Use of bovine pregnancy-associated glycoproteins to predict late embryonic mortality in postpartum Nelore beef cows

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ABSTRACT

The primary objective was to determine if circulating concentration of bovine pregnancy-associated glycoproteins (bPAGs) on Day 30 after artificial insemination (AI) may serve as a marker of late embryonic mortality in *Bos indicus* (Nelore) beef cows. In experiment 1, postpartum Nelore beef cows (n = 56) were artificially inseminated at a fixed time (Day 0) after synchronization of ovulation. Serum samples were collected on Days 0, 21, 24, 27, and 30 after AI. The first significant increase (P < 0.0001) in serum bPAGs after insemination occurred on Day 24 of gestation. In experiment 2, ovulation was synchronized in postpartum Nelore beef cows (n = 1460) and AI was received at a fixed time. Pregnancy diagnosis and blood sample collection were carried out on Days 28 to 30 after insemination. Cows that maintained a pregnancy from Days 28 to 100 of gestation (n = 714) had significantly (P < 0.0001) higher circulating concentrations of bPAGs on Day 28 compared with cows that did not maintain a pregnancy (embryonic mortality [EM]) until Day 100 (n = 89). When Day 28 bPAG concentration was included in a logistic regression model to predict pregnancy maintenance until Day 100 of gestation, there was an increase (P < 0.0001) in the probability of maintaining pregnancy as maternal concentrations of bPAGs increased. A receiver operating characteristic curve was generated to determine bPAG concentrations on Day 28 that should predict embryonic survival or mortality with an accuracy of 95% or more. On the basis of the positive and negative predicative value analysis, at Day 28 of gestation a circulating concentration of bPAGs greater than 7.9 ng/mL was 95% accurate in predicting embryonic maintenance (to Day 100); a concentration of bPAGs less than 0.72 ng/mL was 95% accurate in predicting EM by Day 100. In experiment 3, the preceding model was tested in a separate set of Nelore beef cows to validate whether bPAGs would serve as an accurate measure of late embryonic mortality. Ovulation was synchronized in 650 Nelore cows and received AI at a fixed time. Pregnancy diagnosis and bPAG sampling were performed at Day 28 of gestation. Only pregnant cows were included in the analysis. On the basis of the previously reported bPAG cutoff values, the test was 95% accurate in predicting late embryonic mortality at Day 28 of gestation. In summary, bPAGs seem to be a good marker for predicting EM between Days 28 and 100 of gestation and suggest that this model could help dissect the molecular mechanisms leading to late EM.

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1. Introduction

In the US, the annual cost of reproductive failure to the beef industries is estimated to be $600 million. The exact causes of the preceding reproductive failure include animal management issues, cow infertility, bull infertility, heat stress, and embryonic mortality. Embryonic mortality is thought to be a primary contributor to this loss [1]. During gestation embryonic mortality can occur either early (before Day 28 of gestation) or late (after Day 28 of gestation). Reports of high fertilization rates after a single insemination (∼90% of ovulated oocytes), followed by pregnancy rates of 60% to 70% on Day 28 in cows indicate that early embryonic mortality may be 20% to 30% in beef cows [2,3]. In addition, after Day 28 of gestation late embryonic mortality has been reported to vary from 3.2% to 42.7% [4–11]. The large variation in the incidence of late embryonic mortality may be because of differences in cytoplasmic maturity of the oocyte at ovulation, inadequate preovulatory concentrations of estradiol, reduced postovulatory luteal progesterone secretion, inadequate uterine environment, placental insufficiency, and (or) the source of embryos (*in vivo* fertilized, *in vitro* fertilized, or cloned by somatic cell nuclear transfer). Cytoplasmic maturity of the oocyte, source of embryos, and placenta sufficiency may affect placental function, whereas preovulatory estradiol, luteal progesterone secretion, and inadequate dialogue between the embryo and maternal environment may affect endometrial function [12–15].

