The effects of nutrient restriction on interferon-tau and pregnancy-specific protein-B mRNA during the establishment of pregnancy in beef heifers

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The objectives of this project were to elucidate the effects of maternal nutrient restriction on PSP-B and IFN-τ in placental formation, placental function and establishment of pregnancy. PSP-B and IFN-τ were expressed differentially during early gestation and may be important to the establishment of pregnancy.

Summary

We hypothesize that pregnancy-specific protein-B (PSP-B) and interferon-τ (INF-τ) will be expressed differentially during early pregnancy (days 16 to 50) and will be influenced by plane of maternal nutrition. Commercial Angus crossbred heifers (n = 49; about 16 months of age; body weight [BW] = 713 ± 62 pounds) were maintained on a total mixed ration (TMR) and supplemented with dried distillers grains with solubles. All heifers were subject to 5-day CO-Synch + CIDR estrus synchronization protocol and artificial insemination (AI) to a single Angus sire (day of breeding = day 0). On the day of breeding, heifers were assigned randomly to dietary treatments. One-half were assigned to the control diet (CON) targeted to gain .45 kg/day and the remaining half were assigned to a restricted diet (RES) and received 60 percent of control diets. Heifers were subjected to ovariohysterectomy on days 16, 34 and 50 of gestation. Utero-placental tissues were obtained from the uterine horn ipsilateral (P) and contralateral (NP) to the corpora lutea (CL) and separated into maternal caruncle (CAR); maternal endometrium, inter-caruncle (ICAR); and fetal membrane (FM) After being collected, all tissues were snap-frozen in liquid nitrogen-cooled 2-Methylbutane and stored at minus 80 C. *Pregnancy-specific protein-B* increased ($P < 0.01$) by 18,000-fold as gestation progressed in P-CAR. *Pregnancy-specific protein-B* was increased ($P < 0.01$) on days 34 and 50 (337.3 and 203.2 ± 60.9, respectively), compared with day 16 (10.4 ± 60.9). We found no interactions between stage of gestation and nutritional treatment for PSP-B (P = 0.22). In conclusion, maternal nutri-

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ent restriction only influenced *INF-τ*, but *PSP-B* and *INF-τ* were expressed differentially in maternal and fetal tissues at critical time points during the first 50 days of gestation in beef heifers.

Introduction

Placental development is closely related to fetal growth and is sensitive to maternal nutrient supply from the earliest stages of pregnancy (Reynolds and Redmer, 2001) by facilitating the transfer of nutrient, gas and waste (Ramsey, 1982). Inadequate maternal nutrient supply leads to poor placental development, resulting in compromised fetal growth (Caton and Hess, 2010).

Twenty-one members of the pregnancy-associated glycoprotein (PAG) family have been discovered to date (Green et al., 2000). The assortment of PAG present may provide a multitude of binding sites and perform a variety of functions at and during the formation of the feto-maternal interface (Green et al., 1998).

The binucleated cells and modern PAGs seem to interact extensively with maternal connective tissue that develops during placental villi formation (Wooding et al., 2005). However, the exact functions of both types of PAG remain to be elucidated.

Researchers have speculated that PAGs may be involved in proteolytic activation of growth factors and other molecules specific to pregnancy, protection of fetal tissues from maternal immune response, transport of hormones between fetal and maternal tissues, and cell-to-cell fusion (Wooding et al., 2005). Therefore, we hypothesize that *pregnancy-specific protein-B (PSP-B)* and *interferon-τ (INF-τ)* will be expressed differentially during early pregnancy (days 16 to 50) and will be influenced by plane of maternal nutrition.

Experimental Procedures

All animal procedures were conducted with approval from the Institutional Animal Care and Use Committee at North Dakota State University (A16049). Commercial Angus crossbred heifers (n = 49; about 16 months of age; BW = 713 ± 62 pounds) were transported 142 miles from the Central Grasslands Research Extension Center (Streeter, N.D.) to the Animal Nutrition and Physiology Center (North Dakota State University, Fargo, N.D.).

