



Circulating concentrations of bovine pregnancy-associated glycoproteins and late embryonic mortality in lactating dairy herds

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ABSTRACT

The objectives of these experiments were as follows: (1) to determine the association between circulating concentrations of pregnancy-associated glycoproteins (PAG) and late embryonic mortality (EM) in lactating dairy cattle following fixed-time artificial insemination (TAI) on d 0 or timed embryo transfer (TET) on d 7, (2) to identify a circulating concentration of PAG on d 31 below which late EM would be likely to occur, and (3) to identify when during gestation (d 31–59) late EM is occurring. Cows were diagnosed pregnant on d 31 of gestation based on presence of a fetal heartbeat and reconfirmed to be pregnant on d 59 of gestation. Late EM occurred when a cow had a viable embryo on d 31 of gestation but not on d 59 following TAI or TET. Only pregnant cows on d 31 were included in the analysis (TAI-maintained, $n = 413$; TAI-EM, $n = 77$; TET-maintained, $n = 238$; TET-EM, $n = 47$). Cows that were pregnant at d 31 of gestation and maintained the pregnancy until d 59 had significantly higher circulating concentrations of PAG at d 31 of gestation compared with cows that experienced late EM between d 31 and 59 of gestation in both TAI and TET. To conduct a more stringent test of the effectiveness of a single circulating PAG concentration (d 31) to predict EM, a receiver-operating characteristic curve was generated to identify a PAG concentration on d 31 that would predict EM with $\geq 95\%$ accuracy in cows that received TAI or TET. Based on positive and negative predicative value analysis, a circulating concentration of PAG below 1.4 ng/mL (TAI; minimal detectable level 0.28 ng/mL) and 1.85 ng/mL (TET) was 95%

accurate in predicting EM (between d 31 and 59) at d 31 of gestation, respectively. Following TET, embryonic loss was tracked by Doppler ultrasound, progesterone, and PAG from d 24 to 59 of gestation, with more than 50% of the loss occurring between d 31 and 38 of gestation. In summary, circulating concentrations of PAG on d 31 of gestation may provide a good marker for predicting EM between d 31 and 59 of gestation, and the data suggest that this model could help predict which cows will undergo late EM.

Key words: bovine pregnancy-associated glycoprotein, pregnancy loss

INTRODUCTION

The factors negatively affecting fertility in the lactating dairy cow are multifaceted; however, it is well documented that embryonic mortality (EM) during the first third of gestation plays a large part in reproductive inefficiency. Intensive genetic selection over the past few years has emphasized both milk production and fertility. This increased selection pressure on fertility has led to a notable increase in reproductive success in dairy cows but has not eliminated the embryonic wastage that occurs during early gestation. Multiple reports have demonstrated that early EM (<28 d of gestation) accounts for a large amount of the loss that occurs during gestation (DeJarnette et al., 1992; Dalton et al., 2001; Sartori et al., 2002). Loss of pregnancies after d 28 represents a smaller proportion of EM. However, the economic consequences of late EM (>28 d of gestation) are reported to be disproportionately greater than that of early EM, because late EM can cause a significant delay in conception date (Silke et al., 2002). In cattle, late EM has been reported to range from 3.2 to 42.7% (Vasconcelos et al., 1997; Cartmill et al., 2001a,b; Lamb, 2002; Ribeiro et al., 2011; Ribeiro et al., 2012; Aono et al., 2013; Pereira et al., 2013; Ribeiro et al., 2013); however, little is known about the exact causes of these losses. The timing of this loss between

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d 30 to 60 of gestation suggests compromised placental function because the timing corresponds to when establishment of a functional cotyledonary placenta in cattle is taking place (e.g., between d 25 and 40; Pfarrer et al., 2006; Aires et al., 2014).

Because placental insufficiency is a potential cause of late EM, identification of a marker of placental function in the maternal circulation may be a useful tool for predicting late EM. Bovine pregnancy-associated glycoproteins (**PAG**) are secreted by the ruminant placenta (Wallace et al., 2015) into the maternal circulation starting about d 24 of gestation and have been used to diagnose pregnancy in cattle (Sasser et al., 1986; Zoli et al., 1992; Green et al., 2005) and may serve as a marker of placental function (Perry et al., 2005; Breukelman et al., 2012; Pohler et al., 2013). The objectives of these experiments were to examine the relationship between d-31 circulating concentrations of PAG and embryonic survivability in lactating dairy cows undergoing timed AI (**TAI**) or timed embryo transfer (**TET**), along with determining when the majority of late EM is occurring during gestation.