Significant effort has been directed toward understanding the factors causing early embryonic mortality; however, relatively little is known about the causes or mechanisms associated with late embryonic mortality, much of which occurs around the time of placentome formation (Days 35–40 of gestation). Although the incidence of late embryonic mortality is normally less than that of early embryonic mortality, the economic consequences of late embryonic mortality can be significant because late embryonic mortality can cause a prolonged delay in conception date and increases cows culled at the end of the breeding season [7]. Previously it has been shown that bovine pregnancy-associated glycoprotein (bPAGs) may serve as a marker of late embryonic mortality in beef and dairy cattle [16–19]. However, in all the preceding studies *Bos taurus* beef and dairy cows were used; thus, little is known about these relationships in *Bos indicus* cattle. The objectives of these experiments were to characterize basic bPAG profiles early in gestation and determine whether bPAGs were an accurate predictor of late embryonic mortality in these cattle.

2. Materials and methods

Experiments were conducted in a commercial beef farm located in Mato Grosso, Brazil in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching [20]. In all experiments cows were maintained on pastures, specifically *Brachiaria brizantha* with water and mineral salt *ad libitum*. Cows used in all three experiments below were at least 25 days postpartum when the estrus synchronization protocol began. All cows received an intravaginal progesterone (P4) insert containing 1.9 g of P4 (CIDR; Zoetis, São Paulo, Brazil), and 2.0 mg (im) estradiol benzoate (2.0 mL of estrogen; Farmavet, São Paulo, SP, Brazil) on Day −11, CIDR withdrawal, 25 mg (im) dinoprost tromethamine (PGF; 5.0 mL of Lutalyse; Zoetis, Brazil), 300 iu of equine chronic gonadotropin, and 1.0 mg (im) of estradiol cypionate (0.5 mL; Zoetis, Brazil) on Day −2, and fixed-time artificial insemination (TAI) on Day 0. After TAI, all cows were diagnosed for pregnancy at Days 28 to 30 of gestation. Pregnancy determination was based on the presence of a viable embryo (presence of a heartbeat) as detected by ultrasound scan. After confirmation of pregnancy, a blood sample was collected for quantification of bPAG. All cows were then confirmed pregnant at Day 100 of gestation.

2.1. Animals, treatment, and procedures

2.1.1. Experiment 1

Postpartum Nelore beef cows (n = 56) were artificially inseminated at a fixed time after synchronization of ovulation (Day 0) by using the protocol described previously. Before TAI on Day 0, the size of the ovulatory follicle was also determined by ultrasound. Serum samples were collected on Days 0, 21, 24, 27, and 30. All samples were harvested by venipuncture into a 10-mL vacutainer tube and allowed to clot at room temperature for 1 hour before being placed in a 4 °C refrigerator for 24 hours. After centrifugation, serum was collected and stored at −20 °C until measurement of bPAGs was performed.

2.1.2. Experiment 2

Synchronization of estrus and TAI in postpartum Nelore beef cows (n = 1460) was conducted as described. In this experiment, there were both primiparous (n = 240) and multiparous cows (n = 1220). A subset of the cows (n = 720) was artificially inseminated at a fixed time with semen from eight Angus sires (n = 90 cows per sire) to assess the effects of sire on pregnancy rate after TAI and Day 28 bPAG concentrations. All other cows were randomly assigned to be inseminated with semen from Angus sires of proven fertility. Serum samples were collected from all cows on Day 28 after insemination as explained in experiment 1.

2.1.3. Experiment 3

Ovulation was synchronized in primiparous postpartum Nelore beef cows (n = 689) as described previously and received TAI on Day 0. Cows were inseminated randomly from Angus sires of proven fertility. In addition, Estrotect heat detector patches were scored on a scale of 0 to 4 (0, lost patch; 1, <25% activated; 2, <50% activated; 3, <75% activated; and 4, >75% activated). Serum samples were collected from all cows on Day 28 after insemination as explained in experiment 1.

2.2. Assays

Serum concentrations of progesterone were quantified by RIA with Coat-a-Count RIA kit (Diagnostic Products Corporation, Los Angeles, CA) as described previously [21,22]. Intra-assay coefficient of variation was 5% and the assay sensitivity was 0.08 ng/mL for the progesterone RIA. Serum concentrations of bPAGs were determined by a monoclonal-based bPAG ELISA similar to that described by

Green et al. [23] and used previously to monitor bPAGs [18,24]. Each assay was run with a standard curve, a sample from a pregnant cow from Day 60 of gestation and a pooled sample from a nonpregnant cow.

