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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