The heifers were housed in pens, with six heifers per pen, and individually fed daily in an electronic head gate facility (American Calan; Northwood, N.H.) at 8 a.m. Heifers were maintained on a total mixed ration (TMR) (48.4 percent dry matter [DM], 5.3 percent crude protein [CP], 29.4 percent neutral detergent fiber [NDF], 6.8 percent ash), supplemented with dried distillers grains with solubles (87.5 percent DM, 31.3 percent CP, 53.4 percent NDF, 8.2 percent ash), and granted ad libitum access to water.

All heifers were subjected to 5-day CO-Synch + CIDR estrus synchronization protocol and artificial insemination (AI) to a single Angus sire (day of breeding = day 0). On the day of breeding, heifers were assigned randomly to dietary treatments. One-half were assigned to control diet (CON) targeted to gain .45 kg/day and the remaining half were assigned to the restricted diet (RES) and received 60 percent of control diets.

Heifers were subjected to ovariohysterectomy on days 16, 34 and 50, as previously described, McLean et al., (2016). Thus, experimental design for the pregnancy analysis was a 2 × 3 factorial design. Nonbred, nonpregnant control heifers (NP; n = 6) were ovariohysterectomized on day 16 of the luteal cycle following the synchronization cycle. The NP heifers and heifers that were ovariohysterectomized on days 16, 34 and 50 and fed the CON diet were used in a CRD to address comparisons of pregnancy status and establishment.

Pregnancy was confirmed via trans-rectal ultrasonography day 28 and again on the day of surgery (day beyond 28). During surgery, the left and right uterine arteries, left and right spiral arteries and the cervix were ligated and then the uterus was removed.

Uterine contents were held in place with a 24-centimeter (cm) Crafoord Coarctation Clamp (Integra-Miltex; Plainsboro, N.J.) placed just cranial to the cervical ligatures during and after removal from the body cavity. Following surgery, heifers were kept in individual pens during recovery and stitches were removed 14 days after surgery (McLean et al., 2016), then the animals were returned to the control diets.

Immediately upon removal from the body cavity, tissues were trimmed of excess broad ligament, fat and nonreproductive tissues. Gravid uterus, individual and total ovarian, and CL weights were taken, as well as CL measurements for CL area before fixation, freezing and storage. Utero-placental tissues were obtained from the uterine horn ipsilateral (containing the embryo) to the CL (pregnant uterine horn), maternal caruncle (P-CAR) and maternal endometrium, inter-caruncle, (P-ICAR).

Tissues were obtained in the uterine horn contralateral (opposite the embryo) to the CL (non-pregnant horn), maternal caruncle (NP-CAR) and maternal endometrium, inter-caruncle, (NP-ICAR). Fetal membranes (FM) were collected on days 16, 34 and 50. After being collected, all tissues were snap-frozen in liquid nitrogen-cooled isopentane (Sigma-Aldrich; St. Louis, Mo.) and stored at minus 80 C.

Gene expression was analyzed for threshold cycle using a 7500 Fast

Real-Time PCR System (Applied Biosystems, Grand Island, N.Y.) with SYBR Green Master Mix (Bio-Rad Laboratories, Hercules, Calif.). Gene expression for maternal tissues was calculated using the ΔΔCT method, with *β-actin* as the reference gene and the average of NP expression as the control (set to 1) within each tissue.

Fetal membrane gene expression was calculated using the ΔΔCT method, with *β-actin* as the reference gene and of the ΔCT for uterine endometrium of each individual gene as the control (set to 1) within each tissue.

Statistical analyses for gene expression of *PSP-B* and *INF-τ* were conducted as a 2 × 3 factorial with individual heifer as the experimental unit via the GLM procedure of SAS version 9.4 (SAS Inst. Inc., Cary, N.Y.) to test for a stage of gestation × nutritional plane interaction or the main effects of nutritional plane and stage of gestation. Means were separated using the LSMEANS statement of SAS with differences determined at a *P*-values ≤ 0.05.

Results and Discussion

Factors that influence fetal and placental growth and development include maternal plane of nutrition, number of fetuses, maternal parity and age, maternal and fetal genotype, and maternal stress (Reynolds et al., 2010). The long-term effects of restricted nutrient intake during early gestation may be associated with impaired placental development or poor contact during the establishment of the feto-maternal interface, resulting in intrauterine growth retardation (Zhang et al., 2015).