2.3. Statistical analysis

One-way ANOVA (SAS 9.4) was used to test differences among Day 28 circulating concentrations of bPAGs for beef cows undergoing TAI that maintained pregnancy and those that established a pregnancy, but did not maintain it. The LOGISTIC procedure in SAS (9.4; SAS Institute Inc., Cary, NC) was used to determine the probability of pregnancy maintenance based on a single Day 28 serum concentration of bPAG. Receiver operating characteristic (ROC) curves were generated with the MedCal software package, setting embryonic mortality as the "true positive." After the generation of an ROC, the resulting true positive and false positives were subjected to positive and negative predictive value analysis to determine a concentration of bPAGs on Day 28, below which 95% of cows would experience embryonic loss by Day 100. Analysis of breakpoints was conducted by using PROC NLIM [25] in SAS and was used to determine the first significant change in the slope of the line.

3. Results

3.1. Experiment 1

Overall pregnancy rate after TAI was 39% (n = 22), and on the basis of break point analysis, the first significant increase (Fig. 1; P < 0.0001) in bPAG concentration occurred at Day 24 of gestation. In addition, there was no significant effect of ovulatory follicle size (P = 0.44) at Day 0 on circulating concentrations of bPAGs at Day 28 or any relationship between circulating P4 (P = 0.37) and bPAG concentrations on Day 28.

3.2. Experiment 2

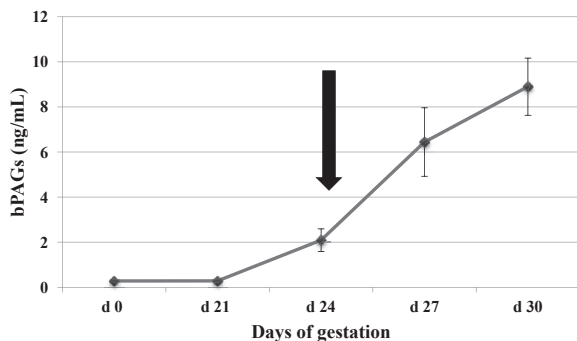The Day 28 pregnancy rate was 55% (n = 803); pregnancy was confirmed based on a viable fetal heartbeat

Fig. 1. Serum concentrations of bovine pregnancy-associated glycoproteins (bPAGs) in pregnant Nelore cows (mean ± SEM; n = 22) from Days 0 to 30 of gestation in experiment 1. First significant increase (P < 0.0001) in circulating bPAG concentrations occurred on Day 24 of gestation. Arrow head represents the first significant increase in circulating bPAG concentrations.
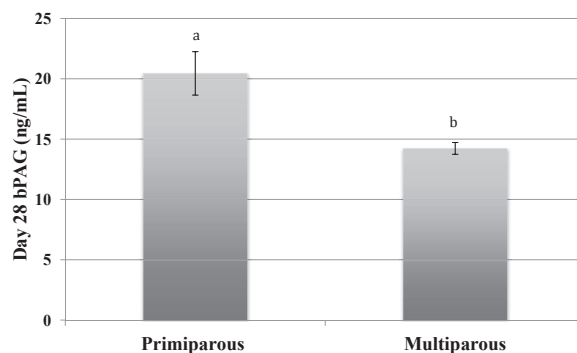

Fig. 2. Serum concentrations of bPAGs (mean ± SEM) in postpartum pregnant Nelore beef cows (primiparous, n = 116; multiparous, n = 687), which received TAI on Day 0 and had a viable embryo on Day 28 of gestation. Primiparous Nelore beef cows had increased (P < 0.05) circulating concentrations of bPAGs on Day 28 compared with multiparous cows independent of body weight. a,b denote a significant difference.

