Fetal programming: maternal-fetal interactions and postnatal performance

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Abstract

Placental and fetal development is sensitive to direct and indirect effects of maternal environment. Environmental stimulus or insult during critical periods of development (e.g. fetal period) can program lifelong production characteristics of animal, irrespective of their genotype. Several environmental factors negatively impact placental development and blood flow during pregnancy and hinder offspring vigor. Timing of environmental insult or therapeutic intervention during pregnancy is critical, as developmental sensitivity of fetoplacental unit changes from early to late pregnancy. Association between poor growth performance of offspring and decreased blood flow to uterus and placenta during pregnancy was established. Nutrient restriction during early to midpregnancy decreased uterine artery blood flow, leading to compensatory growth of placental blood vessels during exponential fetal growth. Although many studies investigated offspring phenotypes following maternal nutrient restriction or overfeeding during pregnancy, fewer concentrated on enhancing postnatal performance in livestock based on offspring body weight. Survival rate of offspring born at average weight was higher than those with below-average birth weight. In this regard, dietary supplements promoting uterine blood flow increased postnatal growth and weaning weights. Elucidating consequences of specific supplements on continual plasticity of placental functional capacity will allow us to determine important mediators of offspring growth and development.

Keywords: Cattle, development, fetus, nutrition, placenta, sheep

Introduction

Irrespective of animal's genotype, environmental stimulus or insult during a critical period of development can impact phenotype. Exposure to environmental stimulus or insult may establish a permanent postnatal phenotype, resulting in adverse consequences for milk production, carcass yield, feed efficiency, and/or reproductive function.[1,2] Process of permanently altering animals' phenotype through environmental stimuli is referred as 'developmental programming hypothesis'.[2] For example, 2 animals with similar genotype but raised in different environments are expected to have differing lifelong phenotypic characteristics, which is further explained by developmental plasticity. Importantly, magnitude of phenotypic change is vastly different between these 2 animals with similar genotype, if their exposure to different environments occurred while they were embryos, fetuses, calves, weaned heifers, or mature cows. Changes in animals' developmental trajectory with lasting consequences were greatest in embryos and fetuses, with decreasing developmental plasticity with increasing animal age. Study of developmental programming during fetal period, an age of high developmental plasticity, is known as 'fetal programming'.

Support for fetal programming concept (strong association between birth weight and lifelong developmental consequences) is growing.[3,4] For example, low birth weight offspring are at increased risk of morbidity and mortality, slowed postnatal growth, poor body composition (increased fat and reduced muscle growth), metabolic disorders, cardiovascular pathologies, and dysfunction of several organs (ovaries, testes, mammary gland and gastrointestinal tract).[4,5] Livestock are specifically at risk due to poor nutritional environments during pregnancy (e.g. breeding young, growing, peripubertal dams that are competing for nutrients with fetus). In addition, poor pasture conditions or environmental heat stress in relation to seasonal breeding can decrease nutrient availability for both dam and fetus during critical periods of development.[6] Although these initial fetal programming studies focused exclusively on offspring (fetal or birth) weight, we now understand that multiple measurements of offspring size at birth can predict developmental trajectory. Therefore, phenotypic changes in livestock production as a result of

fetal programming may be independent of birth weight[7-9] with environmental insults during early pregnancy altering phenotypic changes (production) despite no change in birth body weight.[10]

Insufficiencies during pregnancy, resulting in reduced fetal growth and development, are detrimental to livestock, where newborns represent next generation of meat and milk producing animals. Several animal models of fetal and placental growth restriction (e.g. maternal nutritional plane, maternal age, heat stress, hypoxic stress, and fetal number) were developed to better unravel relationships among uterine blood flow and offspring development.[11-14] Establishment of functional fetal and placental circulation is one of the earliest events during conceptus development[15,16] and exponential increase in placental exchange is vital for maintaining remarkable growth and development of fetus during last half of pregnancy.[17] Therefore, understanding impacts of maternal environment on placental function is especially relevant to these proceedings, as majority of mammalian livestock raised for red meat production spend 30 - 40% of their life being nourished by placenta. Percent time in each phase of beef production from conception to harvest is depicted in Figure 1. In addition, amount of developmental plasticity of offspring varies during their lifespan, with maximal influences of developmental programming occurring during embryonic and fetal stages (Figure 1).

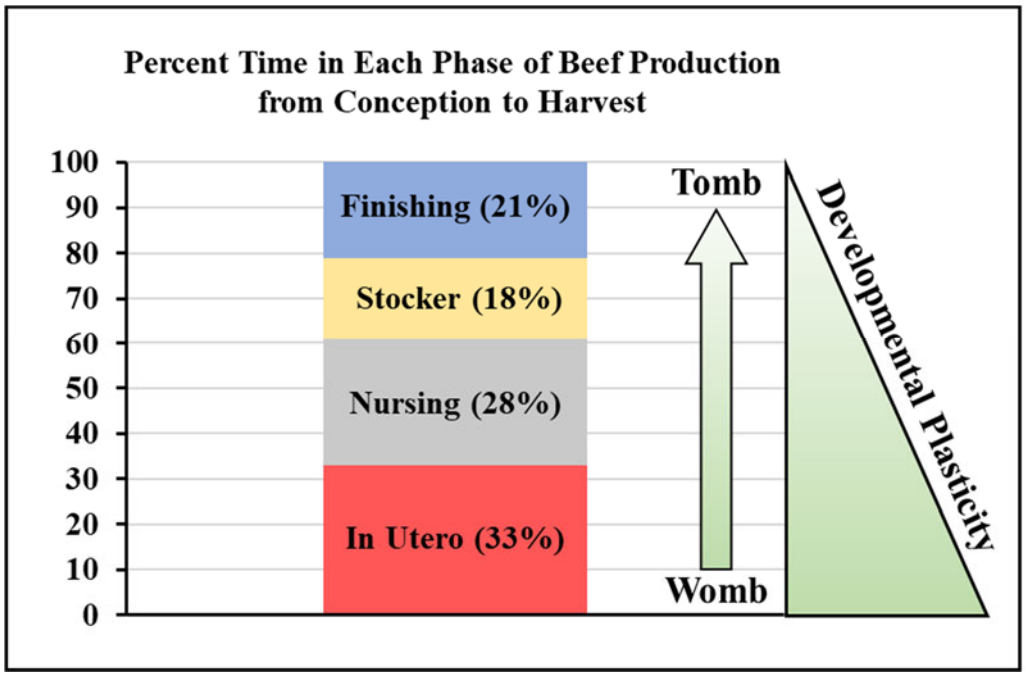

Figure 1. Percent time in each phase of beef production from conception to harvest (womb to tomb), note: nearly one-third of life is spent developing in utero when fetus is most vulnerable to environment, due to increased developmental plasticity.

