EFFECTS OF MATERNAL NUTRITION, INTRAUTERINE GROWTH RESTRICTION (IUGR), AND ESTROGEN (E2) SUPPLEMENTATION ON PLACENTAL AND FETAL INTESTINAL GROWTH AND DEVELOPMENT IN SHEEP

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Effects of Maternal Nutrition, Intrauterine Growth Restriction (IUGR), and Estrogen (E2) Supplementation on Placental and Fetal Intestinal Growth and Development in Sheep

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

Animal Sciences

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ABSTRACT

The placenta and fetal intestines are two key nutrient transport organs that sustain and nurture growing fetus. Insufficient placental development and consequently inadequate fetal nutrient supply can lead to IUGR resulting in low birth weight offspring. Our experimental objectives were to investigate the effects of elevated maternal nutrition, IUGR, and E2 supplementation during mid-gestation (in an attempt to rescue IUGR offspring) on placental and fetal intestinal cell proliferation, angiogenic gene expression, and vascularity. Limited responsiveness in placental development and vascularization to E2 supplementation was observed, likely due to inappropriate timing or dose of E2. However, maternal E2 supplementation increased fetal small intestinal length and *GUCY1b3* mRNA expression, suggesting that E2 supplementation has positive effects on IUGR fetal intestinal growth. In conclusion, understanding molecular mechanisms associated with IUGR and possible effects of E2 supplementation in rescuing IUGR may lead to enhanced human health and livestock production efficiency.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS

ADG.....Average daily gain

AFRC.....Agriculture and Food Research Council

Akt.....Serine/threonine-specific protein kinase

ANGPT1.....Angiopoietin 1

ANGPT2.....Angiopoietin 2

APA.....American Pregnancy Association

BCS.....Body condition score

BMI.....Body mass index

BMJ.....British Medical Journal

BW.....Body weight

B12.....Cobalamine

CAD.....Capillary area density

CAR.....Caruncle

cDNA.....copy deoxyribonucleic acid

CON.....Control

COT.....Cotyledon

CL.....Corpus luteum

CND.....Capillary number density

CSD.....Capillary surface density

c-Src.....Cytoplasmic protein with tyrosine-specific protein kinase activity

CpG.....Cytosine-phosphate-Guanine

CVD.....Cardiovascular disease

d.....day

DNA.....Deoxyribonucleic acid

DNMT.....DNA Methyl Transferase

E2.....Estradiol-17β

ERα.....Estrogen receptor alpha

ERα AF-1.....Estrogen receptor alpha activation factor-1

ERβ.....Estrogen receptor beta

ER46.....Estrogen receptor 46 isoform

FBW.....Fetal body weight

FGF.....Fibroblast growth factor

FGFR2.....Fibrobalst growth factor receptor

EGF.....Epidermal growth factor

FLT1.....Fms-related tyrosine kinase 1

GCs.....Glucocorticoids

GLP-2.....Glucagon like peptide-2

GPR30/GPER.....G-protein-coupled receptor 30/G-protein coupled estrogen receptor 1

GUCY1B3.....Soluble guanylate cyclase

GI.....Gastrointestinal

h.....hour

hCG.....human chorionic gonadotropin

HGF.....hepatocyte growth factor

HI.....high

HIF1A.....Hypoxia-inducible factor 1, alpha subunit

HPA.....Hypothalamic-pituitary-adrenal

H19.....gene that is known as Adult Skeletal Muscle (ASM) and Beckwith-Wiedemann syndrome (BWS)

HSD11B.....11β-hydroxysteroid dehydrogenase type

IgA.....Immunoglobulin A

IgG.....Immunoglobulin G

IGF-I.....Insulin-like growth factor-I

IUGR.....Intrauterine growth restriction

KDR.....Kinase insert domain receptor

LWG.....Live weight gain

MBW.....Maternal body weight

ME.....Metabolizable energy

MOD.....March of Dimes

mGLUR.....Metabotropic glutamate receptor

mRNA.....Messenger ribonucleic acid

MTHFR.....Methionine Tetrahydrofolate Reductase

NO.....Nitric oxide

NOS3.....Endothelial nitric oxide synthase 3

NK.....Natural killer

NLM.....National Library of Medicine

NR3C1.....Nuclear receptor subfamily 3, group C, member 1

NRP1.....Neuropilin 1

NRP2.....Neuropilin 2

NVSR.....National Vital Statistics Reports

PGF.....Placental growth factor

PIH.....Pregnancy-induced hypertension

PI3K.....Phosphoinositide-3 kinase

PBS.....Phosphate buffer saline

PDAR.....Redeveloped assay reagent

PCNA.....Proliferating cell nuclear antigen

P450.....cytochrome P450 (aromatase)

RT-PCR.....Reverse transcription-polymerase chain reaction

SAM.....S-Adenosine Methionine

SAH.....S-Adenosine Homocystein

SLC2A1.....Solute carrier family 2 (facilitated glucose transporter), member 1

SLC2A3.....Solute carrier family 2 (facilitated glucose transporter), member 3

SLC38A1.....Sodium-coupled neutral amino acid transporter 1

SLC38A2.....Sodium-coupled neutral amino acid transporter 2

SLC38A4.....Sodium-coupled neutral amino acid transporter 4

SERM.....Selective estrogen receptor modulators

SH2.....Src Homology 2

SGA.....Small for gestation age

SNAP.....Scottish Needs Assessment Program

T2D.....Type II diabetes

TEK.....Tyrosine kinase, endothelial

tcRNA.....Translational control RNA

THF.....Tetrahydrofolate

UNSD.....United Nations Statistics Division

VEGF.....Vascular endothelial growth factor

VIP.....Vasoactive intestinal peptide

VSM.....Vascular smooth muscle

WHO.....World Health Organization

LIST OF APPENDIX TABLES

CHAPTER I. INTRODUCTION AND LITERATURE REVIEW

Introduction

Developmental programming earlier referred as a "thrifty phenotype hypothesis" (Barker, 1992) is a process where various environmental factors along with maternal diet and age prior and during gestation can affect offspring health and productivity (Wu et al., 2006; Caton and Hess, 2010; Reynolds et al., 2010b; Reynolds and Caton, 2012). One of the primary physiological drivers of developmental programming is abnormal or insufficient placental development which directly affects fetal growth and development (Reynolds and Redmer, 1995; Reynolds et al., 2005a,b). The placenta is a primary nutrient and gas exchange organ and a powerful hormone producing system that affects both mothers and their offspring during gestation. Insufficient placental development leads to restricted fetal growth in utero. Unfortunately, intrauterine growth restricted (IUGR) pregnancies can also result in complications related to improper and immature offspring gut development. Interestingly, the placenta and gut are primarily derived from the embryonic endoderm and have very similar biological function in delivering nutrients for offspring survival (Trahair and Sangild, 2002). Therefore, the placenta and fetal gut will be given special attention in this thesis as two major nutrient exchange organs for fetal and neonatal offspring survival and health.

To investigate developmental programming effects in IUGR pregnancies, a highly controlled ovine overnourished adolescent pregnancy model was developed in the laboratory of Wallace et al. (2001, 2004b). In this model, overnourished adolescent ewes are rapidly growing at the expense of the growing gravid uterus (Wallace et al., 1996, 1999b, 2001) which results in placental growth restriction (30 to 40%) and premature delivery of low birth weight lambs (25 to 30% reduction) when compared to moderately nourished adolescents of the same age. In

addition, this ovine paradigm is also an excellent model to study human IUGR pregnancies (Wallace et al., 2001, 2002, 2003b; Regnault et al., 2002).