visualized by transrectal ultrasonography. The average serum concentration of bPAGs on Day 28 was 15.11 ± 9.92 ng/mL (mean ± SD). Serum concentrations of bPAGs were higher (Fig. 2; P < 0.03) in primiparous cows (n = 116; 20.45 ± 1.80 ng/mL; mean ± SEM) compared with multiparous cows (n = 687; 14.23 ± 0.49 ng/mL; mean ± SEM). There was no relationship between body weight and bPAG concentrations across all cows tested. In addition, cows that maintained a pregnancy from Days 28 to 100 of gestation (n = 714) had significantly (Fig. 3; P < 0.0001) higher circulating concentrations of bPAGs on Day 28 of gestation compared with cows that did not maintain a pregnancy (embryonic mortality) until Day 100 (n = 89). When Day 28 bPAG concentration was included in a logistic regression model to predict pregnancy maintenance until Day 100 of gestation, there was an increase (Fig. 4; P < 0.0001) in the probability of maintaining pregnancy until Day 100 of gestation as maternal concentrations of bPAGs increased. To conduct a more stringent
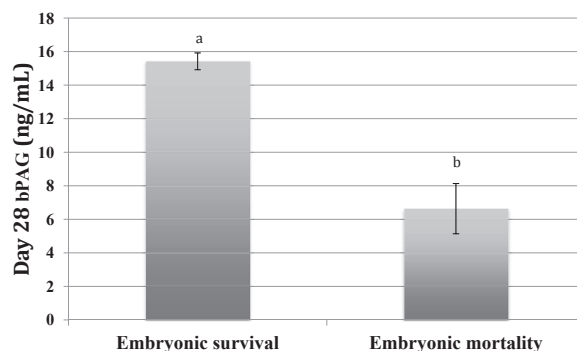

Fig. 3. Serum concentrations of bPAGs (mean ± SEM) in postpartum Nelore beef cows that received TAI on Day 0 and had a viable embryo on Day 28 of gestation (n = 803) and either maintained (embryonic survival; n = 714) or experienced embryonic mortality (n = 89) by Day 100. Nelore cows that experienced late embryonic mortality by Day 100 of gestation had decreased (P < 0.05) circulating concentrations of bPAGs on Day 28 compared with cows that maintained an embryo until Day 100. a,b denote a significant difference.
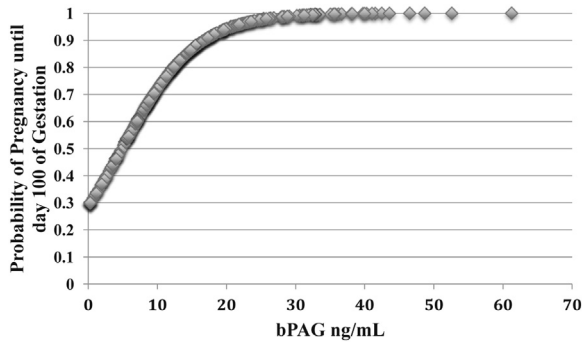
Fig. 4. Probability of pregnancy maintenance after TAI between Days 28 and 100 of gestation based on Day 28 serum concentrations of bPAGs (n = 803). Increased serum concentrations of bPAGs on Day 28 significantly increased (P < 0.05) the probability of pregnancy maintenance until Day 100 of gestation in Nelore beef cows after TAI.

Fig. 6. Serum concentrations of bPAGs on Day 28 of gestation from cows with pregnancies sired by sires 1 to 8. Although there was variation in the pregnancy rate after TAI among sires (44%–64%), there was no linear relationship between the pregnancy rate by sire and circulating concentrations of bPAGs. However, there were significant differences in circulating concentrations of bPAGs among sires.

test of the effectiveness of a single circulating bPAG concentration to predict embryonic survivability and/or mortality, an ROC (Fig. 5) curve was generated to determine bPAG concentrations on Day 28 that should predict embryonic survival or mortality with an accuracy of 95% or more. On the basis of the positive and negative predicative value analysis, a circulating concentration of bPAGs greater than 7.9 ng/mL was 95% accurate in predicting embryonic maintenance (until Day 100) and a concentration of bPAGs
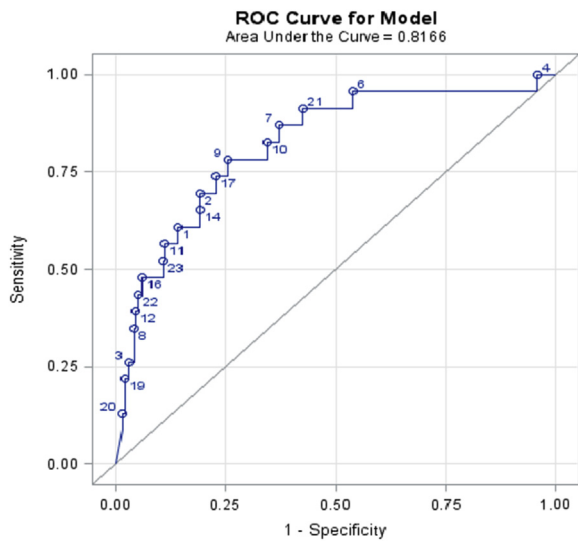
less than 0.72 ng/mL (minimal detectable level, 0.28 ng/mL) was 95% accurate in predicting embryonic mortality (between Days 28 and 100) at Day 28 of gestation. In addition, P4 concentrations were not significantly associated with bPAG in circulation or predictive of late embryonic mortality.