Fetal growth and organ development

Embryonic period in cattle is defined as time from conception (single cell embryo, as zygote) to completion of organogenesis. This embryonic period typically extends from days 1 - 42 of pregnancy, with highest percent pregnancy wastage occurring during this interval. Fetal period is defined as the remainder of pregnancy from days 42 - 280 at organ differentiation completion (Figure 2).[18] Characterization of bovine fetal growth throughout pregnancy allowed researchers to hypothesize phenotypic changes to offspring that experience specific periods of environmental insults that may perturb normal development in utero. For example, nutrient deprivation or heat stress during days 60 - 120 of pregnancy will undoubtedly have different impacts on fetal development compared to similar environmental insults during days 180 - 240 of pregnancy. This is where timing becomes a critical component of fetal programming outcomes. In addition to extrinsic environmental effects altering fetal growth, several inherent intrinsic mechanisms are associated with fetal growth and development. During

days 70 - 100 of pregnancy, fetal weight across several breeds of cattle increased ~ 10 grams per day.[19] Further along in pregnancy (days 200 - 250), rate of fetal growth increased to ~ 200 - 300 grams per day; however, absolute growth of late term fetus declined to 100 grams per day, which may be due to inherent function of fetus exceeding capabilities of uteroplacental exchange near term. Alternatively, fetus may be secreting or altering its own hormone profiles near term, favoring proper maturation and differentiation of organs overgrowth.

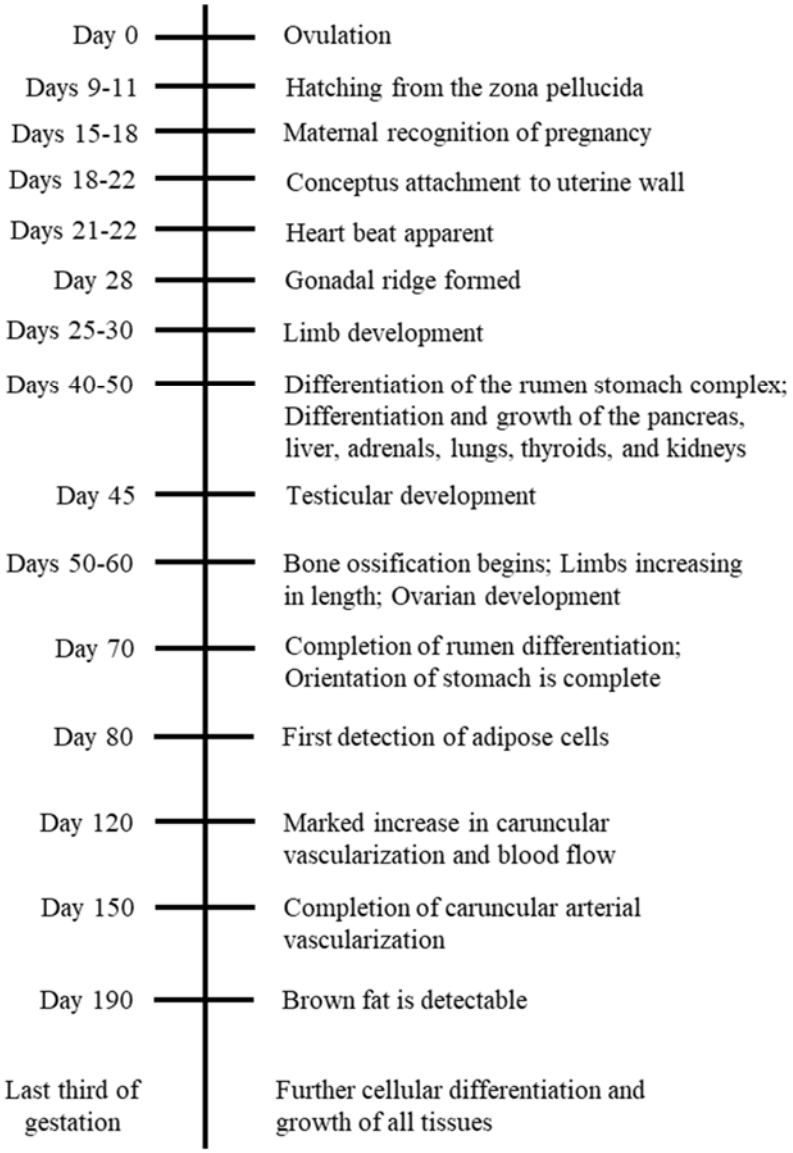

Figure 2. Timeline of bovine fetal development.[18]

Bovine fetal growth typically lags allantoic fluid volume and expansion of chorioallantoic membrane.[19,20] Nutrient partitioning by uteroplacenta and continuous supply of nutrients is a prerequisite for fetal growth. Chorioallantois expansion over endometrium allows for maximal surface area for nutrient exchange during development, which should support acceleration in fetal growth. There was significant positive correlation for total amniotic fluid volume and fetal weight, but no correlation for total allantois fluid volume and fetal weight.[19] Cloning of cattle by somatic cell nuclear transfer allowed researchers to further define this association between fetal fluid homeostasis and fetal and placental development.[21] Cloned bovine fetuses with abnormal placentation are usually associated with excessive fluid accumulation in fetal sacs (hydrops syndrome). In this experimental model, cloned embryos with

hydrops syndrome surviving until midpregnancy have overgrowth of placentomes, fetal liver, and fetal kidney compared to normal pregnancies.[21] In addition, organomegaly of offspring's liver, kidney, and heart is typically accompanied with polyhydramnios (in several mammalian species studied). Moreover, underlying mechanisms of fluid sac homeostasis are not fully understood in bovine pregnancy and further research is needed to elucidate cause and effect relationships.