Both in human and livestock, IUGR pregnancies present not only health related complications but also result in costly economic problems. In humans, preterm and low birth babies are at risk of suffering respiratory distress and long-term neurodevelopmental, metabolic, and cardiovascular complications (Arnon et al., 2001; Adams-Chapman, 2006; Sholtz and Philips, 2009). In addition, hospitalization charges increase as birth weight and gestation age decreases (Cuevas et al., 2005). According to Russell et al., (2007), the cost for hospitalization of preterm and low birth weight infants in the US totaled $ 5.8 billion, which is almost half of the costs spent for all infant hospitalizations. In addition, for moderately preterm/low birth weight babies hospital costs average $15,100, and for extremely preterm infants costs average almost $65,600, whereas term babies hospital costs average $600 (Russell et al., 2007).

Similarly, in livestock, IUGR offspring result in poor postnatal growth, metabolic disorders, and dysfunction of specific organs, as well as altered body composition and growth efficiency (Wu et al., 2006; Reed et al., 2007; Caton et al., 2009a). Moreover, as reported by Reynolds et al., (2010a), the neonatal mortality rate in domestic animals is approximately 10%. Furthermore, the decrease in animal growth and productivity costs millions of dollars for feedlot producers annually (Smith et al., 1995; Gardner et al., 1998). Therefore, understanding placental development and developmental programming of a growing fetus is critical and has both long-term health and economic implications.

As reported by Reynolds and Redmer (1995) placental growth and proper vascularization are crucial for optimal fetal growth. Moreover, Wallace et al., (2008) demonstrated a high correlation between fetal birth weight and maternal circulating E2 levels during gestation. Most

importantly, E2 is known to play a critical role in angiogenesis (Niklaus et al., 2003), cell proliferation, and overall growth and vascular development in ovine placenta (Wallace et al., 2000; Reynolds, 2009). Therefore, placental size and vascular development determine prenatal growth trajectory of the fetus which ultimately affects postnatal viability and long-term health. Determining mechanisms and developing therapies to improve placental and subsequently fetal growth may help to manipulate and enhance both human health and livestock productivity.

Developmental programming in livestock and humans

Developmental programming, earlier introduced by Barker (1992) as a "thrifty phenotype hypothesis", also known as developmental origin of health and disease (DOHD) is the process where various factors including maternal nutrition prior to and during gestation have long-term effects on offspring health and productivity. In addition to maternal factors, there are other environmental stressors during fetal development and neonatal life that "program" the function and morphology of fetal organ growth (Godfrey and Barker, 2000; Barker, 2004). Moreover, this developmental programing can lead to increased susceptibility to metabolic syndrome and cardiovascular disease later in life (Barker, 1992; McMillen and Robinson, 2005). Depending on stage of gestation, maternal nutrition can affect fetal growth and development of specific organs. For instance, development of brain and the cardiovascular system is most sensitive during embryogenesis, whereas kidney development and function are most sensitive during placental development, and adipose tissue formation first appears around mid-gestation (Symonds et al., 2007). One of the key nutrients that may affect long-term developmental consequences for fetal life is an optimal supply of glucose transferred from mother to fetus during gestation (Symonds et al., 2012). Adequate availability of glucose along with other macro and micronutrients transferred from mother to fetus may insure optimal embryonic, placental, and fetal growth. It

has been previously established that alterations in macro and micronutrient composition of maternal diet either before gestation or different stages of gestation can have a pronounced effect on the embryo, placenta, and fetus (McMillen and Robinson, 2005). In sheep, reduced maternal food intake contributes to preterm birth (Bloomfield at al., 2003), while overnourishing adolescent sheep during pregnancy results in IUGR (Wallace et al., 1999, 2004b) and often preterm birth as well. In addition, prenatal growth is strongly dependent on maternal dietary intake from the early stages of embryonic life (Robinson et al., 1999). Therefore, it is very important to understand the mechanism driving developmental programing in both humans and livestock.

Several studies have demonstrated that periconceptional and neonatal periods are one of the most susceptible periods for developmental programing (Dolinoy et al., 2007; Fraga et al., 2005). In humans, the Dutch Hunger Winter (1944-1945) study related maternal undernourishment during pregnancy with increased incidence of offspring developing chronic metabolic disease and increased stress responsiveness (Painter et al., 2006a,b; Ravelli et al., 1999; Stein et al., 2006). However, animal models are the optimal models of choice in attempts to completely understand the physiological and molecular mechanisms of developmental programming (Barry and Anthony, 2008). As pointed out by Wu et al., 2006 and Meyer et al., 2012, epigenetic mechanisms are likely to be significantly involved in alteration of gene expressions leading to developmentally programmed fetal outcomes.

Epigenetics literally means in addition to genetics. Maternal and environmental factors modify DNA via direct DNA methylation/demethylation/acetylation/deacetylation or histone modification. In both instances, the normal “healthy” gene expression is “hijacked” by environmental factors by way of depleting or overstimulating gene expression. In mammals,

DNA methylation occurs on the C-5 of the pyrimidine ring of the cytosine base that is followed by guanine also referred as CpG dinucleotide (Rakyan et al., 2001). Methylation of CpG dinucleotides suppresses gene expression, whereas demethylation results in over expression (Urnov, 2002). Therefore, it has been proposed that, depending on nutrient availability including vitamins and nutrients participating in 1-carbon metabolism, the balance between methylation and demethylation processes can be obtained (Delcuve et al., 2009; Niehrs, 2009; Figure 1.1). Therefore, dietary nutrients are essential for the proper methylation and demethylation before, during, and after embryogenesis that affects changes in health later in life.

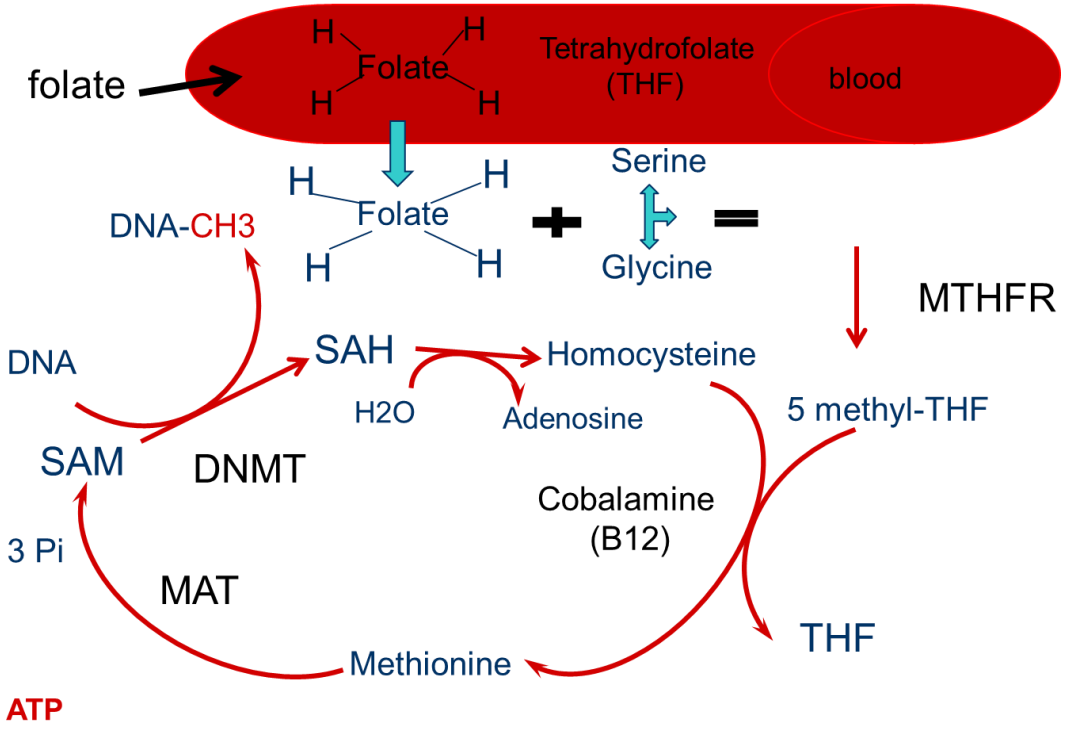

Figure 1.1. One-Carbon metabolism. Acronyms used are: MTHFR = Methylene tetrahydrofolate reductase; B12 = Cobalamine; MAT = Methionine adenosine transferase; SAM = *S*-adenosine methionine; DNMT = DNA methyl transferase (DNMT); DNA-CH3 = methylated DNA fragment; SAH = *S*-adenosine homocysteine.

