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Amino acid concentrations in uterine fluid during early pregnancy differ in fertile and subfertile dairy cow strains

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ABSTRACT

The objective of this study was to determine if free AA concentrations in uterine luminal fluid (ULF) and plasma differed between dairy cow strains that differ phenotypically for fertility and to evaluate the effect of the presence of a conceptus on ULF AA concentrations. Uterine luminal fluid was obtained postmortem from cows characterized on the basis of genetic ancestry as fertile (n = 11) or subfertile (n = 11) strains. At slaughter, cows were at a similar stage of lactation (fertile, 85 ± 1 d and subfertile, 87 ± 1 d postpartum, respectively). Cows were slaughtered on either d 17 of the estrous cycle [nonpregnant (n = 10): fertile n = 5; subfertile n = 5] or d 17 of pregnancy [10 d after embryo transfer, which was undertaken 7 d after estrus (n = 12, pregnant): fertile n = 6, subfertile n = 6]. Uterine luminal fluid was collected from each uterine horn of the pregnant (gravid and nongravid horns) and nonpregnant (horn ipsilateral and contralateral to the corpus luteum) cows. Plasma harvested on the day of slaughter and ULF samples were analyzed for AA determination using HPLC. The main effects of genetic strain, reproductive status, and their interactions on ULF and plasma AA content were tested. Additionally, the effect of uterine horn on ULF AA was tested for the pregnant and nonpregnant cows. Reproductive status had the greatest effect on AA concentrations in ULF. The concentrations of Leu, Met, Phe, Val, 1-methyhistidine, Asp, essential, ketogenic, and branched-chain AA, and those AA classified as both glucogenic and ketogenic were greater in the ULF collected from pregnant cows, with taurine being lower. Additionally, we observed effects of uterine horn and genetic strain × uterine horn interaction for ULF AA concentrations. Concentrations of the essential AA plus Met and Phe were greater in the ULF from the gravid horn, irrespective of strain. The ULF from the gravid horn of fertile cows contained the greatest concentrations of nonessential, glucogenic, branched-chain AA, and Leu, Thr, Ala, Ser, and Asp. With the exception of Asp, plasma AA profiles were not different in fertile and subfertile strains. These data support the hypothesis that reproductive status modifies the AA profiles of the ULF and that these profiles differ in fertile and subfertile genetic strains. Successful pregnancy depends on the complex interactions between the developing conceptus and uterine environment. Understanding the mechanisms contributing to maternal–conceptus communication using models with divergent fertility phenotypes could provide information regarding novel mechanisms to improve dairy cow fertility.

Key words: amino acid, uterine fluid, plasma, genetic strain

INTRODUCTION

Successful pasture-based dairying is dependent on a seasonal calving pattern so that peak pasture demand aligns with peak pasture availability (Holmes et al., 2002; Verkerk, 2003). Studies comparing North American (**NA**) and New Zealand (**NZ**) Holstein-Friesians (**HF**) report that, as a group, the NA HF strain has reduced pregnancy rates (Macdonald et al., 2008; Coleman et al., 2009) and conception rates (Horan et al., 2005) compared with the NZ strain in pasture-based systems. The underlying physiological differences that might explain differences in reproductive outcomes between NZ and NA HF strains remain poorly defined. Previous studies have reported differences between the NZ and NA strains, with lactating cows and postpubertal heifers from the NA (subfertile) strain reported to have longer luteal phase and estrous cycle compared with the NZ (fertile) strain (McNaughton, 2003; Meier et al., 2009b). Others have reported differences among these fertile and subfertile strains in either luteal or follicular characteristics (Horan et al., 2005), embryo quality (de Feu et al., 2008), and maternal environment (endometrial fatty acid content; Meier et al., 2009a). Additionally, genes implicated in immune tolerance to the embryo and luteolysis and genes that promote embryo growth and development were downregulated

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in the endometrium from NA HF cows compared with endometrial gene expression of NZ HF cows (Walker et al., 2012). Collectively, these data indicate that the uterine environment, early embryo growth, and signaling mechanisms between the preimplantation conceptus and the maternal environment contribute to differences in fertility of these genetic strains.