Failure of proper organ development occurs in cloned calves that die shortly after birth. Using this experimental model, researchers have examined 8 developmentally important genes in 6 organs (heart, liver, spleen, lung, kidney, and brain). Of these developmental genes, kidney was the least affected organ associated with calf mortality, whereas heart was most affected by gene dysregulation compared to normal offspring.[22] Of these developmental genes, vascular endothelial growth factor (VEGF) was upregulated in cloned offspring associated with early neonatal mortality. Vascular endothelial growth factor was implicated in stimulating vasculogenesis and angiogenesis to restore oxygen supply to tissues when blood circulation is inadequate. Appropriate concentrations of VEGF during organogenesis and early organ development are vital for proper establishment of fetal cardiovascular system. Downregulation of VEGF could decrease fetal organ angiogenesis, with fatal consequences to fetus (pregnancy wastage) or neonate (calf mortality); however, upregulation may result in organomegaly or fetal cardiac dysfunction, with similar fatal consequences to newborn.

Placental development and growth

Placenta has major role in fetal growth regulation. Ruminant placenta is morphologically classified as cotyledonary and histologically as syndesmochorial. In nonpregnant ruminants, caruncles are organized in 2 dorsal and 2 ventral rows that run length wise along uterine horns. Chorioallantois has flat surface that becomes irregular when it starts to cover caruncles, due to growth and expansion of conceptus within uterine lumen. This process is followed by recognition of cotyledons.[23] Caruncular-cotyledonary unit is called a placentome and is formed from growth and interdigitation of fetal villi and caruncular crypt adopting a convex shape.[24] Contact surface area is enhanced as cotyledon's finger-like projections enter crypts formed in caruncles. Placentomes vary in size; however, they are bigger at uterine horn base and decrease in size close to tip.[23] Placentome is the primary functional area of physiological exchanges between mother and fetus.

Efficiency of placental nutrient transport is directly related to uteroplacental blood flow.[25] All nutrients and wastes that are exchanged between maternal and fetal systems are transported via uteroplacenta.[25-26] Establishment of functional fetal and uteroplacental circulations is one of the earliest events during embryonic/placental development.[15-16] To support exponential increase in fetal growth during last half of pregnancy, proper growth and development of uteroplacental vascular bed must occur during first half of pregnancy.[25,27] Understanding factors that impact uteroplacental blood flow will directly impact placental efficiency and thus fetal growth. However, despite much research in placental-fetal interactions area, regulators of placental growth and vascularization, including uteroplacental blood flow, are still largely unknown, particularly in cattle.

Our laboratory has been investigating blood perfusion and blood flow of reproductive tract using Doppler ultrasonography (applies Doppler Effect principle, named after Austrian physicist Christian Doppler; change in frequency of a wave for observer moving relative to source of respective wave). Example of this principle is perception of sound or differences in pitch one hears from stationary siren as person driving past the siren. Another example is that you are the stationary object and a car with a siren is driving past you. In this example, differences in pitch from moving siren will change as the car is driving towards you, the stationary object, and then away from you. In Doppler ultrasonography, stationary object is the transducer of ultrasound machine, which is detecting shift in frequency of red blood cells moving past stationary object.[28] Color can be assigned to normal B-mode, brightness gray scale image, by selecting color function of Doppler ultrasound machine. This will assign color based on directional flow (e.g. blood moving towards transducer probe is displayed in red and blood moving away from it is displayed in blue).

Good example of 2 vessels in close proximity and moving in opposite directions is illustrated in Figure 3A. Note the head and body of fetal sheep resting in amniotic sac at ~ day 40 of pregnancy. Top red arrow is pointing to umbilical cord, which is surrounded by color box and is showing a difference in directional blood flow from umbilical vein and umbilical artery. At this stage of pregnancy, umbilical cord is very small; however, it is still large enough to measure vessel diameter. Apart from color images, Doppler ultrasonography can estimate velocity of blood moving through a vessel. Figure 3B illustrates measurements taken while using Doppler mode. Sample gait cursor (green I) is placed on top of umbilical cord of a sheep fetus at day 90 of pregnancy (Figure 3B). Results are graphed in Figure 3C, where pulsatile umbilical artery cardiac cycle waveforms are visible above green x-axis line. Y-axis is detecting velocity (centimeters per second) of blood flow, whereas x-axis is depicting time in seconds. In addition, each pulsatile waveform of umbilical artery matches up with 1 fetal heart beat, enabling fetal heart rate calculation.

In addition to umbilical cord blood flow examination, our laboratory has used Doppler ultrasonography to examine uterine artery blood flow during mid to late pregnancy in cattle. Figure 3D illustrates a Doppler ultrasonography image of maternal uterine artery of a pregnant Holstein heifer ~ 180 days of pregnancy. Pulsatile cardiac cycles of uterine artery represent maternal heart rate. Moreover, these Doppler data allow calculation of velocity of blood flow during peak systolic and diastolic contractions of heart. Numbers on y-axis are in centimeters per second and are negative as red blood cells are moving away from transducer. In comparison, we also examined blood flow through hepatic portal vein of lactating dairy cows (Figure 3E). In this example, blood is flowing towards transducer, thereby giving a positive velocity on y-axis. In addition, nonpulsatile blood flowing through hepatic portal vein has an average velocity of ~ 50 centimeters per second. Data generated using Doppler ultrasonography are comparable to previous experiments estimating umbilical, uterine and hepatic blood flow with dye dilution techniques.[7,8,18]

Data on development of bovine placentome capillary bed are limited. Cotyledonary growth progressively increases throughout pregnancy in cattle.[29,30] Histological analysis of capillary bed development was performed in mid and late bovine pregnancies.[30] During this period, capillary area density, a measure related to blood flow, decreases ~ 30% in caruncular tissue, but increases ~ 186% in cotyledonary tissue. Also, number of capillaries increases ~ 150 and 80% in caruncular and cotyledonary tissue, respectively. Capillary surface density, a measurement related to nutrient exchange, increased in both caruncular and cotyledonary tissues (32 and 172%, respectively), whereas capillary size decreases 67% in caruncular tissue and increases 71% in cotyledonary tissue from mid to late pregnancy. Pattern of capillary development is very different between maternal and fetal portions of placentome, possibly due to energy demands of these independent tissues that share a similar function, delivery of nutrients to developing fetus. Placenta has fundamental role in supporting metabolic fetal demands. Although placental growth slows during last half of pregnancy, placental function increases dramatically to support exponential fetal growth rate.[20,31] For example, in sheep and cattle, uterine blood flow increases ~ 3 - 4 fold from mid to late pregnancy.[25,32-34] Relationship between uteroplacental blood flow and conceptus size throughout pregnancy is further defined below.

