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Hepatic steroid metabolizing enzyme activity during early, mid, and late bovine pregnancy

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ABSTRACT

The objective was to examine hepatic steroid inactivating enzymes throughout gestation and determine the effect of early to mid-gestation maternal nutrient restriction followed by realimentation on the activity of these enzymes. On day 30 of gestation, cows were assigned to dietary treatments: control (CON; 100% National Research Council; n = 18) and restricted (RES; 60% National Research Council; n = 30). On day 85, cows were slaughtered (CON, n = 6 and RES, n = 6), remained on control (CC, n = 12) and restricted (RR, n = 12), or were realimented to control (RC, n = 11). On day 140, cows were slaughtered (CC, n = 6; RR, n = 6; RC, n = 5), remained on control (CCC, n = 6; RCC, n = 5), or were realimented to control (RRC, n = 6). On day 254, all remaining cows were slaughtered. Jugular blood samples were collected before the slaughter for steroid analysis. At slaughter, maternal liver samples were collected for hepatic enzyme activity analysis. Activity of cytochrome P450 3A decreased ($P = 0.05$) from mid- to late-gestation. Peroxisome proliferator-activated receptor alpha DNA binding activity was increased ($P < 0.01$) on day 140 and 254 of gestation vs day 85. Concentrations of estradiol-17β (E2) increased ($P < 0.01$) as gestation proceeded, whereas progesterone concentrations (P4) tended to increase ($P = 0.06$) from mid- to late-gestation. Activity of cytochrome P450 1A and 2C were decreased ($P < 0.05$) in nutrient restricted cows vs control, whereas concentrations of E2 were increased ($P < 0.05$) in nutrient restricted cows vs control. A longer period of nutrient realimentation from mid- to late-gestation increased ($P < 0.05$) aldo-keto reductase 1C activity and decreased ($P < 0.05$) P4 concentrations compared with the shorter period of nutrient realimentation. In addition, significant negative correlations were observed for cytochrome P450 3A activity vs E2 ($r^2 = -0.30$; $P < 0.05$) and aldo-keto reductase 1C activity vs P4 ($r^2 = -0.29$; $P < 0.05$). The present study implicates hepatic steroid inactivation in the partial modulation of peripheral concentrations of E2 and P4 during gestation.

1. Introduction

Pregnancy is known to influence xenobiotic metabolism via alterations in expression and activity of cytochrome P450 (P450) enzymes [1,2]. Hepatic protein expression and activity of P450s are consistently lower in pregnant vs nonpregnant rats of similar age [3,4]. Similarly, pregnancy typically decreases P450 expression and activity in mice, which is strongly correlated with peroxisome proliferator-activated receptor alpha (PPARα) [2]. In addition to xenobiotic metabolism, P450 enzymes are involved in a number of pathways including endogenous vitamin D3 activation, metabolism of cholesterol to bile

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acids, and metabolism of all major classes of steroid hormones [5]. Apart from characterization studies in rodents, limited studies have examined hepatic activity of P450s in pregnant livestock species, which could have direct implications in modulating peripheral concentrations of endogenous hormones.

Progesterone (P4) is required for the maintenance of pregnancy as it blocks uterine contractions from occurring creating a quiescent uterine environment until parturition [6–8]. In ruminants, pregnancy loss may be because of decreased concentrations of P4 [9]. It is important to note that both production from the corpus luteum and/or hepatic steroid inactivation impacts peripheral concentrations of P4 [10]. Cattle with an elevated dry matter intake have increased blood flow to the digestive tract and liver. This in turn leads to an increased delivery rate of steroids to the liver, and thus, increases metabolism of these substrates [11]. Excessive hepatic steroid inactivation contributes to decreased peripheral concentrations, which can alter reproductive performance. Previous studies have observed a decrease in peripheral concentrations of P4 and estradiol-17β (E2) in mid- to late-pregnant ewes fed 140% of nutrient requirements vs control [12]. In contrast, peripheral concentrations of P4 and E2 were increased in ewes nutrient restricted from mid- to late-gestation [12]. This variation in steroid bioavailability during mid- to late-gestation could alter placental nutrient exchange or uterine blood flow, which are partially controlled by P4 [13] or E2 [14], respectively.