Others have suggested that maternal nutrition alters growth and development (including vascularization) of key nutrient exchange tissues, including placenta and fetal small intestine,

which can lead to compromised fetal and offspring growth and development (Reynolds and Redmer, 1995; Caton et al. 2009a; Meyer et al., 2010a). Besides maternal nutrition, there are other stressors including maternal thermoregulatory capacity (Ziskin and Morrissey, 2011) and maternal age (adolescents) during gestation which may affect placental and fetal growth (Wallace et al., 1996, 1999a, 1999b, 2000, 2001).

The placenta, as a main nutrient and gas exchange organ and a powerful hormone producing system affects both maternal and fetal functions. Therefore, when proper placental vascularization and fetal nutrient availability are limited, adverse intrauterine environment and compromised offspring are likely to result. For the past two decades it has been demonstrated that compromised pregnancies leading to a restricted intrauterine environment results in preterm and low birth weight offspring. These offspring have a higher risk of developing health complications including cardiovascular and metabolic disorders, and compromised mental health not only in infancy but also later in life (Thorn et al., 2011; Schlotz and Phillips, 2009). In humans, many epidemiological studies confirmed the strong association between preterm delivery, low birth weight, and exposure to stress-related hormones with developing various diseases as adults (as reviewed by Reynolds and Caton, 2012).

Moreover, developmental programming affects livestock production as well. It was demonstrated by many researchers (Caton et al., 2007, Greenwood et al., 1998, 2000a, Wu et al., 2006, Larson et al., 2009, and Caton and Hess, 2010) that fetal growth restrictions due to maternal undernutrition or overnutrition have negative impacts on growth efficiency and body composition of offspring. Adverse developmental programming is likely prevalent in regions of the western U.S. (mainly due to limited forage quantity and quality) and in other similar environments throughout the world. Particularly, in the U.S. a poor maternal nutritional

environment is more likely to occur when breeding dams that are still growing (adolescents), or due to increased litter size, or increased maternal milk production to meet energy demands for lactation, which consequently diminishes energy supply for a growing fetus (Reynolds and Caton, 2012). These factors can result in fetal growth restrictions in utero and can lead to alterations in postnatal metabolism causing potential challenges for livestock producers. Therefore, understanding developmental programming and its effects will potentially increase the profitability for animal producers. In addition, as reported by Wu et al., (2006), compromised pregnancies result in increased neonatal mortality and morbidity, poor postnatal growth, reduced ADG, weaning weights, metabolic disorders, and dysfunction of specific organs including ovaries and testis, which by itself can impact livestock productivity. In fact, Wu et al., (2006) and Reynolds et al., (2010b) reported that in the U.S. domestic animal neonatal mortality rate is approximately 10% with mostly occurring in the first week after delivery. Therefore, understanding developmental programming and the critical windows before and during gestation can help us to manipulate and enhance animal growth and production potential.

For the past decade numerous epidemiological studies demonstrated the strong association of inadequate nutrition during gestation with increased incidence of low birth weight and preterm delivery in humans. Preterm and low birth weight babies are more likely than term babies to suffer complications such as respiratory distress (Arnon et al., 2001; Engle and Kominiarek, 2008), suffer brain injuries resulting in long term neurodevelopmental problems (Adams-Chapman, 2006), require intensive and prolonged hospitalizations resulting in high medical costs (Arnon et al, 2001; Ramachandrappa and Jain, 2009; Wang et al., 2004), and developing common diseases including obesity, cardiovascular disorders (CVD), type II diabetes (T2D), hypertension, asthma, mood disorders and schizophrenia, immune disorders later in life

and other pathologies (Schlotz and Phillips, 2009). Therefore, maternal nutrition is essential for proper placental development that will secure optimal nutrient, waste and gas exchange between the fetus and mother. Malnourished *in utero* fetuses tend to adapt to the restricted nutrient environment and appear to do better if exposed to similar nutritional conditions postnatally and in adulthood. However, if there is a mismatch between prenatal and postnatal environments, diseases are more likely to develop, according to the concept of "predictive and adaptive responses" (Gluckman and Hanson, 2006). It is important to notice that predictive and adaptive responses are induced only during crucial developmental windows that are specific to various organs. For instance, a study examining 500 men and women born in Helinski during 1924-1933 demonstrated the correlation of developing insulin resistance in those individuals who were thin at birth, remained thin till childhood, and rapidly gained weight in adulthood (Erikson et al., 2002). The probable explanation to this correlation as reported by Hales and Barker (2001) is that during fetal growth restriction, the number of pancreatic cells is most likely reduced, however the limited number of endocrine cells in pancreas cannot meet metabolic demands of an overweight adult. This mismatch of prenatal and postnatal environment altered physiological and metabolic homeostasis that interrupted normal energy metabolism, and in the case mentioned above, resulted in developing insulin resistance.

The role and importance of placenta in developmental programming has been studied by numerous researchers (Godfrey, 2002; Reynolds and Redmer, 1995; Reynolds et al., 2010 a,b; Gluckman and Hanson 2004a,b,c, 2006) using epidemiological studies as well as animal models. Placental insufficiency is a primary physiological key in developmental programming and leads to altered fetal growth and development. It is also important to note, that even though placental size is established by mid-gestation, its function continues to develop throughout gestation

depending on fetal needs and nutritional requirements (Reynolds and Redmer, 1995). For example, in response to certain fetal needs, placenta can alter its amino acid and transporter protein expression, which can alter placental blood supply. As reported by He et al., (2011) piglets had altered lipogenesis, amino acid and protein metabolism due to intrauterine growth restriction of placenta. Moreover, disturbed epigenetic regulation of the placenta during preimplantation and further in gestation results in IUGR (Nelisson et al., 2010). In addition, Thorn et al., (2011) reported reduced oxygen, isoleucine, insulin, IGF-I, and amino acid uptake in growth restricted sheep fetuses due to placental insufficiency. Therefore, one of the most important organs of developmental programing is the placenta.

