Fresh in vitro XY-semen (%)	29.85	29.23
Fresh in vitro Y-semen (%)	1.49	6.15
Fresh in vitro X-semen (%)	2.99	1.54
Vitrified in vitro XY-semen (%)	5.97	7.69
Fresh in vivo XY-semen (%)	28.36	20.00
Frozen in vivo XY-semen (%)	28.36	29.23
Artificial Insemination XY-semen (%)	2.99	6.15
Fetal gender by ultrasonography (n)[i]	–	–
Female (%)	52.24	44.62
Male (%)	47.76	55.38

[a] Chronological age of nulliparous heifer at the time of amniocentesis procedures ± standard deviation of the mean.
[b] Breed distribution across study groups.
[c] Average number of services per conception.
[d] Dairy-based body condition score scale 1–5.
[e] Average CL tissue area measured on day-5 post estrus detection .
[f] Average gestation length at which amniocentesis was performed.
[g] Distribution of embryo breeds across the study groups.
[h] Embryo stage and quality based on the International Embryo Technology Society guidelines.
[i] Determination of fetal gender performed by ultrasonographic observation of the genital tuberculum at day 60 of gestation.

Since the main objective of the study was to determine the effects of prophylactic antimicrobial vs. no-antimicrobial use on subsequent pregnancy losses post amniocentesis procedures, an equivalence study approach was carried out to test the hypothesis whether pregnancy losses are not different across groups. Amniocenteses were approved by the Institutional Animal Care and Use at The Ohio State University, approval identification 2020A00000019.

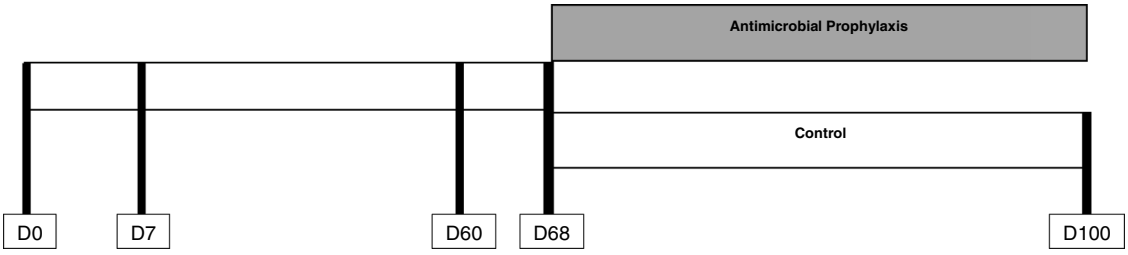

Fig. 1. Schematic illustration of the study period. Enrolled nulliparous heifers were bred either by artificial insemination or embryo transfer techniques. Chronological sequence starts at D0 which corresponds to the reference heat. Moreover, animals not being bred during D0 were eligible for embryo transfer at D7. Fetal gender and subsequent amniocentesis sessions were performed at D60 and at D68, respectively. In this study, $n = 67$ heifers were treated with prophylactic antimicrobials and, $n = 65$ remained untreated as the control group. After amniocenteses, all animals were closely monitored during the following days to observe any spontaneous abortion. Animals had one last pregnancy check by ultrasonography ~ 4 weeks post amniocentesis procedures which corresponded to ~100 days of gestational age.

Amniocentesis procedure

The range of gestational age at the time of amniocentesis was between 67 and 74 days which falls into the most appropriate window of time for genotyping purposes in cattle. Prior to amniocentesis, pregnancies were screened at day 60 of gestation via transrectal ultrasonography with a 5–9 MHz linear transducer connected to an EVO II platform (E.I. Medical Imaging®, Loveland CO, USA) to determine fetal gender by observing the genital tuberculum as well as assessing the overall soundness of the pregnancy. Amniocentesis procedures were performed according to published methods from Garcia and Salaheddine (1997); Kamimura et al. (1997); Makondo et al. (1997) and Gonzales da Silva et al. (2016), with some modifications. Briefly, each heifer was safely restrained in a squeeze chute followed by gentle massage of the ventral vulvar area to stimulate urination. Epidural block was achieved by administering 5 mL of lidocaine 2% (Aspen Veterinary Resources®, Liberty MO, USA) into the inter-coccygeal space between the first and second vertebrae. Additionally, to minimize stress 10 mg of Xylazine (Rompun®, Shawnee Mission, Kansas, USA) was administered intravenously as sedative. A vaginal lavage was performed by infusing 60 mL of 0.9% sodium chloride solution while rectal contents were being emptied to allow manipulation of the broad ligament. After appropriate aseptic procedures, a convex transducer 5–10 MHz connected to an EVO II platform (E.I. Medical Imaging®, Loveland CO, USA) was introduced into the vaginal canal up to the fornix and by means of transrectal manipulation of the broad ligament, the amniotic space was juxtaposed against the vaginal wall in order to permit the entrance of a 20 G X 2″ needle (WTA, College Station, TX, USA) without the risk of injuring a vital point such as the umbilical cord, placentomes or the fetus. The needle guide was connected to a 1.4 m tubing (WTA, College Station, TX, USA) subsequently connected to one port of a 3-way stopcock (MILA International, Florence KY, USA) and, 2 syringes connected to the remainder two ports. Upon needle entrance into the amniotic cavity, 5-mL of fluid was aspirated into a 20 mL luer-lock syringe to prime the line. The port was then switched immediately to collect approximately 40-mL final volume into a 50 mL luer-lock syringe (Air-Tite Products Co., Inc. Virginia Beach, VA) for subsequent laboratory purposes. Post procedure, animals were provided with pain management medication Flunixin meglumine at a dosage of 50 mg per 50 Kg of body weight intravenously (Vetameg™ Aspen Veterinary Resources®, Liberty, MO, USA) and Meloxicam at a dosage of 50 mg per 50 Kg of body weight orally (Unichem Pharmaceuticals Inc., East Brunswick, NJ, USA), followed by random allocation of the 2 experimental groups: antimicrobial prophylaxis with Ceftiofur (Excede®, Kalamazoo, MI, USA) 300 mg per 50 Kg of body weight subcutaneously in the fat pad of the ear versus the control group which did not receive any antimicrobial but did still receive pain management. Amniocentesis procedures were performed by 2 experienced clinicians.