Steroids are highly lipophilic and therefore difficult for animals to excrete through the urine and feces. Several hepatic enzymes contribute to steroid inactivation by adding polar groups to the cyclopentanoperhydrophenanthrene nucleus. These enzymes include cytochrome P450 1A (CYP1A), 2C (CYP2C), 3A (CYP3A), aldo-keto reductase 1C (AKR1C), and uridine diphosphate-glucuronosyltransferase (UGT) [15–17]. Currently, these enzymes have not been characterized throughout bovine gestation, and little is known about how hepatic enzyme function may contribute to reproductive performance via substrate bioavailability. Moreover, a paucity of information exists on bovine hepatic transcription factors that may regulate steroid metabolic enzymes during gestation. Therefore, our primary objective was to characterize hepatic steroid inactivating enzymes throughout gestation as well as the hepatic transcription factor PPARα. In addition, we also examined the effect of maternal nutrient restriction followed by realimentation at different stages of gestation on hepatic steroid inactivating enzymes. We hypothesized that maternal nutrient restriction would decrease hepatic steroid inactivating enzyme activity and increase peripheral concentrations of P4 and E2. Moreover, a shorter duration of realimentation following nutrient restriction would result in increased concentrations of P4 and E2 during mid- to late-gestation.

2. Materials and methods

2.1. Animals, diets, and breeding

Animal care and use was approved by the North Dakota State University Animal Care and Use Committee. Animal management, breeding, and experimental design were previously published [18]. Briefly, multiparous crossbred beef cows (initial body weight = 620.5 ± 11.3 kg and body condition score = 5.1 ± 0.1) predominately of Angus breeding were synchronized using Select Synch plus P4 insert and fixed-time artificial insemination. On day 27 and 28 post-insemination, pregnancy was confirmed via transrectal ultrasonography (500-SSV; Aloka, Tokyo, Japan) using a linear transducer probe (5 MHz). Nonpregnant cows restarted the same breeding protocol; cows were allowed to be artificial insemination only twice during the experiment. On day 30 of gestation, cows were randomly assigned to dietary treatments (Fig. 1): control (CON; 100% NRC; n = 18) and nutrient restriction (RES; 60% NRC; n = 34). On day 85, cows were slaughtered (CON, n = 6 and RES, n = 6), remained on control (CC; n = 12) and restricted (RR; n = 12) treatments, or were realimented to control (RC; n = 11). On day 140, cows were slaughtered (CC, n = 6; RR, n = 6; RC, n = 5), remained on control (CCC, n = 6; RCC, n = 5), or were realimented to control (RRC, n = 6). On day 254, all remaining cows were slaughtered (CCC, n = 6; RCC, n = 5; RRC, n = 6).

Diet composition and nutrient analysis were previously published [18]. Briefly, the control diet consisted of grass hay fed to meet 100% net energy recommendations for maintenance and fetal growth [19] and to meet or exceed metabolizable protein, mineral, and vitamin recommendations. Nutrient analysis determined the grass hay to consist of 11.8% ash, 8.1% crude protein, 69.2% neutral detergent fiber, and 41.5% acidic detergent fiber on a dry matter basis. Nutrient restricted cows received 60% of the same control hay diet. Cows were individually fed once daily in a Calan gate system and had ad libitum access to water. Vitamin and mineral supplement was top dressed 3 times per week at a rate of 0.18% of hay dry matter intake to meet or exceed vitamin and mineral requirements relative to dietary net energy intake [19]. The vitamin and mineral supplement (Trouw Dairy VTM with Optimins; Trouw Nutrition International, Highland, IL) consisted of 10% Ca, 5% Mg, 5% K, 2.7% Mn, 2.7% Zn,

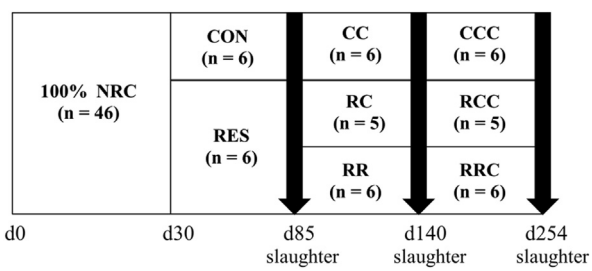

Fig. 1. Experimental design consisted of training 46 beef cows to the Calan gate feeding system from day 0 to 30 of gestation [18]. On day 30, cows were randomly assigned to 1 of 2 dietary treatments: control (CON; 100% NRC; n = 18) and nutrient restriction (RES; 60% NRC; n = 30). On day 85, cows were slaughtered (CON, n = 6 and RES, n = 6), remained on control (CC; n = 12) and restricted (RR; n = 12) treatments, or were realimented to control (RC; n = 11). On day 140, cows were slaughtered (CC, n = 6; RR, n = 6; RC, n = 5), remained on control (CCC, n = 6; RCC, n = 5), or were realimented to control (RRC, n = 6). On day 254, all remaining cows were slaughtered (CCC, n = 6; RCC, n = 5; RRC, n = 6).