Intrauterine growth restriction (IUGR) in livestock and humans

Intrauterine growth restriction is a condition where the fetus does not reach its full growth potential. The term IUGR is not the same as small for gestational age (SGA). A small for gestation age (SGA) fetus is the one who fully reaches it growth potential, but is statistically small when compared to a given population (Walther and Ramaekers, 1982). The most common definition of IUGR in humans is a fetal weight that is below the 10th percentile for gestational age as determined through an ultrasound (American Pregnancy Association, 2012). There are two types of IUGR: symmetric (primary) and asymmetric (secondary). Symmetric IUGR is characterized by all internal organs being reduced in size (about 20 to 25% of all IUGR cases in humans). Asymmetric IUGR is characterized by the head and brain being normal in size, but the abdomen is smaller (typically can't determine until the third trimester; American Pregnancy Association, 2012). In livestock, according to Wu et al. (2006), IUGR is defined as reduced fetal body weight relative to gestational age that is accompanied with impaired embryo/fetal growth and development. Although, defining IUGR in livestock is rather challenging due to the different

species and breeds. In our study, IUGR fetuses were defined based on their birth weights which were at least two standard deviation below the means of controls (3,591 g). However, if livestock IUGR was defined the same way as in humans, most of livestock IUGR offspring would not survive. For instance, approximately 20 to 65% of fall-born lambs that weigh less than 2.8 kg do not survive (Shelton, 1964). Moreover, as reported by Swalha et al. (2007), the mortality rate for lambs weighing less than 3.5 kg is between 5 to 45 % (Figure. 1.2). And even though those lambs that survive they experience increased morbidity and slow postnatal growth.

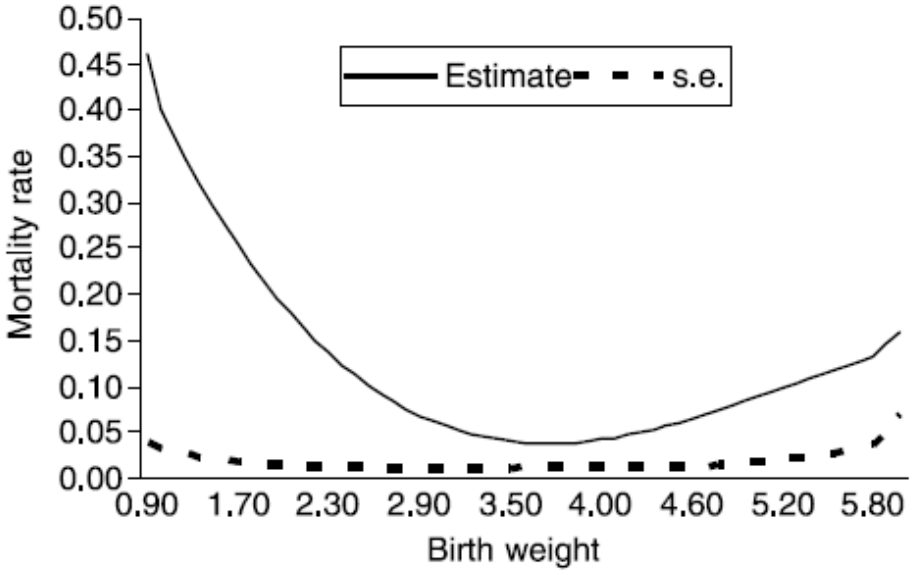

Figure 1.2. Relationship of mortality rate at birth with birth weight (kg). (Adapted from Sawalha et al., 2007)

Moreover, majority of IUGR pregnancies, both in livestock and humans, are due to reduced placental nutrient transport function, vascular development, and uteroplacental blood flows (reviewed by Reynolds et al., 2010a, b). In fact, IUGR remains one of the main causes of increased mortality and morbidity both in livestock and humans. In addition, IUGR potentially presents challenges not only in areas of proper growth and development and maintenance of health but also becomes costly economic problem.

Livestock

In livestock, intrauterine growth retardation and impaired growth and development of the fetus during pregnancy are a major concern for domestic animal production (Wu et al., 2006). Most IUGR neonates in livestock die in the first week of life. In the U.S. across all livestock species the rate of neonatal mortality is approximately 10% (Wu et al., 2006). Moreover, domestic animals are often bred at immature BW in order to increase animal productivity. However, several studies have demonstrated that lambs, calves, piglets, and foals from immature dams weigh 10 to 15% less compared to offspring born from mature dams (Bellows and Short, 1978; Quiniou et al., 2002; Wilsher and Allen, 2003). This decrease in fetal body weight compromises normal physiological development. For example, offspring from nutrient compromised dams can have reduced body composition, ADG, and growth efficiency (Reed et al., 2007; Caton et al., 2009a). Annually, the decrease in animal growth and suboptimal carcasses cost feedlot producers millions of dollars (Smith et al., 1995; Gardner et al., 1998). In addition, efficient, healthy, and quality livestock production is essential especially considering an increasing need of animal-source foods worldwide.

Humans

In humans, 50% percent of non-malformed stillbirths are due to IUGR (McMillen and Robinson, 2005). Moreover, infants weighing less than 2.5 kg at birth have perinatal mortality rates that are 5 to 30 times higher than those with average birth weights, while infants weighing less than 1.5 kg have mortality rates that are 70 to 100 times higher (McMillen and Robinson, 2005). In the U.S. there is a high incidence of pre-term delivery and low birth weight mostly due to IUGR leading to increased infant mortality. In fact, according to the Center of Disease Control the U.S. is 29th in terms of infant mortality rate worldwide, and the second worst in the

developed countries. However, the global burden of death and disability as a result of impaired fetal development is huge and affects both developed and developing countries (WHO, 2006). In fact, many factors including parental (or prenatal) genetics, maternal nutrition and age, and other environmental factors affect fetal growth. For instance, adolescent pregnancies are at risk of developing IUGR. Moreover, the adolescent population in the U.S. is rapidly increasing from 34.9 million to 40.7 million between 1990 to 2000, and expected to increase up to 53.2 million by 2050 (National Adolescent Health Information Center, 2003). In the U.S., adolescent pregnancy rates have reached 750,000 per year (Kost et al., 2010). The annual public cost associated with adolescent childbearing care has been estimated at $ 9.1- $10.9 billion annually (Hoffman, 2006; The National Campaign to Prevent Teen and Unplanned Pregnancy, 2011).

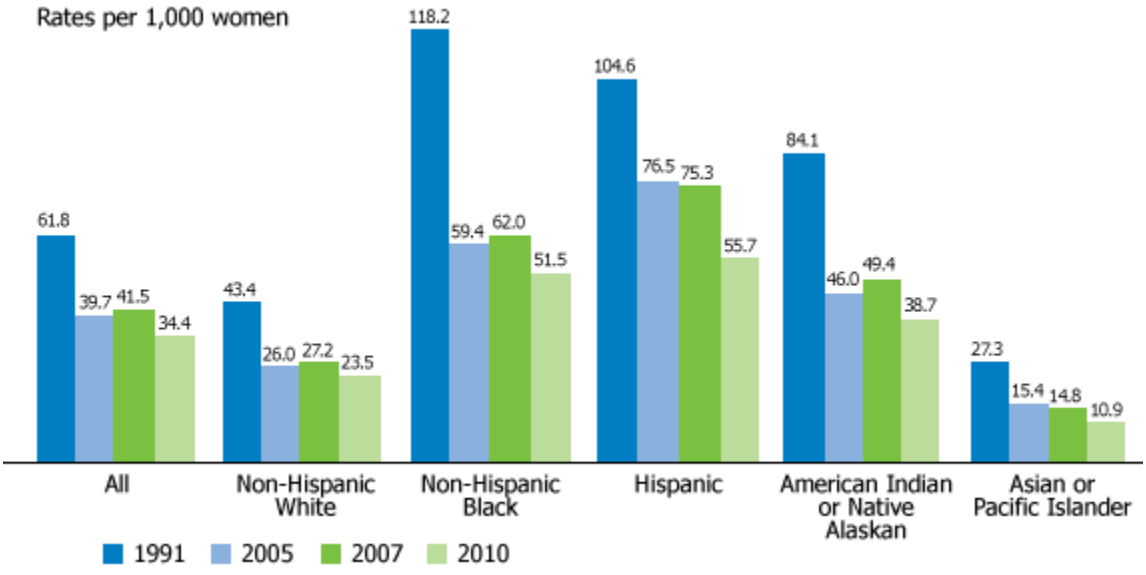

Figure 1.3. Birth rates for U.S. women ages 15 to 19, by race and ethnicity. (Adapted from Hamilton and Ventura, 2012).