A subset of cows in this experiment (n = 720; pregnant at Day 30, n = 396) were evaluated for a sire effect on bPAG concentrations on Day 30 of gestation. Although there was variation in the conception rate after TAI among sires (44%–64%), there was no linear relationship between the pregnancy rate by sire and circulating concentrations of bPAGs (Fig. 6). However, there were significant differences in circulating concentrations of bPAGs among sires. There were 39 cows that established a pregnancy after TAI and had a viable embryo on Day 28 of gestation but failed to maintain pregnancy until Day 100 of gestation. Three sires in this experiment accounted for more than 70% of the late embryonic mortality. After removing all cows that lost a

Fig. 5. Receiver operating curve (ROC) using Day 28 circulating concentrations of bPAGs to model embryonic mortality between Days 28 and 100 of gestation in Nelore beef cows after TAI. A serum concentration of bPAG less than 0.72 ng/mL resulted in a 95% confidence that embryonic mortality would occur between Days 28 and 100 of gestation with an area under the curve of 81.6 (P < 0.05). The ROC curve graphically displays the relationship between true-positive rate (sensitivity) and false-positive rate (1-specificity) when an increasing cutoff value for the bPAG test was used. When the true-positive rate and the false-positive rate both decrease as the cutoff value is increased, this results in a diagonal line through the center meaning the test is not predictive (50:50 probability). However, when the line is deflected to the left of center the test is useful because it has a relatively high true positive rate and a low false positive rate at a specific cutoff value.
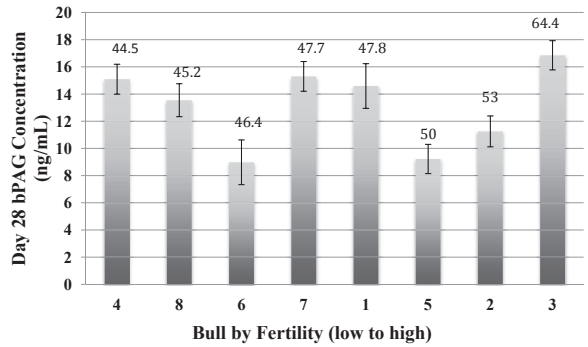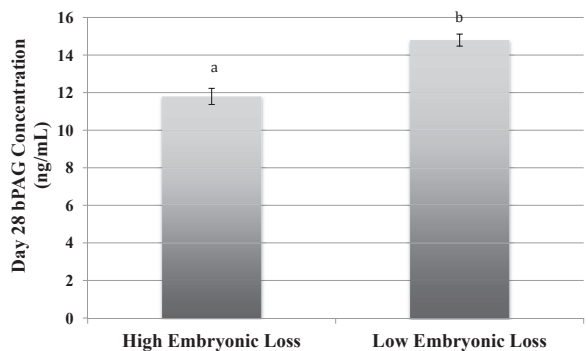

Fig. 7. Serum concentrations of bPAGs on Day 28 of gestation between sires that resulted in high embryonic loss and sires that resulted in low embryonic loss. After removing all cows that lost a pregnancy after Day 28 from the data set, the sires with the highest incidence of late embryonic mortality also were the sires with pregnancies that produced significantly (P < 0.05) lower maternal circulating concentrations of bPAGs on Day 28 of gestation compared with the remaining sires that had pregnancies having low embryonic mortality. a,b denote a significant difference.
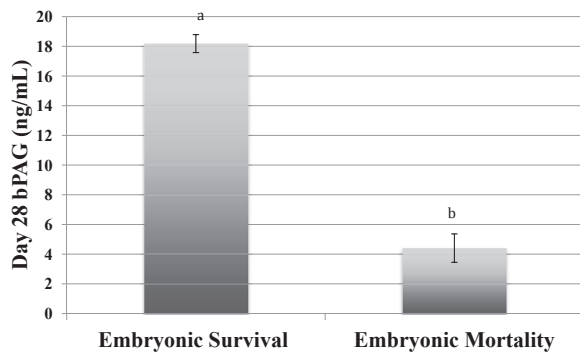
Fig. 8. Serum concentrations of bPAGs (mean ± SEM) in postpartum primiparous Nelore beef cows that received TAI on Day 0 and had a viable embryo on Day 28 of gestation (n = 303) and either maintained (embryonic survival; n = 285) or experienced embryonic mortality (n = 18). Nelore beef cows that experienced late embryonic mortality by Day 100 of gestation had decreased (P < 0.05) circulating concentrations of bPAGs on Day 28 compared with cows that maintained an embryo until Day 100. a,b denote a significant difference.