Cardiovascular adaptations during pregnancy

Physiologic state of dam is associated with significant but reversible alterations to metabolic demand and alterations to endocrine and cardiovascular systems. Maternal cardiovascular functional capacity changes dramatically during pregnancy, whereby systemic arterial blood pressure and vascular resistance decreases and cardiac output, heart rate, stroke volume and blood volume increase.[35] Although not all variables determined during bovine pregnancy, several mammalian species (including sheep) have decrease in mean arterial pressure in early pregnancy that persists throughout pregnancy. Moreover,

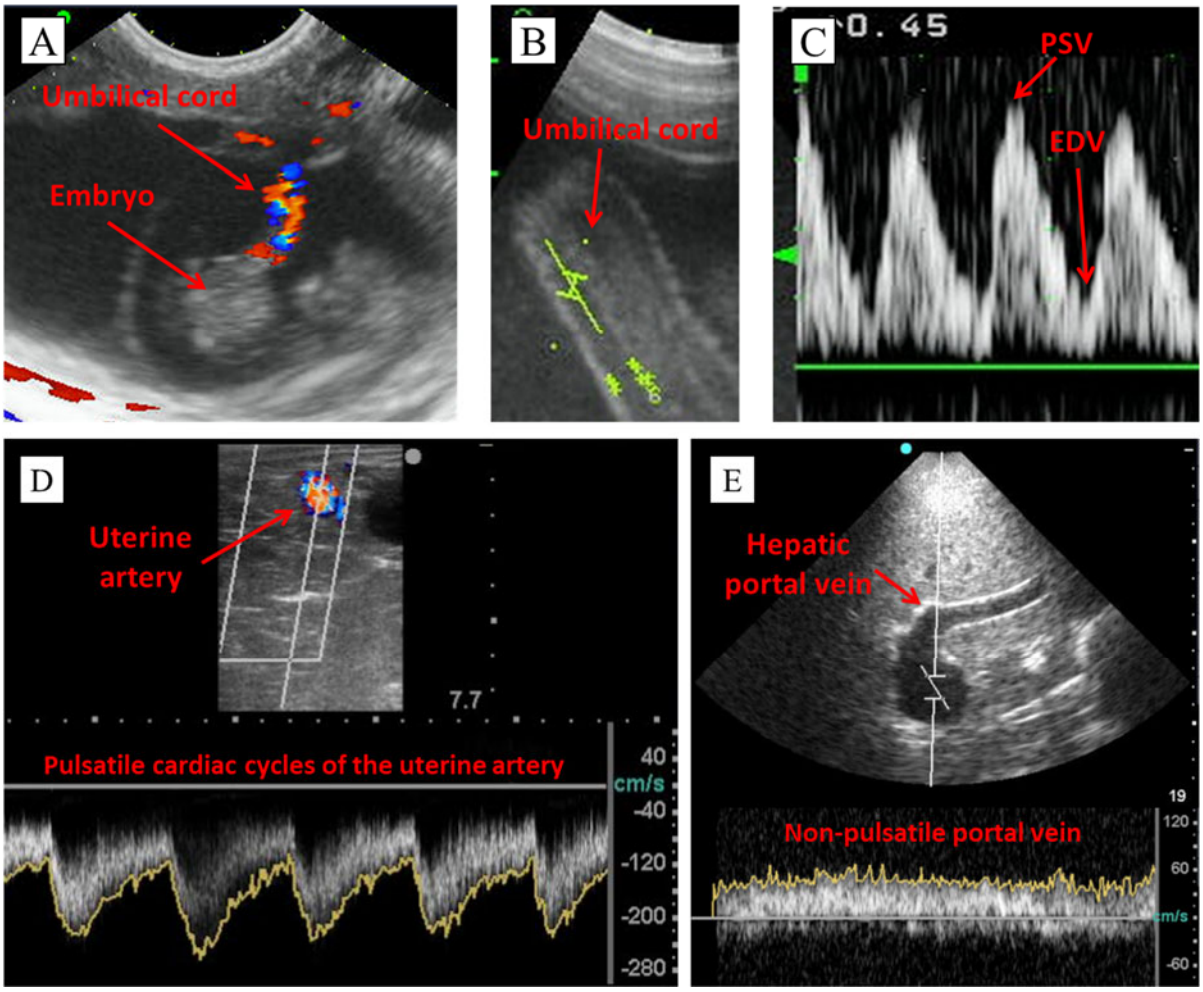

Figure 3. Doppler ultrasonography images of ovine umbilical cord at ~ day 40 (A) and day 90 (B) of pregnancy. Cardiac cycle waveforms of ovine day 90 umbilical artery (C) showing peak systolic blood velocity (PSV) and end diastolic blood velocity (EDV). Doppler ultrasonography image of bovine uterine artery and corresponding cardiac cycle waveforms (D) at day 180 of pregnancy. Doppler ultrasonography image of nonpulsatile bovine hepatic portal vein (E) of a lactating nonpregnant dairy cow.

decrease in arterial pressure (~ 5 - 10% decrease) is minor compared to ~ 20 - 30% decrease in total peripheral vascular resistance. Maternal cardiac output increases 30 - 40% in nonpregnant versus pregnant ruminants. Therefore, increase in cardiac output is associated with dramatic decline in systemic vascular resistance, allowing researchers to characterize pregnancy as a state of systemic vasodilation, resulting in profound increases in total systemic flows to all vascular beds. Most of these studies characterize this relationship by the equation; systemic vascular resistance = mean arterial pressure/cardiac output.