For the past 70 years for which national data are available on adolescent childbearing, only in 2009 the birth rate for adolescents was lower (Martin et al., 2010; Hamilton et al., 2010; Ventura et al., 2001) comparing to birth rates in previous years (39.1 and 48.7 births per 1,000

females aged 15–19, respectively; Stephanie et al., 2011; NVSR, 2001). Although the births to adolescents fell in 2009 to 409,840 compared to previous years (37% less from 1991), it still remains the highest among industrialized countries (UNSD, 2009). The latest statistics available on adolescent childbearing reported by Hamilton and Ventura (NCHS Data Brief 89; 2012) in the U.S. is shown in Figure 1.3.

What is more alarming is that adolescent girls have increased risk of delivering premature and low birth weight babies when compared with mothers who are older than 20 (MOD 2002: NLM, 2002). Poor pregnancy outcomes in adolescent girls (aged 12 to 19 years) is due to several factors including poor socio-economic status, gynecological immaturity, or the growth and nutritional status of the mother at the time of conception (Fraser et al., 1995). Poor socio-economic environment of adolescent pregnancies are strongly linked to early sexual activity, poor knowledge of reproductive health, low educational expectations, age of partner, parental illiteracy, sexual and domestic abuse, disrupted or non-existent family structure, and ethnicity (as reviewed by Wallace et al., 2005a).

Furthermore, the rate of spontaneous miscarriage is the greatest in girls aged 13 to15 years (SNAP, 1994). Similarly, in a large population-based study involving over 300,000 pregnancies, the rates of very pre-term births (<32 weeks) increased significantly with decreasing maternal age and almost entirely explained the increased risk of neonatal and post-neonatal mortality in the 13 to 15 age group (Olausson et al., 1999). Moreover, when analyzing term births between 39 and 41 weeks of gestation, the younger the mothers (12 to 16 years old) the smaller their infants comparing with both older adolescents (17 to19 years old) and adult women (20 to 29 years; Kirchengast et al., 2003). As reviewed by Wallace et al., (2005a), very young mothers (12 to 16 years old) have a higher risk of delivering IUGR infants weighing less

than 2.5 kg. Additionally, the adolescent female population is also at high risk of developing pregnancy related complications including a four-fold increase in maternal mortality rates when compared to women aged 20 to 24 years. Therefore, it is evident that the adolescent girls and their gynecological immaturity during gestation negatively affects fetal development and puts mothers themselves at risk of developing pregnancy related complications. These complications are mainly due to the fact that adolescent girls are still growing and are competing with a growing fetus (Wallace et al., 2004b). In summary, it is clear that there is a crucial role of nutrient availability and proper placental development for optimal fetal growth and development.

Ovine models and IUGR

The importance of proper placental function is described above. However, to perform detailed experiments on placental function and IUGR pregnancies in humans presents ethical issues; therefore, to better understand the driving mechanisms of IUGR pregnancies and possible therapeutical approaches to rescue those fetuses, the overnourished adolescent sheep model was developed in the laboratory of Wallace et al., (2001, 2004b). This unique and highly controlled experimental paradigm has been developed showing that overnourishing singleton-bearing adolescent ewes results in rapid maternal growth and mostly acquisition of maternal adipose tissue at the expense of the growing gravid uterus (Wallace et al., 1996, 1999b, 2001). In this model, ewes become obese. Moreover, overnourishing adolescent ewes results in major placental growth restriction (30 to 40%), leading to the premature delivery of low birth weight lambs (25 to 30% reduced in birth weight) when compared with moderately nourished adolescents of the same gynecological age. Therefore, maternal nutrition and dietary intake in the still growing adolescent ewes during pregnancy affects pregnancy outcomes.

Numerous studies have demonstrated that this pregnant adolescent ewe model is an excellent model to study human IUGR pregnancies (Wallace et. al., 2001, 2002 and 2003b; Regnault et al., 2002; Figure 1.4).

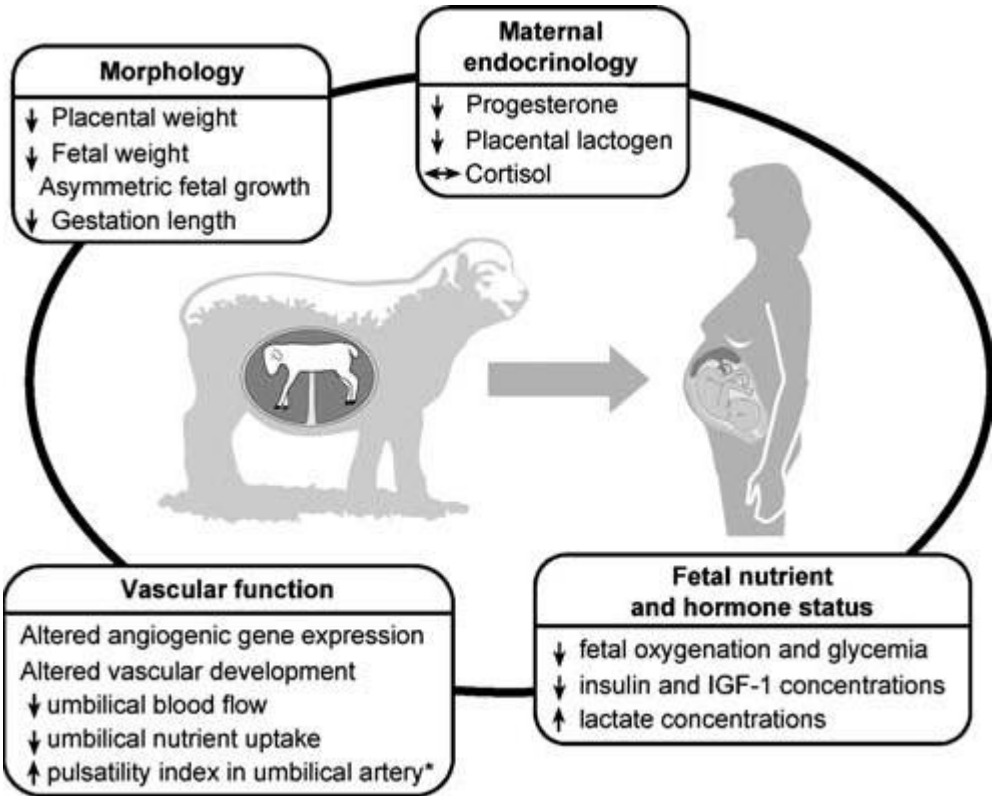

Figure 1.4. Features similarities in fetal growth restriction due to placental insufficiency in the human and in two contrasting ovine paradigms, manly the hyperthermic adult and the overnourished adolescent sheep. (Adapted from Wallace et al., 2005a).