pregnancy after Day 28, the three sires with the highest incidence of late embryonic mortality were the sires whose pregnancies produced significantly (P < 0.05; Fig. 7) lower maternal circulating concentrations of bPAGs on Day 28 of gestation compared with the five remaining sires with pregnancies that experienced lower embryonic mortality.

3.3. Experiment 3

Primiparous Nelore beef cows underwent pregnancy diagnosis at Day 30 of gestation and the average bPAG concentration for all pregnant cows was 17.42 ± 10.80 ng/mL (n = 303; mean ± SD). As observed in experiment 2, there was a significant difference in Day 30 bPAG concentrations between cows that successfully established and maintained (n = 285; 18.18 ± 0.61 ng/mL; mean ± SEM) a pregnancy compared with those that established and failed
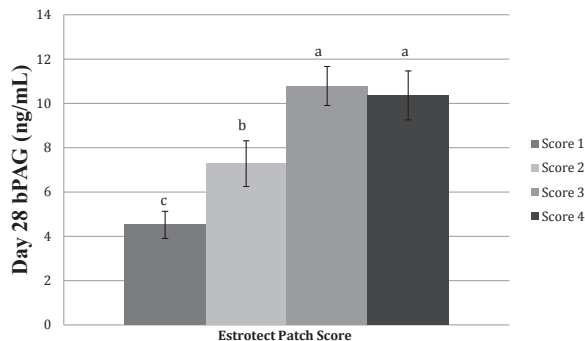

Fig. 9. Serum concentrations of bPAGs (mean ± SEM) in postpartum primiparous Nelore beef cows that received TAI on Day 0 and had a viable embryo on Day 28 with different levels of Estrotect patch activation on Day 0 (0, lost patch; 1, <25% activated; 2, <50% activated; 3, <75% activated; and 4, >75% activated). As intensity of estrus expression increased, as determined by Estrotect patch score at TAI, there was a significant increase in circulating bPAG concentrations on Day 28. a,b denote a significant difference.

to maintain a pregnancy (n = 18; 4.41 ± 0.95 ng/mL; mean ± SEM; P < 0.05; Fig. 8). In addition, as the intensity of estrus expression increased, as determined by Estrotect patch score at TAI, there was a significant increase in circulating bPAG concentrations on Day 28 (Fig. 9). The cutoff concentration of bPAGs developed in experiment 2 (0.72 ng/mL) was 95% accurate in predicting cows that would experience embryonic mortality in experiment 3. No cows that actually maintained pregnancy fell below the cutoff value for prediction of embryonic mortality. However, on the prediction of embryonic survivability, we were not as successful. On the basis of the cutoff value of 7.9 ng/mL from experiment 2, five cows that ended up undergoing embryonic mortality would have been predicted to maintain pregnancy.

4. Discussion

Bovine PAGs are detected in the maternal circulation beginning around Days 24 to 26 after insemination [18,23] and may serve as a marker for placental function [17]. Bovine PAGs were identified by multiple groups after their purification from placental extracts and their detection in the maternal circulation [26−30]. From that time, the focus of bPAG research has centered on the development of accurate assays for detecting bPAGs in blood and milk for the purpose of pregnancy diagnosis. In the present study, bPAGs were 96% accurate in diagnosing pregnancy in *B indicus* (Nelore) beef cows suggesting that bPAGs can work in crosses of subspecies (*B taurus*×*B indicus*). Currently, there are three commercially based assay platforms that use bPAGs for diagnosis of pregnancy in cattle either by blood or milk [31], and all have been reported to accurately diagnose pregnancy. The assay platform used in these experiments represents the commercially available test; however, it provides quantitative-based measurements of PAG.