Using above equation, it is apparent that decrease in systemic vascular resistance during pregnancy and increase in cardiac output (total systemic blood flow) help to maintain arterial pressure. In addition, apart from this relationship, increases in blood volume and activation of renin-angiotensin system may also contribute to maintaining blood pressure during this physiological state of substantial vasodilation; however, limited data exists in cattle.[35] To determine specific contributions to increase in cardiac output occurring during pregnancy, we must first examine cardiac output equation, which states that: cardiac output = heart rate x stroke volume, whereby stroke volume equals volume of blood pumped from 1 ventricle of heart with each beat. In majority of mammalian species, heart rate increases by ~ 15% during pregnancy which does not fully explain 30 - 40% increase in cardiac output. Therefore, stroke volume may increase 30 - 35% during pregnancy and is one of the major contributors to this

increase in cardiac output. In sheep treated chronically with estrogen, induced increase in left ventricular heart dimensions and enlargement were similar to pregnancy; therefore changes to endocrine system during pregnancy may help mediate temporal changes to maternal cardiovascular function.[35]

Rise in maternal cardiac output during pregnancy is also associated with increased plasma and blood volume in cows.[18] Increased blood volume varies among species and depends on dam's nutritional status and singleton versus twin pregnancies. Bovine blood volume expands by 10 - 20% during pregnancy, whereas in litter-bearing species, blood volume may expand by 30 - 50% during pregnancy. With increase in plasma volume, dam must maintain a proper balance of water and electrolyte retention; therefore, similar to alterations in maternal arterial pressure, this increase in plasma volume will be integrated with the renin-angiotensin system, which can serve additional purpose as an extrinsic modulator of kidney function and urinary secretion. In addition, to dramatic changes in maternal cardiovascular system during pregnancy, it is even more noteworthy that most mammals return to nonpregnant levels of cardiovascular function within 2 - 5 weeks postpartum.[18] Although lactating, high-producing nonpregnant dairy cattle have a substantial increase in cardiac output compared to nonpregnant and nonlactating counterparts, this redistribution of blood flow during transition period from uteroplacental vasculature towards mammary gland is still a phenomenal physiological feat enabling peak lactation shortly after parturition.

Several animal models of fetal programming were extensively studied in ewes,[14] although extrapolation to cattle should be minimal, due to drastic differences in placental development between sheep and cattle (Figure 4). In ewe, placenta reaches its maximum size during first two-thirds of pregnancy, whereas ~ 90% of fetal growth occurs during last third of pregnancy.[17] In contrast, bovine placenta continues to increase in size exponentially as pregnancy proceeds; however, bovine fetal growth is much greater compared to placental growth (Figure 4).[25] Placenta is involved in transporting nutrients and wastes between maternal and fetal circulations and altered placental function was associated with abnormalities in fetal development. Efficiency of placental nutrient transport is directly related to placental blood flow.[25,26] Key factors affecting placental nutrient transfer capacity are size, nutrient transporter abundance, nutrient synthesis and metabolism, and hormone synthesis and metabolism.[36] Large increases in blood flow to reproductive tract are necessary to support both nutrient and waste exchange between mother and offspring. Several environmental factors negatively impact placental development and blood flow during pregnancy, all of which can hinder offspring health and vigor. Regulators of placental nutrient transport and uteroplacental blood flow are still largely unknown, with most research efforts focusing on rodent models, which are different from livestock species. Elucidating consequences of specific hormonal supplements on the continual plasticity of placental function will allow determination of important endogenous mediators of offspring growth and development.

Maternal nutrient restriction

Poor forage quality in grazing systems can negatively impact nutritional intake of beef cattle. Pregnant beef cows grazing poor forage can alter fetal growth during increased periods of developmental plasticity. Thus, provisions from environment can program these offspring to experience changes in mortality and morbidity rates, slowed postnatal growth, altered carcass weights, and meat quality characteristics.[37] Relationship between maternal nutritional plane during late pregnancy and calf mortality was examined as early as 1975, where maternal nutrient restriction for 100 days prepartum decreased calf birth weight by 7% and increased calf mortality rate by 10%, whereas an additional 20% of calves died between birth and weaning due to scours.[38] Direct effects of nutritional plane on offspring production characteristics are dependent on timing of insult and magnitude of nutrition deprivation in relation to fetal and placental development.[39] In dealing with timing, it is also important to consider separation of prenatal

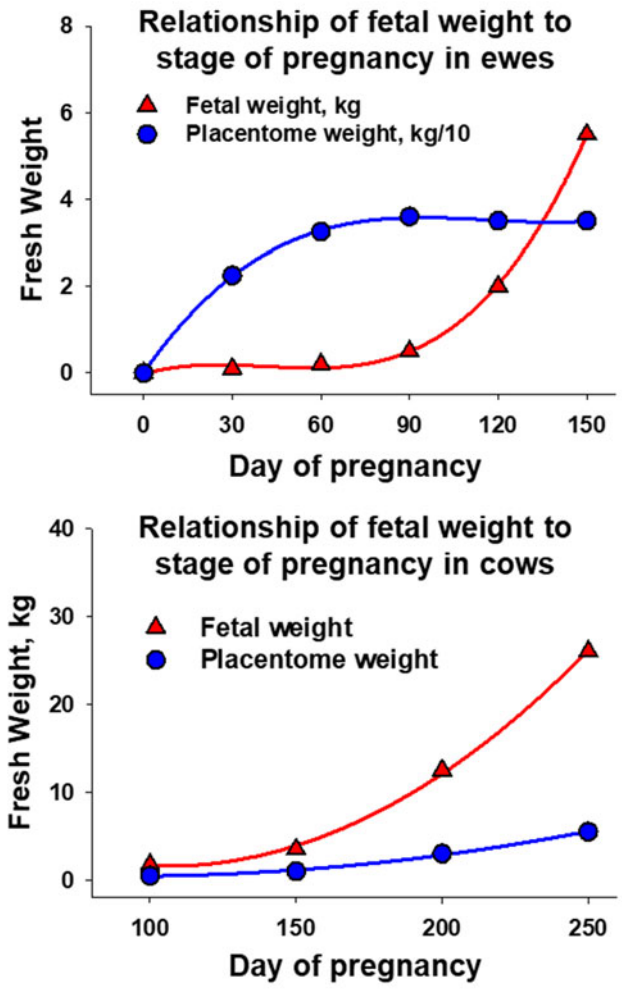

Figure 4. Fetal and placental growth in ewe and cow (redrawn).[17,25]

versus postnatal maternal factors that may influence these developmental programming responses. For example, changes in meat quality of offspring born to dams experiencing late pregnancy maternal nutrient restriction may carry over into early lactation of this dam. Thus, researchers are prevented from identifying important time of maternal nutritional insult that led to negative offspring outcomes. This is vital when considering most economically feasible therapeutic interventions to mitigate negative developmental programming outcomes.