In addition, studies using the ovine model substantial experimental evidence regarding the importance of maternal nutrition, maternal age, placental growth and nutrient transfer in terms of fetal organ growth and development, thereby strengthening our overall biological understanding of IUGR pregnancies (Alexander 1974, Battaglia and Meschia 1988, Ferrel 1989, Robinson, 1990, Bell et al., 1999, Wallace et al., 1999, 2004, 2005, 2006a,b, Reynolds and Redmer, 1995, Caton et al., 2009; Meyer et al., 2010a).

There are many similarities (but also differences between sheep and human pregnancies. For instance, the extended length of gestation, singleton pregnancies, and similar birth weights are observed. Most importantly, there are many similarities with respect to organ development including brain, kidney, reproductive system, pulmonary system, HPA axis, and physiological processes in both humans and sheep. During pregnancy, adult energy requirements increase (Stock and Metcalfe, 1994; Reynolds and Redmer, 1995), and increased dietary intake has been reported to begin at about 12 weeks of gestation in humans (Hirschberg, 1998). Although maternal obesity or excessive weight gain during pregnancy in adult humans do not compromise fetal growth, and may even result in increased birth weight, they do result in fetal growth abnormalities and increased risk of fetal and neonatal morbidity and mortality (Naeye, 1990; Cnattingius et al., 1998; Ogunyemi et al., 1998; Schieve et al., 2000; Abrams et al., 2000; Stephansson et al., 2001; Castro and Avina, 2002; Luke et al., 2003). This is not the case in adolescent girls. Unfortunately, due to a high degree of non-compliance, it is difficult to control and assess the nutritional intake of human adolescents.

Lenders et al. (1997) reported an association between high sugar diets with delivering low birth babies. To further emphasize the importance of the overnourished adolescent ovine model, it was demonstrated that the increased dietary intake during pregnancy is associated with poor pregnancy outcomes, along with the high incidence of obesity among adolescents, especially in very young obese pregnant adolescents resulting in preterm delivery (Perry et al., 1996; Hediger et al., 1998). Therefore, using a similar approach in sheep, multiple studies confirmed that an average 52% of rapidly growing dams produce fetuses that are growth restricted at term (Wallace et al., 2004b). In these pregnancies, as reported by Wallace et al., (2006a), average placental and fetal weights were reduced by 48% relative to controls.

Therefore, this ovine paradigm is an excellent model to test inappropriate nutrient intake and its effects on pregnancy outcomes via alteration of placental development leading to IUGR. Moreover, it is a very consistent model to test therapeutic approaches for rescuing IUGR pregnancies.

Placental growth and angiogenesis, developmental programming, and IUGR.

The placenta is the major organ through which gases, nutrients, and wastes are exchange between maternal and fetal systems (Reynolds and Redmer, 1995; Challis et al., 2000). Therefore, placental size and vascular development (angiogenesis) play crucial roles in determining the prenatal growth trajectory of the fetus, affecting birth weights, postnatal viability, and long-term health (Reynolds and Redmer, 2001). In other words, placental growth, angiogenesis (formation of new blood vessels from existing ones) and function, program fetal prenatal and postnatal growth and development (Wallace et al., 2004; Reynolds and Redmer, 2004). Thus, limited placental growth and hindered nutrient transfer capacity may lead to intrauterine growth restriction which may lead to adverse complications in adulthood.

Normal placental growth and development

The term placenta from Greek *plakuos* means “flat cake” and was named based on its anatomical appearance. It is a materno-fetal organ that starts forming at implantation of the blastocyst and provides nutrient, gas, and waste exchange between the mother and fetus, as well as endocrine and immune support for a growing fetus during gestation (Reynolds and Redmer, 1995; Challis et al., 2000; Godfrey, 2002). The human placenta is a hemochorial placenta in which maternal placental tissue erodes allowing maternal blood to bathe the trophoblast. In other words, the human placenta is an invasive placentation where the fetal embryonic membrane erodes into the maternal endometrium and forms a large single area of contact between the fetus

and mother (Bowen, 2000). In both human and sheep placentation, the trophoblast (outer layer of the blastocyst) and mesoderm (middle portion of the blastocyst) form the chorion. In humans, finger-like projections of the trophoblast (invasive trophoblast) which consists of multinuclear syncytiotrophoblasts and extravillous trophoblasts (Cross et al., 1994; Zhou et al., 1997) insert themselves deeper into the uterine endometrium and inner myometrium. Invasive trophoblast, along with mesoderm (containing blood vessels) extends along the core of each trophoblastic villi to form chorionic villi in contact with uterine vasculature and undergo endothelial-like specialization to replace the smooth muscle layer of spiral arteries (Ishida et al, 2011). These chorionic villi continue to enlarge and branch, forming a complex network within the endometrium. As chorionic villi continue "attacking" maternal endometrium, maternal blood vessels are being eroded, and maternal blood percolates through a small spacing (lacunae) lined with cellular and syncytial trophoblast demonstrated in the Figure 1.5.

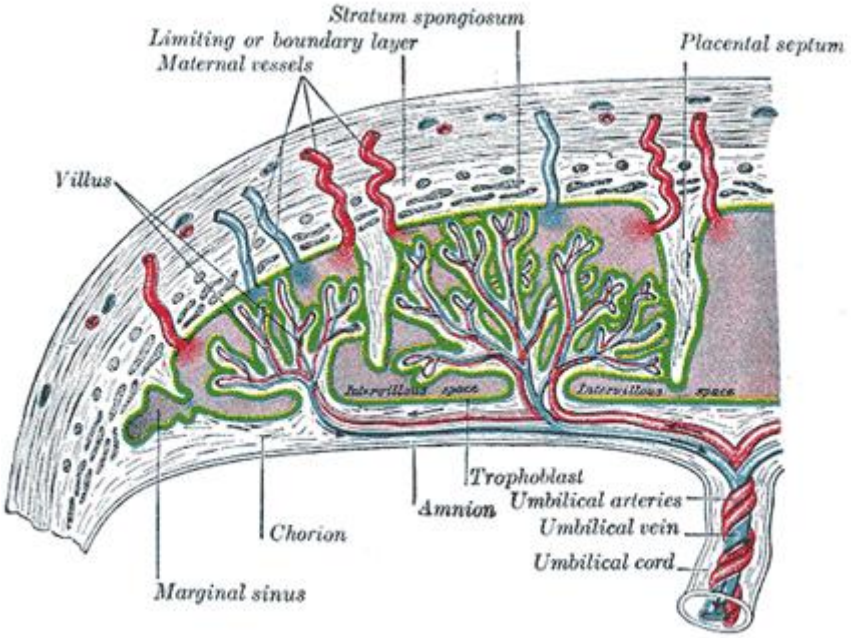

Figure 1.5. Human placentation and vascularization. (Adapted from Grey's Anatomy online source on 05/05/2012)

It is important to note that the level of trophoblast invasion of endometrium and myometrium and the degree of remodeling of spiral arteries can predict the placental function. For instance, Meekins et al., (1994) reported shallow trophoblast invasion and inadequate remodeling of spiral arteries in pre-eclampsia pregnancies which resulted in poor dilation and high resistance capacities in placental blood vessels leading to insufficient blood supply to a growing fetus.

Ruminant placenta are classified as epitheliochorial placenta where the embryonic chorioallantoic membrane forms attachments with patches of endometrial caruncles at about 3 weeks of gestation (Bowen, 2000) forming placentomes that contain maternal (caruncular) and fetal (cotelydon) portions of the placenta shown in Figure 1.6.