Reports of late embryonic and/or fetal mortality in cattle range in the literature from ∼ 3% to 40% depend on the cow type and location [4–9,11]. In the present study, the incidence of late embryonic mortality was ∼11% and 6% for experiments 2 and 3, respectively. The increased incidence of late embryonic mortality in experiment 2 was not surprising because multiparous cows have been shown to have increase late embryonic mortality compared with younger cattle [6,32].

The exact mechanisms that lead to late embryonic mortality have been poorly characterized due in part to the need for a model to identify those cattle that will maintain or not maintain a pregnancy [33]. Multiple reports have demonstrated that circulating concentrations of bPAGs may be associated with late embryonic mortality in cattle [16−18,34,35]; however, other reports have demonstrated no such association [36]. In *B taurus* beef cattle (Angus, Hereford, and so forth) undergoing both TAI and embryo transfer (ET), bPAG concentrations at Days 28 to 30 of gestation have been shown to be significantly increased in cows establishing and successfully maintaining a pregnancy until Days 60 to 72 of gestation compared with those that establish, but do not maintain, a pregnancy during that time period [17,18]. Furthermore, data in dairy cows

showed that an increase in circulating bPAG concentrations at Days 28 to 30 was associated with pregnancy success [16,19,35,37]. Similar results have been shown in sheep pregnancy too [38]. However, there is conflicting data to suggest that bPAGs around Day 30 of gestation are not predictive of late embryonic mortality in high producing dairy cattle [36]. In both the beef studies mentioned previously, along with the dairy studies published by Thompson et al. [19] and Pohler et al. [35], the assay platforms used in those experiments were very similar, in that they used the exact same monoclonal-based sandwich ELISA validated by Green et al. [22]. These data suggest that the bPAG assay platform has the potential to have a major impact on the usefulness of bPAG measurements for diagnosing pregnancy and predicting late embryonic mortality.

In the present study, we aimed to develop a cutoff model by using the same sandwich ELISA platform that has shown utility in previous reports [18,22,23]. In this experiment similar results were observed. Cows undergoing late embryonic mortality between Days 28 and 100 of gestation had significantly decreased circulating concentrations of bPAGs at Day 28 of gestation compared with cows that successfully maintain a pregnancy. In addition, on the basis of the ROC curves and positive and negative predictive value analysis, we were able to determine a circulating concentration of bPAG at Day 28 of gestation that was predictive of embryonic mortality or survivability. Previous studies have shown associations between bPAG concentrations and late embryonic mortality; however, this model has allowed for prediction of pregnancy success during Days 28 to 100 of gestation. In experiment 3, this model was tested in a separate set of cows to validate its ability to detect late embryonic mortality. In primiparous Nelore beef cows, the model was 95% accurate in predicting late embryonic mortality for a concentration less than 0.72 ng/mL; however, it was not 95% accurate in predicting embryonic maintenance. One possible explanation for this is that primiparous cows were shown in experiment 2 to have significantly increased circulating concentrations of bPAGs at Day 28 of gestation, which could explain the higher cutoff value for predicting embryonic survivability although primiparous cows were included in the original model construction in experiment 2. Another possible explanation is that bPAGs have been shown to be only predictive of pregnancy loss between Days 28 and 40 of gestation [18]; therefore, evaluating embryonic loss from Days 28 to 100 of gestation may encompass too much time. Indeed, Pohler et al. [35] suggest that bPAGs are really only predictive of embryonic mortality between Days 28 and 45 of gestation and do not take into account the possibility of ovarian failure or other types of pregnancy loss that may occur after Day 45 of gestation.