Adaptations of placenta during maternal nutrient restriction are incomplete and less is known about specific differences amongst breeds of cattle. Normal physiology such as pregnancy length, fetal growth, placentome weight, even uterine blood flow can differ substantially between breeds of cattle.[33,34] Therefore, magnitude of fetal programming is expected to be breed dependent and further research is needed to identify when and with what breeds should interventions be sought. In a recent study, we examined the effect of early to mid pregnancy nutrient restriction on uterine blood flow and fetal development in Brahman and Angus heifers.[40] Heifers were restricted to 60% of net energy requirements for pregnant cattle from days 50 - 180 of pregnancy. This early to mid pregnancy nutrient restriction decreased uterine artery blood flow and fetal weight at day 180 of pregnancy irrespective of heifer breed (Table 1). Moreover, efficiency of uterine artery blood flow relative to fetal weight was improved in nutrient-restricted versus adequate-fed dams (Table 1). A similar response was observed in Brahman heifers, irrespective of nutrient restriction, signifying a lesser amount of uterine blood flow needed to

grow similar weight fetus from a Brahman versus Angus dam (Table 1). A portion of these responses have been associated with increased placental efficiency in nutrient-restricted dams and Brahman dams.[40] Using a novel imaging technique to ascertain macroscopic blood vessel density of cotyledons (Figure 5), we observed significant increases in blood vessel density in Brahman versus Angus dams. As noted in the Figure 5 illustration, cotyledonary blood vessel density is heterogenous, with areas of high versus low vascularity within the same placentome. Moreover, nutrient restriction and decreased uterine artery blood flow increased cotyledonary blood vessel density, which we believe to be a compensatory mechanism within fetal membranes.[40] Angus and Brahman heifers subsets were allowed to calve and postnatal growth monitored though weaning (unpublished observations). Most postnatal measurements of growth were unaffected by maternal nutrient restriction from days 50 - 180 of pregnancy. However, heart girth increased in calves born to nutrient restricted versus adequate-fed dams, which may have in utero overcompensation of fetal growth when nutrient-restricted dams are re-alimented to adequate nutrition during late pregnancy.

Table 1. Uterine artery blood flow (BF) at day 175 of pregnancy and fetal flow at day 180 of pregnancy in nutrient-restricted (RES) or control (CON) fed Angus and Brahman heifers. Nutritional treatments were applied from days 50 to 180 of pregnancy. Data reported are main effect of nutritional plane and main effect of breed. Results adapted from.[40]

				P value
Item	CON	RES	SEM	Trt
Nutritional treatment				
Uterine artery BF, liter/minute	5.04	2.33	0.73	0.035
Fetal weight, kg	9.56	7.35	0.61	0.047
Uterine artery BF by fetal weight, liter/minute/kg of fetus	0.52	0.29	0.07	0.058
				P value
Item	Angus	Brahman	SEM	Breed
Breed				
Uterine artery BF, liter/minute	4.66	2.71	0.76	0.119
Fetal weight, kg	8.04	8.88	0.61	0.388
Uterine artery BF by fetal weight, liter/minute/kg of fetus	0.53	0.28	0.07	0.054

Apart from nutritional management during pregnancy, we also examined heifer development practices and season on uterine artery blood flow during mid to late pregnancy.[41] For example, beef producers opting for low-input forage-based replacement heifer management programs have lighter weights at breeding, with some heifers reaching only 50 - 55% of expected mature body weight at breeding versus a traditionally recommended target weight of 60 - 65% of expected mature body weight. We concluded that heifers developed on low0input management schemes until confirmation of pregnancy (days 30 - 45) had no compromise in uterine blood flow or calf birth weights compared to conventionally developed heifers. Moreover, volume of late pregnancy uterine artery blood flow relative to maternal body weight was significantly increased in low-input versus conventionally developed heifers, which may be a compensatory mechanism to safeguard fetal growth and development.[41] In addition to low-input heifer development programs, we also examined effect of calving season on uterine artery blood flow, as differences in postpartum anestrus interval, conception rates, and weaning weights were reported between fall and spring calving herds.[42,43] A portion of these responses could be programmed in utero via changes in nutrient and waste exchange between dam and fetus. In these initial studies, we observed an increase in uterine artery blood flow in the last third of pregnancy, consistent with exponential growth of fetus in spring- versus fall-calving heifers.[41] Although cattle are considered nonseasonal breeders, seasonal