The pregnancy-associated glycoproteins (PAGs) have been found only in binucleate trophoblast cells (Wooding et al., 2005). *Pregnancy-specific protein-B* increased ($P < 0.01$) by 18,000-fold as gestation progressed in P-CAR (Figure 1A).

In P-ICAR, the expression of *pregnancy-specific protein-B* was increased ($P < 0.01$; Figure 1B) on days 34 and 50, compared with day 16. In NP-CAR, the expression of pregnancy-specific protein-B increased ($P < 0.01$) by 317-fold as gestation progressed in NP-CAR (Figure 1C). The expression of *PSP-B* was increased ($P = 0.02$) on days 34 and 50, compared with day 16, in NP-ICAR (data not shown).

The PAGs seem to interact extensively with maternal connective tissue that develops during placental villi formation (Wooding et al., 2005). Researchers have theorized that PAGs may be involved in proteolytic activation of growth factors and other molecules specific to pregnancy, protection of fetal tissues from maternal immune response, transport of hormones between fetal and maternal tissues, and cell to cell fusion (Wooding et al., 2005).

The increase in expression of PSP-B in our data supports functions for cell-to-cell fusion and transport of hormones at the feto-maternal interface. The interaction of maternal caruncles and fetal cotyledons is the most intimate contact between maternal and fetal tissues and could add to the evidence to support

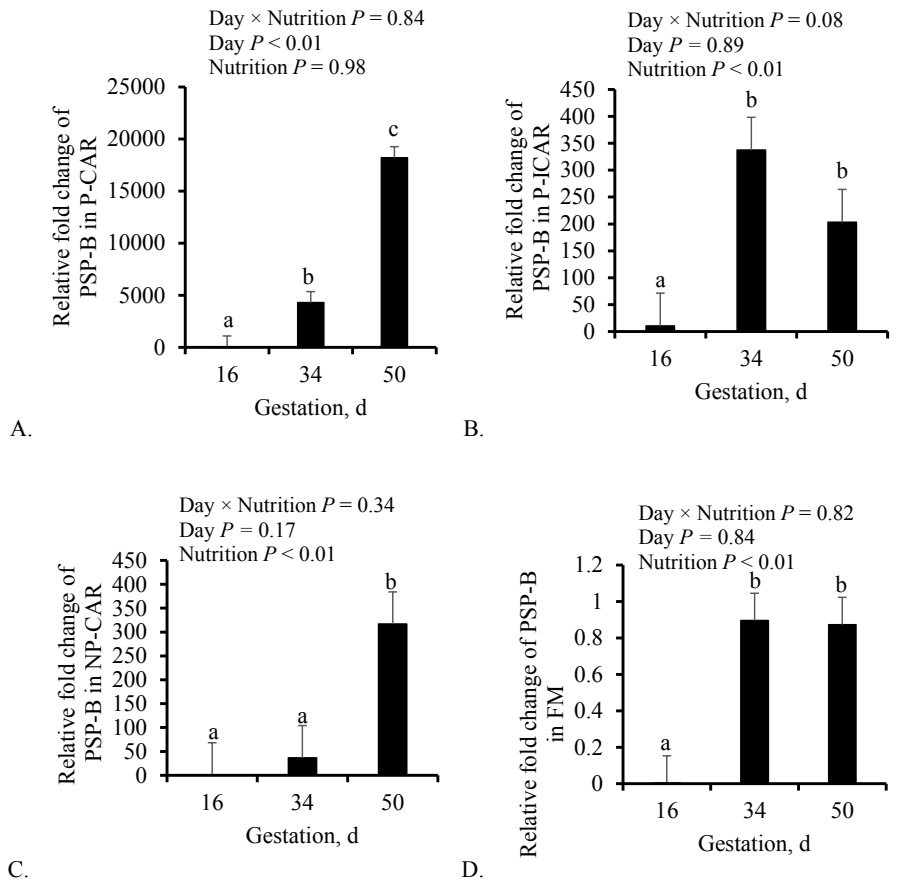

Figure 1. Expression of PSP-B in utero-placental tissues during early gestation. A) pregnant uterine horn caruncle (P-CAR) B) pregnant uterine horn endometrium (P-ICAR) C. non-pregnant uterine horn caruncle (NP-CAR) D. expression in fetal membranes. Data presented as a $2^{-\Delta\Delta CT}$ fold change normalized to *β-Actin* and the average of NP.

