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Growth of the conceptus from day 33 to 45 of pregnancy is minimally associated with concurrent hormonal or metabolic status in postpartum dairy cows

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ABSTRACT

A hypothetical explanation for pregnancy loss in postpartum dairy cows is that the metabolic environment of the cow inhibits the growth of the conceptus and places the pregnancy at risk for loss. The objective of the current study, therefore, was to model the association between cow-level metabolic indicators and conceptus growth during early pregnancy (day 33–45 after AI) and to determine if an association (if present) is large enough to cause pregnancy loss. Metabolic indicators included milk production, changes in body weight and body condition score, parity, and concentrations of circulating hormones and metabolites (glucose, non-esterified fatty acids, growth hormone, IGF1, progesterone, and pregnancy-associated glycoproteins). One-hundred cows were enrolled. Cows that became pregnant with single conceptus pregnancies (n = 53) weighed more ($P < 0.007$) and had fewer uterine polymorphonuclear neutrophils (uterine health indicator; $P < 0.051$) compared with cows that failed to become pregnant. The embryo and amniotic vesicle were measured by using ultrasound on day 33, 35, 38, 40, 42, and 45 of pregnancy. Most of the cow-level indicators that were included in the model of conceptus growth failed to achieve statistical significance. Day of pregnancy had the largest effect on conceptus growth (size and cross-sectional area of the embryo and amniotic vesicle; $P < 0.001$). There were effects of sex of fetus (male fetuses larger than female), insulin (negative association), and body weigh change (positive association) on embryo length and cross-sectional area but these effects were small when compared with the range in conceptus length or area that we observed. The conclusion was that the capacity of the cow to become pregnant was associated with body weight and uterine health but we failed to find a large association with metabolic status on conceptus growth from day 33 to 45 of pregnancy in lactating dairy cows.

1. Introduction

Pregnancy rate is an important economic metric on dairy farms. While increased production can contribute to decreased pregnancy rates, infertility is a complex

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issue with many contributing factors (Lucy, 2001). These factors include transition cow disease, metabolic imbalances, immunosuppression, abnormal estrous cyclicity, poor estrous expression, low conception rates, and pregnancy loss. Whereas abnormal estrous cyclicity, poor estrous expression and low conception rates can be partially overcome by using timed AI programs, there is no effective treatment to date to prevent pregnancy loss. In over 4800 lactating dairy cattle examined between day 28–58 after AI, pregnancy loss ranged from 3.2% and 42.7% in dairy herds, with an average value of 12.8% (Santos et al., 2004). Estimates of pregnancy loss in dairy cows from the more-recent literature are similar to those reported by Santos et al. (2004) and indicate that the incidence over time has not changed (Ricci et al., 2015). Based on the magnitude of the loss across the industry, there is a compelling need to understand the underlying mechanisms that lead to pregnancy loss; particularly during the period of early pregnancy (approximately day 32–45 after AI).

Potential mechanisms that contribute to pregnancy loss are found during the early postpartum period as well as during the breeding period (60–100 days after calving). Uterine disease early postpartum, for example, is a risk factor for pregnancy loss (Santos et al., 2009). In their recent work, Ribeiro et al. (2013) and Machado et al. (2015) found that cows with metritis were more than twice as likely to undergo pregnancy loss. Likewise, mastitis can cause the release of pro-inflammatory molecules that negatively affect the pregnancy (Sheldon, 2015).