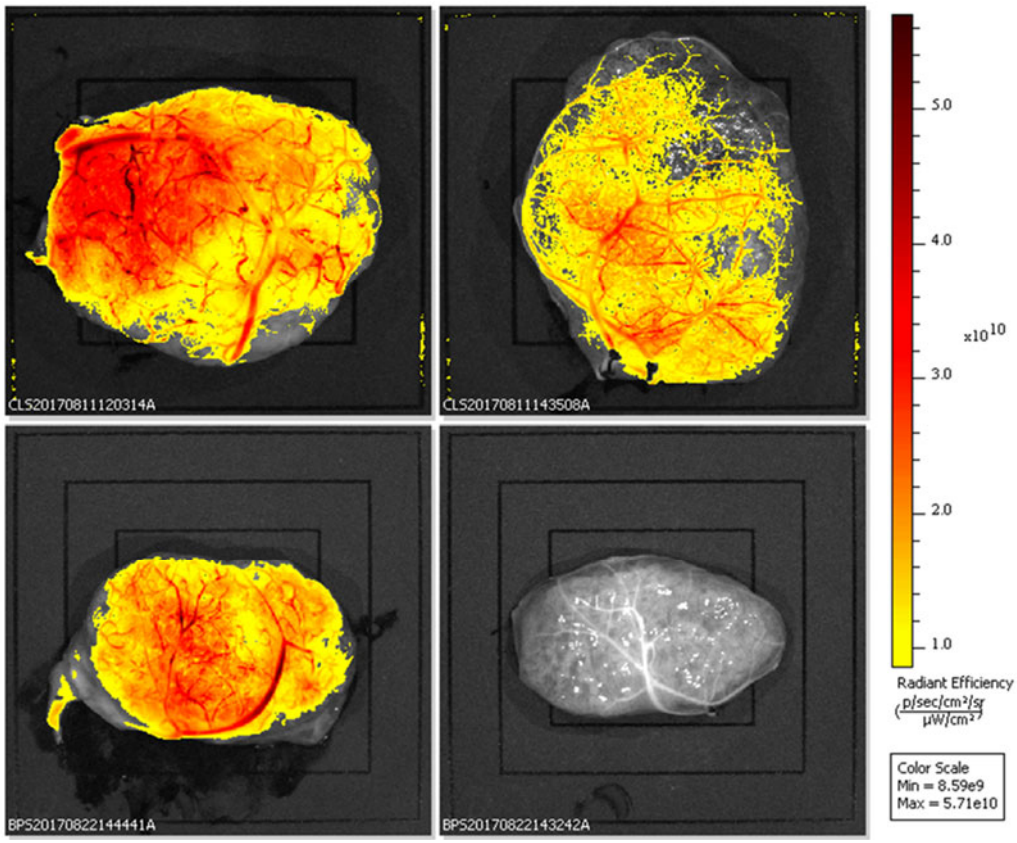

Figure 5. Fluorescent detection of macroscopic cotyledonary blood vessel density in placentomes collected at day 180 of pregnancy. Three representative images and one negative control placentome with cotyledonary surface up are shown.[40]

changes in photoperiod, thermal stress, and nutrient availability can influence numerous performance and reproductive traits. Changes in hormone concentrations, as a result of photoperiod, may influence blood distribution to the reproductive tract, specifically changes in melatonin-modulated cardiovascular function.[44]

Maternal melatonin supplementation

Amplitude of melatonin secretion has been associated with improved oxidative status and altered hormone metabolism in rats and sheep, and altered cardiovascular function in several mammalian species.[45-47] In several studies, melatonin partially regulated blood pressure and blood flow.[48] Melatonin has both direct and indirect effects on the cardiovascular system and may cause either arterial vasodilation or vasoconstriction, depending on the origin of the blood vessel under investigation. Taking into account the above physiological responses, which can be partially altered by peripheral concentrations of melatonin, our research team examined effects of melatonin supplementation on uteroplacental development and functional capacity. Similar to other fetal programming models, our initial studies focused on pregnant ewe lambs. Using this sheep model of intrauterine growth restriction, we supplemented dietary melatonin as a potential therapeutic during mid to late pregnancy.[49] In our sheep model, ewes were supplemented with 5 mg of melatonin or no melatonin and allocated to receive 100% (adequate) or 60% (restricted) of nutrient requirements from days 50 - 130 of pregnancy. Using Doppler ultrasonography, we observed an increase in umbilical artery blood flow at day 130 in ewes supplemented with dietary melatonin, whereas uterine artery blood flow was unaffected by maternal melatonin supplementation.[49] At day 130, uterine artery blood flow decreased in nutrient-restricted versus adequate-fed ewes. Although melatonin supplementation failed to rescue fetal weight in restricted-fed ewes, we

observed similarities between fetal size and measurements of uterine and umbilical blood flow during mid to late pregnancy.

Recently, we examined uterine artery blood flow in Holstein heifers supplemented with 20 mg of dietary melatonin from days 190 - 262 of pregnancy.[7] Uterine artery blood flow increased by 25% in melatonin-treated versus control heifers (Table 2). Surprisingly, calf birth weights were not different between treatments; however, calf body weight at 9 weeks of age increased in calves born to melatonin-supplemented dams versus control dams.[8] Therefore, similar to other pregnancy models, an increase in uteroplacental blood flow during mid to late pregnancy is associated with alterations in postnatal offspring growth and development. This is apparent in the dairy heifer study, as calves were removed from dams, managed identically and fed a similar milk replacer and starter diet prior to weaning at 8 weeks of age. Therefore, postnatal maternal factors were removed from this dairy project, allowing us to propose direct fetal programming responses. Due to observed differences in dairy calf body weights, we replicated a similar experiment in beef cows. In this follow-up study, heifers and cows were assigned to 1 of 2 treatments: melatonin implants (MEL; n = 29) or no melatonin implant control (CON; n = 28) starting on day 180 of pregnancy and ending on day 270.[9] As expected, uterine artery blood flow increased in commercial beef heifers and cows supplemented with melatonin during the last third of pregnancy (Table 2). Similar to the dairy heifer study, beef calf birth weights were not different; however, a 26 kg increase in weaning weight was observed in calves born to melatonin-supplemented versus control dams.[9] Although results were similar to dairy study, it is important to note that postnatal maternal factors (e.g. postpartum cow health, colostrum composition, and lactational performance) could be contributing to increased weaning weights of calves born to melatonin-supplemented dams.

Table 2. Uterine artery blood flow (BF), calf birth weight, and calf weaning weight at 9 weeks of age from dairy heifers treated with (MEL) or without (CON) dietary melatonin from days 190 - 262 of pregnancy.[7,8] Uterine artery BF, calf birth weight, and calf weaning weight at 28 weeks of age from beef cows treated with (MEL) or without (CON) dietary melatonin from days 180 - 270 of pregnancy.[9]

				P value
Item	CON	MEL	SE	Trt
Dairy heifers				
Uterine artery BF, liter/minute	5.73	7.16	0.35	0.02
Birth weight, kg	37.4	35.8	2.4	0.63
Weaning weight, kg	86.0	99.1	2.4	0.01
				P value
Item	CON	MEL	SE	Trt
Beef cows				
Uterine artery BF, liter/minute	5.76	7.87	0.57	0.01
Birth weight, kg	31.1	31.8	1.8	0.77
Weaning weight, kg	195.5	221.5	7.3	0.01

Taken together, results we observed in both cattle studies following melatonin supplementation allowed our research group to speculate on potential circadian alterations of reproductive tract during pregnancy. For example, rhythms generated from circulating concentrations of melatonin could mediate circadian rhythms in placenta and developing fetus during pregnancy. Potentially this could indicate a natural 24 hour rhythm in uterine artery blood flow, which may be influenced by exogenous melatonin supplementation. Therefore, from a livestock production standpoint, alterations in circadian rhythms during specific windows of pregnancy may lead to changes in offspring body composition; however, these fetal programming data are lacking. Nevertheless, the potential of establishing and/or disrupting these circadian rhythms during specific time points of pregnancy may alter production characteristics of offspring, which warrants further investigation.