MATERIALS AND METHODS

All animal procedures followed the recommendations of the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 1999). Cows that were past the voluntary waiting period (40 d) and not pregnant received the following protocol: an intravaginal progesterone (**P4**) insert containing 1.9 g of P4 (Zoetis, São Paulo, Brazil) and 2.0 mg (i.m.) of estradiol benzoate (2.0 mL of Estrogin, Farmavet, São Paulo, SP, Brazil) on day -11, 25 mg (i.m.) of dinoprost (PGF; 5.0 mL of Lutalyse, Zoetis) on day -4, intravaginal-P4-insert withdrawal and 1.0 mg (i.m.) of estradiol cypionate (0.5 mL of E.C.P., Zoetis) on day -2, and fixed TAI on d 0 (study 1) or TET on d 7 (study 2 and 3). In study 1 and 2 only pregnant cows at d 31 were used, based on presence of a viable embryo (presence of a heartbeat) detected by ultrasound scan. In study 3 only pregnant cows were used, based on the presence of a vascularized corpus luteum (**CL**) at d 24 of gestation, evaluated with a color Doppler ultrasound, an increased circulating concentration of P4 on d 24 (>1.0 ng/mL), and circulating d-24 PAG greater than the baseline of the assay.

Experiment 1—TAI

Timed AI was performed (d 0) by using commercial frozen-thawed semen from 13 different sires. This study used a total of 490 pregnant lactating Holstein cows. At the beginning of the experiment (d -11), cows aver-

aged 147 ± 4.19 DIM, yielded 29.5 ± 0.3 kg of milk per day, and had a BCS of 2.84 ± 0.02 [in a 1 (emaciated) to 5 (obese) scale (Wildman et al., 1982)]; 264 were primiparous (= 1); 209 were multiparous (≥ 2); and cows had been bred 1.96 ± 0.11 times.

Experiment 2—TET

Timed embryo transfer was performed (d 7) by using fresh embryos produced in vitro from 76 donors and commercial frozen-thawed sexed semen (X-bearing sperm) from 9 sires. This study used a total of 285 pregnant lactating Girolando cows (~three-quarters Holstein—*Bos taurus* \times *Bos indicus*). At the beginning of the experiment (d -11), cows averaged 135 ± 4.9 DIM, yielded 18.9 ± 0.4 kg of milk per day, and had a BCS of 2.86 ± 0.03 [in a 1 (emaciated) to 5 (obese) scale (Wildman et al., 1982)]; 90 were primiparous (= 1); 148 were multiparous (≥ 2), and cows had been bred 2.07 ± 0.15 times.

Experiment 3—Profiling of Embryonic Loss Following TET

The TET was performed (d 7) by using fresh embryos produced in vitro from 24 donors and commercial frozen-thawed sexed semen (X-bearing sperm) from 8 sires. This study used a total of 101 pregnant lactating Girolando cows. At the beginning of the experiment (d -11), cows averaged 109 ± 10.6 DIM, yielded 22.9 ± 0.8 kg of milk per day, and had a BCS of 3.08 ± 0.04 [in a 1 (emaciated) to 5 (obese) scale (Wildman et al., 1982)]; 50 were primiparous (= 1); 51 were multiparous (≥ 2); and cows had received an embryo 2.27 ± 0.18 times.

Transrectal Ultrasonography

The uteri of all cows in experiments 1 and 2 were examined on d 31 of gestation following TAI or TET and at d 59 of gestation to determine pregnancy status (viable fetal heartbeat) and incidence of late embryonic or fetal mortality. In experiment 3, cows were examined by ultrasonography on d 24 by using a Doppler ultrasound probe to determine blood flow to the CL. Cows were considered to be pregnant on d 24 if they met the following criteria: (1) a well-vascularized CL, as detected by ultrasound, on the same ovary that possessed a CL at the time of embryo transfer, (2) increased circulating concentrations of P4 (>1.0 ng/mL) on d 24, and (3) the presence of elevated d-24 PAG. Normal transrectal ultrasonography as explained above was conducted on d 31, 38, 45, 52, and 59 of gestation in all cows. Only cows bearing singletons were used in this study.

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