[a,b]Means without a common superscript differ ($P < 0.05$)

a role in immune protection and further implicate PSP-B in cell-to-cell fusion functions. The secretion of *INF-τ* from the trophoblast is widely accepted as the ruminant signal for pregnancy recognition secreted from the trophoblast (Spencer et al., 2007). Nutritional treatment also influenced *INF-τ* mRNA expression, with RES heifers having decreased (P = 0.05) *INF-τ* expression, compared with CON feed heifers (Figure 2A).

In NP-ICAR, the nutritional plane tended (P = 0.09) to influence INF-τ expression, with greater expression in CON heifers, compared with RES heifers (data not shown). In FM, *INF-τ* had an interaction of stage of gestation × nutritional plane (P < 0.01). Fetal membranes on day 16 were greater than all other days, and RES heifers were greater, compared with CON heifers (Figure 2B).

Fetal membrane expression of *INF-τ* had an interaction between stage and nutritional plane on day 16 that was greater than all other days, and RES heifers were greater, compared with CON heifers. The expression of *INF-τ* in P-CAR was influenced by nutritional treatment and *INF-τ* expression in FM was increased in RES heifers more during the time of maternal recognition (about day 16), compared with CON heifers. This may be a compensatory mechanism to try to establish pregnancy successfully in a slower-growing embryo.

In conclusion, 50 day of 40 percent nutrient restriction may not be severe or long enough of a restriction to influence most expression PSP-B or *INF-τ* in utero-placental tissues. Our previous data and this work confirmed differential expression of PSP-B and *INF-τ* in maternal and fetal tissues during the first 50 days of gestation.

These differences in expression are at critical time points during the establishment of pregnancy, specifically maternal recognition (day 16), completion of fetal adhesion (day 34) and rapid placental development (day 50). While exact functions during early gestation remain to be elucidated for PSP-B and *INF-τ*, they appear to complete vital steps for the successful establishment of pregnancy in beef heifers.

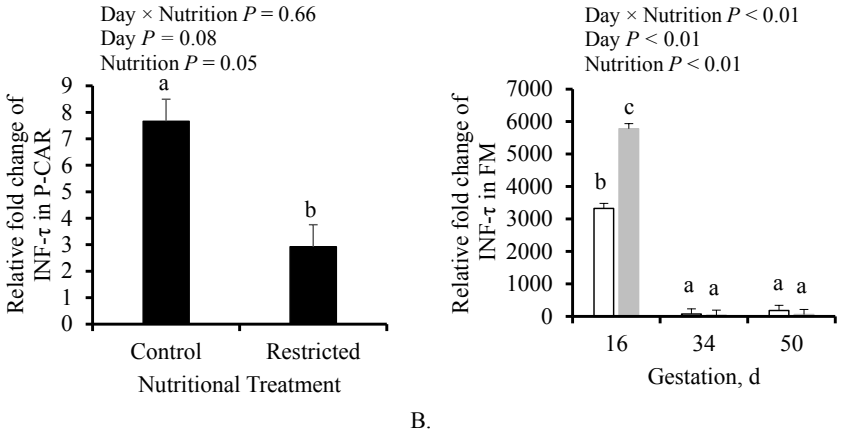

Figure 2. The effects of nutrient restriction and day of gestation on IFN-τ expression in utero-placental tissues B) the effects of nutritional plane on mRNA of IFN-τ in pregnant uterine caruncle (P-CAR). and B) IFN-τ in fetal membranes (FM) where white bars outlined in black represent control heifers and gray bars represent restricted heifers Data presented as a $2^{-\Delta\Delta CT}$ fold change normalized to *β-Actin* and the average of NP.

[a, b]Means without a common superscript differ (P < 0.05)

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