Circulating concentrations of bPAGs have been reported to increase in maternal circulation around Day 24 of gestation until about Day 36 in *B taurus* cattle and subsequently decrease until about Day 60 of gestation; circulating concentrations of bPAGs then begin to increase again between Days 60 and 90, and they steadily rise throughout gestation until reaching a peak around the time of parturition [18]. In experiment 1, a similar rise in bPAGs early in gestation was observed in the *B indicus* cows used in this study. Although there is a large transient rise in bPAGs during early gestation, no clear function has been identified for these proteins; however, many correlations have been reported with circulating concentrations of bPAGs. Pregnancy status and stage, breed, parity of dam, fetal sex and number, fetal birth weight, placental weight, sire, and many more have been shown to be associated to some degree with bPAG concentrations [18,39–41]. In the present study, circulating concentrations of bPAG at Day 28 were influenced by parity of the dam, sire, and breed. In a recent study, Mercadante et al. [24] reported that cows with *B indicus* genetics (similar to Nelore) had increased circulating concentrations of plasma bPAGs early in gestation. We observed similar results in the present study based on comparison of the current data with bPAG data collected at similar stages of gestation in *B taurus* cattle. The exact cause of this increased bPAG concentration early in gestation is not clear; however, Mercadante et al. [24] also reported differences in fetal size and growth rate. Interestingly, parity of the cow also had a large effect on circulating bPAG concentration on Day 28 of gestation independent of overall body weight, which is a good measure of overall blood volume. Similar results have been shown by Kill et al. [32], which reported that *B taurus* heifers had significantly higher circulating bPAG concentrations compared with mature cows. These data suggest that it is not a simple blood dilution effect and that some other mechanism is taking place in these younger animals. Potential explanations could be the half-life of bPAGs in those individual types of animals, the ability of binucleate cells to secrete products into the maternal circulation or maybe even the function or role that bPAGs are playing in these younger animals.

Limited data have been reported on sire effects on bPAG concentrations early in gestation; however, on the basis of the large influence that the sire plays in placental development, we were interested in examining this relationship. Overall, we saw no relationship between circulating concentrations of bPAGs and sire fertility, but there was a large amount of variation across sires and bPAG production. In addition, of eight sires tested three accounted for 70% of the late embryonic mortality reported in the subset of cows in experiment 2. Surprisingly, after removing all the cows that underwent late embryonic mortality after Day 28 from the analysis, those three sires exhibited significantly decreased circulating concentrations of bPAG compared with the other five sires in the study. Taken together, these data suggest that the sire does influence binucleate cell products, such as bPAGs. Indeed, circulating amounts of bPAG may serve as a novel tool for identifying low fertility sires.

Estrus expression at the time of insemination or ET has been directly correlated with pregnancy success in both beef and dairy cattle [17,42,43]. Preovulatory estradiol coordinates a number of physiological events that directly affect pregnancy establishment and maintenance including gamete transport and preparation of the uterine environment [15]. In beef cattle, Perry et al. [17,43] reported that beef heifers and cows exhibiting estrus within 24 hours of TAI have greater fertility compared with cows that do not exhibit standing estrus. In addition, increased preovulatory concentrations of estradiol and increased postovulatory progesterone production were observed with increased

pregnancy success [17,43]. In a recent study by Pereira et al. [42], lactating dairy cows undergoing TAI or ET had increased fertility and decreased embryonic morality if they exhibited estrus versus those that did not exhibit estrus. Furthermore, lactating dairy cows that experienced pregnancy loss had decreased circulating concentrations of bPAGs early in gestation [35], similar to the present study. In the present study, there was an increase in bPAG concentrations on Day 28 of gestation when comparing Estrotect patch scores at TAI (Day 0). Surprisingly, results of previous work have not reported an association with preovulatory and/or postovulatory estradiol or progesterone production with bPAG production early in gestation [18]. Thus, the current data suggest that cows that exhibit estrus and conceive have increased circulating concentrations of bPAGs on Day 28 and increased likelihood of pregnancy success compared with pregnant cows that did not express estrus at TAI. Future experiments are needed in this area to truly understand this relationship and potential mechanism that is underlying this increase in bPAG production.

4.1. Conclusions

Bovine PAG concentrations increased in circulation on Day 24 of gestation and were successful in diagnosing pregnancy in postpartum Nelore cows. Multiple factors such as parity status and sire were shown to effect circulating concentrations of bPAG at Day 28. Furthermore, bPAGs on Day 28 of gestation were 95% accurate in predicting embryonic mortality during Days 28 to 100 of gestation, which provides a novel model for detecting late embryonic mortality in cattle. This tool could eventually be used to help dissect the physiological and molecular mechanisms that are involved in late embryonic mortality.

Acknowledgments

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