Statistical analyses

Power calculation for this equivalence trial was based on a historical database worth of more than 5000 amniocentesis procedures in bovine species at STgenetics® within a 3-year period with an estimated average 8% pregnancy loss. Therefore, we set the model for a statistical significance $\alpha = 5\%$ and a power $(1\text{-}\beta) = 90\%$ with an abortion difference between groups equal to 2% and with an equivalence limit $d = 8\%$ (Julious, 2009); resulting on a total of 134 procedures (67 per group) required to address the question whether antimicrobial prophylaxis post amniocentesis procedures share a similar risk for pregnancy losses following the probabilistic equation P(1|a)-P(1|b) where 1 represents the response of the category of interest i.e., pregnancy loss and letters (a) and (b) represent antimicrobial use or not, respectively. Descriptive statistics as well as subsequent tests were performed using JMP® version 15 Statistical Discovery™ from SAS Institute Inc. All numerical variables followed a normal distribution. In addition, categorical nominal variables were used for the descriptive statistics such as embryo and recipient heifer breeds and, whether such embryos were originated by in vitro or in vivo fertilization means. To test the hypothesis of whether pregnancy loss was different across groups, a 2-tailed Fisher's exact test was performed followed by a two-sample test for proportions which is based on an adjusted Walt test to measure the probability of equivalence as well as proportion difference including confidence intervals.

Results and discussion

Historically, the amniocentesis technique was established primarily for fetal gender determination purposes in the 1990s (Garcia & Salaheddine, 1997; Kamimura et al., 1997; Makondo et al., 1997). With the advent of better technology and the birth of the genomic era, the amniocentesis procedure has evolved as a diagnostic tool for genetic conditions (Daum et al., 2019) as well as for the discovery of new genetic traits of interest to the livestock industry (Gonzales da Silva et al., 2016). Among all the reported complications from performing this procedure, intra-uterine infections from inadvertently introducing commensal bacteria into the sterile amniotic cavity have been the primary concern (reviewed in Nizard, 2010). Therefore, a total of 151 pregnant heifers were enrolled to test the hypothesis that antimicrobial prophylaxis would yield no significant differences on spontaneous pregnancy losses post amniocentesis procedures to the point of 100 days of gestational age. During the study, 19 animals had to be removed from statistical consideration since the amniotic cavity was punctured 2 times. This additional puncture occurred due to the needle tip being blocked by the amniotic membrane and a second attempt was needed to obtain sufficient sample volume for submission to the reference laboratory. Within those excluded observations there was a twin pregnancy requiring one puncture per amniotic sac. Nonetheless, there were no spontaneous pregnancy losses from double puncturing during collection except for one pregnancy diagnosed by ultrasonography with fetal megalocardia that resulted in an expected abortion two weeks post procedure. Repetitive puncturing during amniocentesis have been associated with a higher likelihood of spontaneous pregnancy losses (Vos, Pieterse, van der Weyden & Taverne, 1990) but in our study no losses were experienced except for the pregnancy with a congenital condition. Further, out of the 132 animals being considered for statistical analyses, there was only one reported spontaneous pregnancy loss within the untreated group (1/65) representing 1.54% of spontaneous abortion post amniocentesis procedures whereas all the 67 treated heifers remained pregnant. Of note, the loss was from a pregnancy originated by a fresh transfer of an in vivo produced embryo. Statistical analyses showed no significant differences across groups and their proportion difference was – 0.015% [95% CI, −0.065%- 0.034%], $P = 0.49$. It is important to mention that the spontaneous abortion rate during the time of the study on a total of 694 pregnant animals within the same facility and, not subjected to amniocentesis procedures from day 60 to ~100 of gestational age was 1.59% which is similar to that of the untreated group. Additionally, such pregnancies corresponded to the transfer of embryos derived from in vivo or in vitro techniques. In a more recent study in humans, an average spontaneous pregnancy loss attributed to the procedure was 1.75% (11/114) which agrees on the average spontaneous pregnancy loss reported in this study. However, cautious interpretation of results needs to be made when extrapolating information from human to cattle studies. One of the main reasons why several references used in the current study belong to the human field is because large databases on cattle amniocentesis procedures are scarce. Although, available reports in the human field have contradictory opinions on the use of antimicrobial prophylaxis (Gramellini et al., 2007 and Giorlandino et al., 2009), based on the results of this study we can only imply that good practices and strict standard operating procedures in place coupled with skilled personnel will reduce and maintain the risk of spontaneous abortions at minimum without the need of antimicrobial prophylaxis. The current prospective study indicates that performing amniocenteses

on cattle have resulted in similar spontaneous pregnancy losses comparable to contemporaneous pregnant heifers without undergoing amniocentesis during the same period and whether antimicrobial prophylaxis was used. It is therefore concluded, unless otherwise indicated, prophylactic antimicrobials may not be required in pregnant cattle undergoing amniocentesis procedures.

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Author's contributions

VAA, RVS, and RB participated in the conceptualization of this work and manuscript writing. VAA and RVS worked on experimental design, data management and, performed all the amniocentesis procedures and statistical analyses.

Declaration of Competing Interest

Authors in this study declare no conflicts of interest.

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