Epigenetic mechanisms of fetal programming

Nearly every cell within an organism retains the entire genetic information or genome; however, cellular differentiation selectively programs different expression rates of genetic code. In contrast to earlier definitions of epigenesis, more contemporary usage of epigenetics refers to "the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence".[50] Therefore, epigenetic processes can program gene activity or expression patterns without altering the genetic code and differentiated adult cells have the capacity to retain epigenetic modifications from fetal development or even previous generations. Two primary mechanisms imparting epigenetic modifications to the genome are: 1) DNA methylation and 2) histone modifications.[51,52] These modifications were implicated as the primary mechanism of developmental programming responses that may lead to later life noncommunicable diseases. An interesting proposal of epigenetic modifications relies on key nutritional cofactors associated with one-carbon metabolism, such as folate, vitamin B12, vitamin B5, choline, S-adenosyl methionine, betaine, and homocysteine.[4] This proposal was shown in rodent studies, where dietary provisions of choline, folate, and vitamin B12 during pregnancy prevented DNA hypomethylating properties of bisphenol A, thereby altering gene expression and mitigating development of obesity in offspring.[53] Therefore, developmental origins of adult disease involve reprogramming the epigenome by environmental factors, e.g. maternal nutrition.[54]

Postnatal outcomes

Animal models were developed and investigated to further our understanding of mechanisms underlying fetal origins of health and disease in humans.[6] For example, maternal nutrient restriction during prenatal period is usually associated with low birth weights (intrauterine growth restriction) in offspring, as well as postnatal development of hypertension, obesity, and diabetes.[55] Lower birth weights were associated with increased postnatal morbidity and mortality,[56] poor postnatal growth, and decreased weaning weight in sheep.[57] However, a portion of these postnatal responses may be related to alterations in mammary gland development, which could lead to postnatal malnourishment and developmental programming responses occurring after the fetal period of development. Differences in birth weight can modestly predict carcass weight irrespective of dietary influences. Magnitude of growth restriction at birth in lambs, pigs, and steers was correlated with carcass yield leaving clear financial implications for intrauterine growth restriction in livestock species.[58] Specifically, in cattle, low birth weight was accompanied with slow postnatal growth, lower weaning weights and significant decreases in carcass weights at 30 months of age.[59] Some of these responses can be related to skeletal muscle development as improved nutritional plane during midpregnancy increased live weight and carcass weight and tenderness in crossbred steers.[60]

Compromised pregnancies, via changes in maternal nutritional plane, were strongly associated with postnatal and later life changes to metabolic and endocrine functional capacity in offspring. Insulin sensitivity was a primary target of fetal programming as epidemiological evidence associated birth weight with an increased risk of developing type II diabetes.[61] In an ovine maternal nutrient restriction model, malnourishment during late pregnancy decreased peripheral insulin sensitivity in young offspring.[62] Similarly, changes to maternal nutritional plane (over or under-feeding) have been associated with increased lipid accumulation in liver of 1 year old sheep, as well as changes in hepatic fatty acid oxidation.[63,64] Other endocrine changes in offspring born to compromised pregnancies were related to multiple hypothalamic-pituitary axes, e.g. those targeting thyroid and adrenal glands.[65] Since information related to adulthood and transgenerational consequences of fetal programming is limited, stringent environmental scenarios over several years are needed to fully relate these later life metabolic changes to fetal period treatments. In sheep, intergenerational programming of a metabolic syndrome phenotype was observed in grandsons and granddaughters of a maternal obesity model.[66] Moreover, these responses were sex specific, as granddaughters (F2 females) had greater insulin resistance compared to F2 males.[66]

Although birth weight continues to be an indicator of potential adverse fetal programming events in offspring, it is important to note that birth weight provides limited information about body composition, morphometric size measurements, or individual organ size.[65] This is especially relevant in

early pregnancy maternal nutrient restriction studies, which usually do not alter birth weight or placental weight if dams are realimented to control diets prior to the exponential increase in fetal growth. Crossbred beef heifers subjected to maternal nutrient restriction from 11 days prior to AI through day 110 of pregnancy had increased concentrations of testosterone during nutrient restriction compared to control-fed heifers.[10] On day 94 of pregnancy, prior to end of nutrient restriction, fetal size was not different between treatment groups; however, the diameter of aortic root increased in fetuses from nutrient restricted dams versus control. In this study, pregnancy length, placental weight, and birth weight were not affected by maternal nutrient restriction.[10] Similarly, postnatal growth of offspring born to nutrient restricted dams was similar to controls until slaughter at 95 weeks of age. However, female offspring born to nutrient restricted dams had decreased ovarian reserves, enlarged aortas and increased blood pressure compared to their control counterparts.[10] Therefore, in cattle, changes in maternal nutrition during first trimester of pregnancy may program female offspring reproductive characteristics and hypertension, which may be related to increased testosterone concentrations during pregnancy.[10] Similar to these lack of changes in birth weight, we stimulated uterine artery blood flow via maternal melatonin supplementation in both dairy heifers and beef cows, with no significant impact on calf birth weight.[7,9] However, postnatal growth and weaning weight increased in offspring of melatonin supplemented versus control dams.[8,9]

Conclusion

Insufficiencies during pregnancy, resulting in reduced fetal growth and development, are detrimental to livestock production. We consistently observed positive associations with uterine and umbilical blood flow in sheep and cattle relative to fetal and postnatal offspring size. Doppler ultrasonography increased our understanding of blood flow and blood perfusion during important reproductive events, enabling producers to apply specific strategies to improve reproductive efficiency of livestock. Early to midpregnancy nutrient restriction increased placental efficiency in both Angus and Brahman heifers. However, late pregnancy nutrient restriction was associated with decreased birth weight, increased mortality and slowed postnatal growth of surviving offspring. Low input heifer development programs resulting in 50% of mature body weight at breeding did not negatively impact uterine artery blood flow and calf birth weight. Spring calving heifers had increased uterine blood flow compared to their fall-calving counterparts, which may be related to environmental differences and even hormonal changes with decreasing daytime length. Furthermore, we consistently identified increased umbilical and uterine blood flows in sheep and cattle during melatonin supplementation. Importance of this pathway in relation to development and transmission of 24 hour rhythms to offspring has not been elucidated in cattle. Negative insults during pregnancy were associated with dysregulation of metabolic and endocrine function in the postnatal period. Moreover, carcass yield and meat quality can be negatively affected by decreased or increased maternal nutrition during specific windows of pregnancy. Studies are needed to further expand these fetal programming responses related to adult offspring and potential transgenerational carryover.

Conflict of interest

There are no conflicts of interest to declare.

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