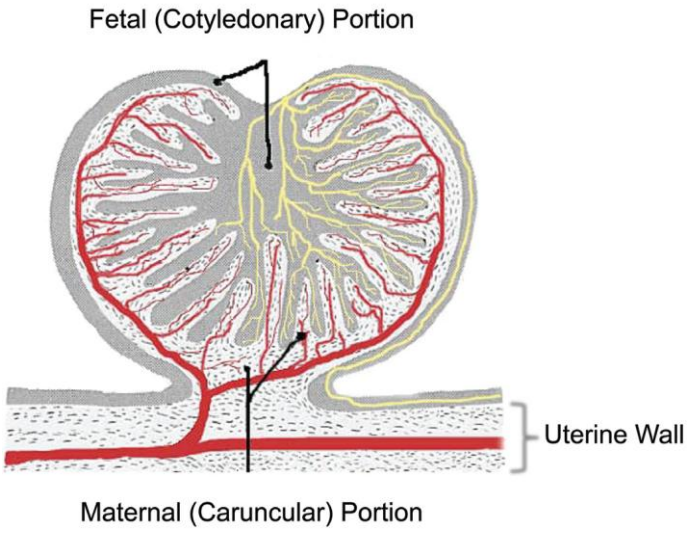

Figure 1.6. Schematic representation of the sheep placentome. (Adapted from Ramsey, 1982.)

Overall, in epitheliochorial placenta, cotyledons (COT) form small villi that extend into caruncular (CAR) epithelium "semi-invading" maternal endometrium in a similar manner as hemochorial placentation but in distinct patches (placentomes). According to Bowen (2000), there are 75 to 125 placentomes per sheep placenta. As reported by Barcroft and Kennedy (1939) sheep cotyledonary placenta are fully formed by day 30 of gestation; however, its full

growth occurs between day 50 and 60 of gestations (Ehrhardt and Bell, 1995). As shown in the Figures 1.7 in sheep, nutrients, wastes, and gases pass through six tissue layers, though some argue, that maternal epithelium is transiently eroded therefore having only five layers to pass through and is referred as syndesmochorial placenta (Reynolds et al., 2005a).

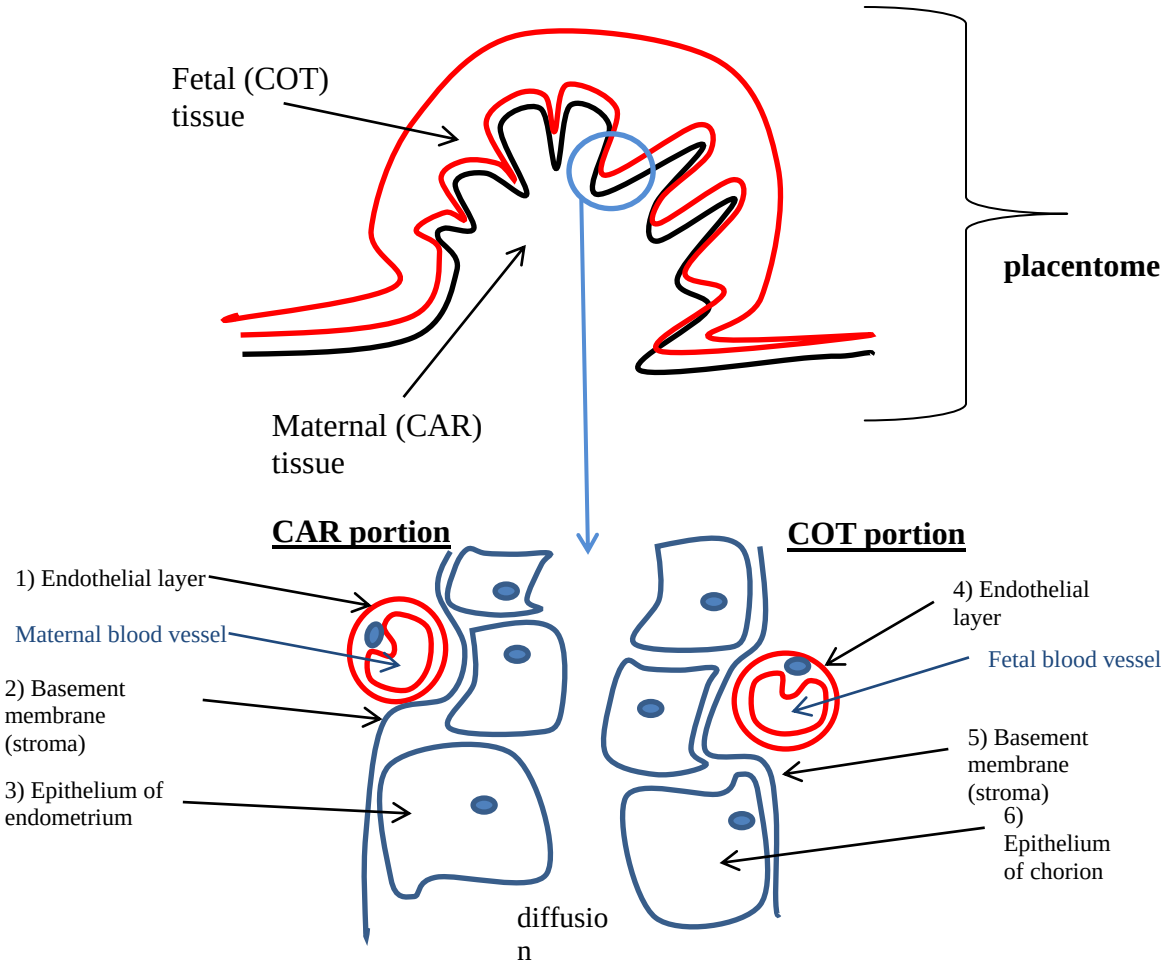

Figure 1.7. Schematic representation of sheep placentome including caruncular and cotyledonary tissues and six tissue layers in the epithelialchorial placenta of the sheep. (Adapted from Senger, 2007)

Contrary to epitheliochorial placentation, in humans due to the invasive implantation of the trophoblast into the endometrium, there are only three cell layers (Figure 1.8) for exchange.

