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Associations between pregnancy-associated glycoproteins and pregnancy outcomes, milk yield, parity, and clinical diseases in high-producing dairy cows

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ABSTRACT

Pregnancy-associated glycoproteins (PAG) are produced by the ruminant placenta and secreted into the maternal circulation throughout pregnancy. The extent to which circulating PAG concentrations predict pregnancy outcomes was examined herein. Also, associations between circulating PAG concentrations and various production parameters and clinical diseases were evaluated. Lactating primiparous and multiparous Holstein cows (n = 345) were bred via timed artificial insemination using a standard Ovsynch protocol. Pregnancy was diagnosed by transrectal ultrasonography at d 32, 46, and 74 of gestation. Blood was harvested at d 32 to determine plasma concentrations of PAG and progesterone. Cows pregnant at d 32 that subsequently lost their pregnancy at d 46 and 74 had reduced PAG concentrations. Both artificial insemination service number and parity were associated with plasma PAG concentrations. Concentration of PAG in plasma was greater for cows pregnant from their second or later breeding than those pregnant from the first breeding postpartum, and was increased for primiparous compared with multiparous. In addition, cows with greater milk yield had increased plasma PAG concentrations. No association was detected between body condition score and plasma PAG concentrations. Cows that experienced clinical metritis, metabolic problems, or left displacement abomasum in the early postpartum period preceding breeding had greater plasma PAG concentrations than cows not experiencing these clinical diseases. Also, cows with multiple clinical diseases had increased odds of pregnancy loss when compared with cows not experiencing clinical diseases. Odds ratio testing detected a tendency in the relationship between reduced milk yield and increased pregnancy loss. Collectively, these associations illustrate one feature of the early developing placenta that may predict pregnancy outcomes in dairy cattle. It is unclear if plasma PAG are actively involved with mediating pregnancy outcomes, but modifications in circulating PAG concentrations due to pregnancy loss, milk yield, parity, and clinical disease implicate placental PAG production or PAG release as being responsive to various physiological stimuli.

Key words: placenta, pregnancy loss, clinical disease, milk yield

INTRODUCTION

Pregnancy-associated glycoproteins (**PAG**) are aspartic proteases released in the maternal bloodstream from binucleate cells (**BNC**; also known as trophoblast giant cells) of the ruminant placenta throughout most of pregnancy (Sasser et al., 1986; Green et al., 1998; Telugu et al., 2009). The PAG are implicated for playing roles in placental-endometrial adhesion and fetal antigen sequestering (Wooding et al., 2005), but they are best known for their use in pregnancy diagnosis in cattle and other ruminants (Sasser et al., 1986; Green et al., 2005). Moreover, reductions in circulating PAG concentrations during early pregnancy also are associated with pregnancy failures in dairy and beef cattle (Thompson et al., 2010; Pohler et al., 2013; Ribeiro et al., 2014).

Lactation-induced infertility is one of the greatest costs associated with the US dairy cow industry (Bellows et al., 2002). Pregnancy losses are rampant in lactating dairy cows, ranging from 20 to 50% of all fertile matings (Santos et al., 2004). Infertility is a multifaceted problem. High-producing dairy cows contain reduced circulating steroid hormone concentrations

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(Santos et al., 2004), are more susceptible to heat stress (Wilson et al., 1998), poorly exhibit estrus expression (Lucy, 2001; Sartori et al., 2002), and have reduced oocyte competency and embryo quality (Sartori et al., 2002).

Cow health and successful metabolic transition from a nonlactating to lactating state at parturition are also key components of fertility. Cows that make this transition without adverse clinical disorders (e.g., retained placentae, metritis, severe negative energy balance, metabolic diseases) usually are among the highest producing cows in the herd, and these high-producing cows usually are more fertile than moderate and low producers, which exhibit at least one clinical disorder in the early postpartum period at a higher likelihood than the high-producing cows (Lucy, 2001; Vasconcelos et al., 2011).