In addition to disease, the hormonal and metabolic status of the cow may impinge upon the developing pregnancy and perhaps affect its development (Lucy et al., 2014). The development of the preovulatory follicle and circulating progesterone (P4) concentrations within three weeks after breeding are critical to the establishment and maintenance of pregnancy (Bridges et al., 2013). Less is known about the potential for hormones and metabolites that circulate concurrently with pregnancy to affect the growth of the conceptus. Green et al. (2012) found that lactating cows compared with non-lactating cows had smaller conceptuses on day 28, 35, and 42 of pregnancy. The authors proposed a hypothetical link between pregnancy loss in lactating cows and the presence of a smaller conceptus. In a subsequent paper, the same laboratory reported that lower circulating glucose in lactating (compared with non-lactating) cows was associated with less glucose in the fetal fluids collected from lactating cows (Lucy et al., 2012). Glucose is the primary substrate for growth of the conceptus (Battaglia and Meschia, 1978). Perhaps glucose constrains the growth of the conceptus, leading to a smaller conceptus that is predisposed to loss (Lucy et al., 2014).

We designed a study to examine the association of known metabolic indicators with the growth of the conceptus between day 33 and 45 of pregnancy (a known period of pregnancy loss). The metabolic indicators included milk production, changes in body weight (BW) and body condition score (BCS), and concentrations of circulating hormones and metabolites. The hypothesis was that measurements made at the cow level would be associated with the growth of the conceptus between day 33 and 45 of pregnancy. Furthermore, slower growth would be found in cows with pregnancy loss. This new information could potentially reveal mechanisms leading to pregnancy loss in postpartum cows.

2. Materials and methods

2.1. Estrus synchronization and pregnancy diagnosis

One-hundred lactating Holstein dairy cows at the University of Missouri Foremost Research Center were enrolled in the Presynch Ovsynch_56 protocol beginning at approximately 42 days postpartum (Fig. 1). The presynchronization protocol consisted of an IM injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 5 mL Lutalyse; 25 mg dinoprost tromethamine, Zoetis, NJ) with a second $PGF_{2\alpha}$ injection 14 days later. The Ovsynch_56 protocol began 14 days after presynchronization with an IM injection of gonadotropin-releasing hormone (GnRH; 2 mL Factrel; 100 μg gonadorelin, Zoetis, NJ), followed 7 days later by a $PGF_{2\alpha}$ injection, followed 56 h later by a GnRH injection, and completed with timed AI 16 h later. Pregnancy diagnosis was performed by transrectal ultrasonography using a Sonosite Edge equipped with a variable MHz linear probe (SonoSite Inc., Bothell, WA) 32 days after AI. Pregnancy was determined by the presence of an embryo with a visible heartbeat. Animals diagnosed as non-pregnant at examination were re-enrolled in the Ovsynch_56 protocol on the same day as diagnosis. Animals that were not pregnant to first or second AI were removed from the study.

2.2. Conceptus measurements

There were 65 pregnant cows (65%) after two inseminations. Five cows pregnant with twins were removed from the study because of the likely effect of twin pregnancy on the growth of individual conceptuses. An additional 7 cows were removed because of pregnancy loss (n = 2) or being culled from the herd (n = 5). The remaining pregnant cows (n = 53; 122 ± 18 days of lactation at pregnancy diagnosis) had singleton pregnancies that were examined by transrectal ultrasonography on day 33, 35, 38, 40, 42, and 45 of pregnancy using an Aloka 900 ultrasound with a 7.5 MHz transducer (Hitachi Aloka Medical Ltd., Wallingford, CT). The embryo and amniotic vesicle were imaged with the ultrasound. The ultrasound frame was frozen when a clear image of the conceptus was obtained that included the crown-rump length of the embryo as well as the entire amniotic vesical. Measurements of the crown-rump length (l) and width (w) of the embryo and the l and w of the amniotic vesicle were made using electronic calipers within the ultrasound. Fetal sex was determined by using transrectal ultrasonography between day 60 and 80 of pregnancy and confirmed at birth.

2.3. Cow measurements

An endometrial sample was collected for cytological examination by using a cytobrush (Cytobrush Plus cell collector, Cooper Surgical, CT) 1 ± 2 days after the first GnRH injection of the initial Ovsynch_56 protocol approximately 70 days postpartum (Fig. 1). The vulva was cleaned