The survival of the embryo and the growth of the preimplantation conceptus, including its subsequent production of IFN-τ (Heap et al., 1979; Bazer et al., 1986; Robinson et al., 2006), are crucial in the successful establishment of pregnancy. The preimplantation conceptus receives its early nutrition from uterine secretions known as the histotroph (Roberts and Bazer, 1988). Amino acids are an important component of the histotroph, contributing to protein synthesis, acting as signaling molecules, and regulating embryo development and implantation (Steeves and Gardner, 1999; Van Winkle, 2001; Martin et al., 2003). Altering AA availability in culture modifies the subsequent production and use of AA by the embryo (Steeves and Gardner, 1999; Morris et al., 2002). Additionally, AA concentrations in the uterine luminal fluid (**ULF**) differ during the estrous cycle and with the presence of the preimplantation conceptus (Hugentobler et al., 2007; Groebner et al., 2011a). Groebner et al. (2011a) reported the effect of pregnancy on AA concentrations in the ULF at 12, 15, and 18 d, with significant effects from d 15 of pregnancy onward. Together, these studies have identified the patterns of depletion and production of AA through the most critical stages for pregnancy recognition in cattle.

Aspects of embryo quality have been reported to differ between NZ and NA strains (Meier et al., 2009a); more recently, Walker et al. (2012) reported a downregulation of IFN-τ responsive genes, genes involved in preventing luteolysis and supporting embryo growth in the endometrium collected from NA cows. It was hypothesized that the reported effect of HF genetic strain (NZ and NA) on endometrial gene expression reported by Walker et al. (2012) is associated with differences in the composition of the ULF supporting the preimplantation conceptus. Therefore, the objectives of this study were (1) to determine if free AA concentrations in ULF differed among dairy cow strains, and (2) to evaluate the effect of pregnancy on ULF AA concentrations. Additionally, concentrations of plasma AA were evaluated.

MATERIALS AND METHODS

Animals and Management

Twenty-two of 27 Holstein-Friesian cows were used in this study (see Figure 1). Cows were in their second to fifth lactation and were from 2 genetic strains [<23% NA genetics (fertile; n = 11) or >92% NA genetics (subfertile; n = 11)]. The 22 cows were in 2 reproductive states: d 17 of the estrous cycle, termed nonpregnant (fertile n = 5; subfertile n = 5) or having received an embryo by implant 10 d earlier, 7 d after estrus, termed pregnant (fertile n = 6; subfertile n = 6), from which a conceptus was retrieved in the uterine flushings after slaughter (see Figure 1). Cows were managed as a single herd, grazing fresh pasture in an intensive rotational manner as described previously (Roche et al., 2006). Cows were allocated a pasture allowance of >40 kg of DM/cow per day (measured to ground level). Pasture grazing residuals were used to ensure adequate pasture allowance: postgrazing residuals of >1,800 kg of DM/ha were targeted during spring. All procedures were undertaken with the approval of the Ruakura Animal Ethics Committee (Hamilton, New Zealand) in accordance with the New Zealand Animal Welfare Act (1999).

An average BW was calculated from daily BW measures taken over 4 consecutive days during the week before slaughter. An average BCS was calculated from individual scores of 6 body sites (on a scale of 1 to 10, 1 being emaciated, and 10 being obese; Roche et al., 2004). A composite milk sample obtained from the p.m. and a.m. milkings immediately before slaughter was analyzed for milk yield, fat, CP, and total solids (FT120, Foss Electric, Hillerød, Denmark). Table 1 provides a summary of the phenotypic descriptions of the dairy cows used, including ancestry, age, BW, BCS, DIM, milk yield, milk composition, and pedigree information at the time of slaughter.

Reproductive Management

All animals enrolled in the study were free of purulent vaginal discharge following the assessment of the vaginal contents (Metricheck device; Simcro Tech, Hamilton, New Zealand; McDougall et al., 2007) before the initiation of the estrous synchrony program, and had not been treated for mastitis during that lactation. Cows were grouped by calving date, and estrous cycles were synchronized at similar DIM (fertile: 60 d; subfertile: 58 d; nonpregnant: 58 d, pregnant: 60 d: SE = 4.6 d) using a controlled intravaginal drug-release device containing progesterone (1.38 g; CIDR-B, Zoetis, Auckland, New Zealand) inserted for 8 d (day of insertion = d −8). On the day of insertion, cows were injected with 2 mL of estradiol benzoate (2 mg i.m., Cidirol Bomac Laboratories Ltd., Auckland, New Zealand). All cows received an injection of sodium cloprostenol (500 μg, EstroPlan, Parnell Laboratories NZ Ltd., Auckland, New Zealand) on the morning and afternoon of d 6 and