The objectives of this study were to (1) determine the association between plasma PAG concentrations collected at d 32 of pregnancy with subsequent pregnancy loss in lactating dairy cattle, (2) determine associations between plasma PAG concentrations with production variables and peri- and postpartum diseases, and (3) define associations between peri- and postpartum diseases with pregnancy loss.

MATERIALS AND METHODS

All animal experimentation was completed in accordance with and with the approval of the Institute of Food and Agricultural Sciences Animal Care and Use Committee at the University of Florida.

Experimental Design

The study was conducted at the University of Florida Dairy Research Unit (Alachua) during 2 time periods: (1) August 2010 to February 2011, and (2) October 2011 to January 2012. Lactating primiparous and multiparous cows were maintained on a diet to equal or exceed protein and energy requirements for maintenance and milk production in accordance with the NRC (2001). The diet was based on alfalfa hay, corn silage, whole cottonseed, soybean hulls, corn grain, citrus pulp, soybean meal, and a mineral-vitamin premix, and designed to contain approximately 16.8% CP, 5.2% ether extract, 1.7% NE_L, and 35.9% NDF. Cows did not receive glucogenic or calcium supplementation postpartum. Cows were milked twice daily throughout lactation.

A Presynch-Ovsynch-56 protocol was used to synchronize the first postpartum timed AI (Pursley et al., 1997). Presynch was conducted by administering $PGF_{2\alpha}$ (25 mg; Lutalyse, Zoetis Inc., Madison, NJ) twice at a 14-d interval. At 11 d after the second $PFG_{2\alpha}$ injection, GnRH was provided (100 μg; Factrel, Zoetis Inc.) followed 7 d later by $PGF_{2\alpha}$ and 56 h thereafter by a second GnRH injection. Between 16 to 20 h later, AI was performed (average = 82 d postpartum). A total of 18 sires were used over the course of the study. Transrectal ultrasonography was completed to diagnose pregnancy at d 32 post-AI by using a portable US scanner (Easi-Scan, BCF Technology, Livingston, UK). The Ovsynch-56 protocol and timed AI was completed in second and subsequent services for cows diagnosed as nonpregnant at d 32 post-AI. In most cases, the semen from the same bull was used for repeated breedings of the same cow.

Blood was collected by coccygeal venipuncture immediately after pregnancy diagnosis (d 32 post-AI; EDTA-coated Vacutainer; Becton Dickinson, Franklin Lakes, NJ). Only pregnant cows on d 32 after AI (n = 345) were enrolled in the experiment. Samples were placed in ice, transported to the laboratory, and centrifuged for plasma separation (1,500 × *g*, 15 min, 4°C). Plasma was frozen at −20°C until analysis of progesterone (**P4**) and PAG. Pregnancy retention was examined at d 46 and 74 post-AI by using transrectal ultrasonography. Pregnancy maintenance to term was determined by monitoring calving. Cows that lost their pregnancy were not used again if they were confirmed pregnant on a subsequent cycle.

Progesterone and PAG Analyses

Plasma P4 concentrations were determined by a solid-phase RIA (Coat-A-Count Progesterone kit, DPC Diagnostic Products Corp., Los Angeles, CA; Seals et al., 1998). The standard curve dilution consisted of duplicate uncoated tubes for total counts and nonspecific binding, and a 100-μL aliquot of increasing progesterone concentrations (0.1, 0.25, 0.5, 1, 2, 5, 10, and 20 ng/mL). Control samples were included multiple times on each assay. The intra-assay coefficients of variation were 1.8 and 3.7% for samples analyzed in the first and second period of the study, respectively. The same control samples were analyzed in both periods.

Circulating plasma PAG concentrations were determined by ELISA as described previously (Green et al., 2005) with slight modifications in Jonathan Green's laboratory at the University of Missouri, Columbia. A pool of 3 anti-PAG monoclonal antibodies recognizing different binucleate cell-specific PAG (bPAG4, 6, 7, 16, 20, and 21) was used as trapping antibodies. A polyclonal antiserum with broad specificity for PAG was used as the primary antibody, and an alkaline phos-