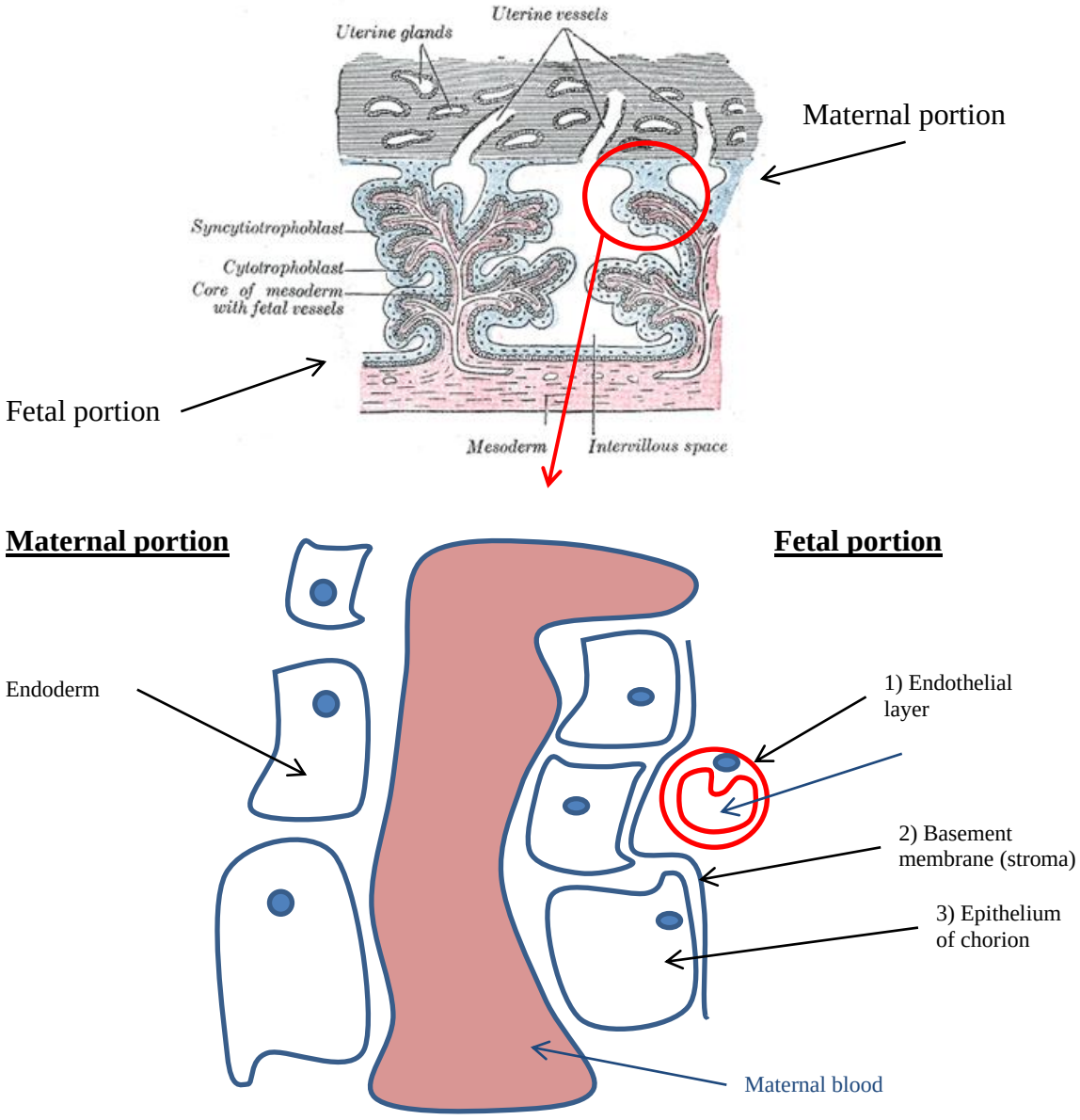

Figure 1.8. Schematic representation of human placenta including three tissue layers in the hemochorial placenta of human. (Partially adapted from Segner (2007) and Grey's Anatomy online resource).

In addition, both in human and sheep placenta maternal and fetal blood is not in direct contact. In humans, the diffusion occurs between maternal blood filling the lacunae and fetal blood flowing within the chorionic villi (Martini et al., 2006; Fig. 1.5) while in sheep, due to its non-invasive nature, there is absolutely no contact between fetal chorionic villi and maternal blood. In both humans and sheep, deoxygenated blood flows from the fetus to the placenta via

paired umbilical arteries and returns oxygenated via a single umbilical vein. Nutrients from the mother including O2 and glucose leave maternal blood vessels and enter fetal placental vessels. Similarly, fetal waste travels via the placenta only in a reverse direction.

During the implantation period in both humans and sheep, nutrients absorbed by the trophoblast can easily reach the blastodisc via simple diffusion. However, as the embryo and trophoblastic complex become larger, the distance between the two increases and simple diffusion of nutrients is not sufficient for a growing fetus. As a result, the chorionic villi penetrate the uterine caruncular endometrium to link the trophoblast with embryo. Recent studies of mouse mutants with disrupted placental development indicate that signaling interactions between the placental trophoblast and embryonic cells play a key role in placental morphogenesis (Rossant and Cross, 2001). By week four of development in human, the embryo, amnion, and yolk sac are suspended in the fluid-filled sac. The body stalk forms the connection between the embryo and chorion and contains blood vessels that carry blood to and from placenta. At first the blastocyst is surrounded by chorionic villi. But as placental organization starts developing, chorionic villi disappears on the thin portion of the endometrium that covers the embryo and separates it from the uterine cavity (decidua capsularis). By the end of the first trimester, the fetus moves further away from placenta and remains connected to placenta via an umbilical cord, which contains the allantois, placental blood vessels, and the yolk stalk (the narrow connection between the endoderm of the embryo and the yolk sac; Martini et al., 2006).

Moreover, the placenta synthesizes hormones that affect maternal as well as fetal tissues. For instance, in humans within first days of implantation, human chorionic gonadotropin (hCG) is released to stimulate the corpus luteum (CL) to continue production of progesterone during the early stages of gestation. In sheep, around day 12, interferon τ secreted by the embryo signals

maternal recognition of pregnancy (Spencer and Bazer, 2004). However, during the second and third trimester, in both humans and sheep, the placenta itself releases progesterone to maintain pregnancy. Additionally, placenta releases estrogens, placental lactogen, and relaxin, all of which are synthesized by the trophoblast.

Furthermore, placental growth slows down by the third trimester while fetal growth is exponential. Therefore, to keep up with the metabolic needs of a growing fetus, placental transport capacity also increases (Reynolds and Redmer, 1995). Both in humans and sheep, uterine and umbilical blood flow increases significantly as gestation proceeds (Reynolds, 1986). Similarly, fetal glucose uptake also increases throughout gestation (Reynolds, 1986). It is clear that placental development, its vascular growth and angiogenesis, along with nutrient transport capacity are major players for optimal fetal growth and development.

Placental function and angiogenesis

The placenta produces hormones that affect fetal and maternal physiology and metabolism. The growth of the placenta precedes that of the fetus and a strong positive association exists between placental mass and size at birth in all species studied, including sheep and humans (Reynolds and Redmer, 1995). Placental efficiency is often determined by the ratio of neonate weight to placenta weight, which is 6:1 in humans and 10:1 in sheep (Luther et al, 2007). Moreover, during early pregnancy, placenta synthesizes glycogen, cholesterol and fatty acids that serve as energy sources for the fetus (Ishida et al., 2011). Thus, the size and nutrient transfer capacity of the placenta play a central role in determining the prenatal growth trajectory of the fetus and hence birth weight, postnatal viability, and long-term health.

Proper placental vascular growth and development is pivotal for optimal placental function including delivering nutrients and oxygen to fetus, as well as balancing hormones in

fetal and maternal systems. In humans, the amount of circulating blood in the mother during pregnancy increases 40 to 45% (Pritchard, 1965; Whittaker et al., 1996). In sheep, uterine and umbilical blood flow increases significantly during pregnancy (Reynolds, 1986). This observation alone indicates the importance of the vascular system in placental function. Alterations in vascularity are thought to contribute to poor placental function and therefore poor pregnancy outcome (Reynolds and Redmer, 1995). Thus, it is not surprising that fetal growth restriction in a number of experimental paradigms is highly correlated with reduced placental growth and development (Reynolds and Redmer, 1995, 2001). Establishment of functional fetal and placental circulations is one of the earliest events during embryonic/placental development (Ramsey, 1982). It has been shown that the large increase in transplacental exchange, which supports the exponential increase in fetal growth during the last half of gestation, depends primarily on the dramatic growth of the placental vascular beds during the first half of pregnancy (Meschia, 1983; Reynolds and Redmer, 1995; Redmer and Reynolds et al., 2004).

Vascular density of maternal caruncles in sheep increases substantially from day 40 until mid-gestation, and then slows down as reviewed by Luther et al., (2007). In contrast, vascular density in fetal cotyledons remains relatively constant until mid-gestation, and dramatically increases along with the exponential growth of the fetus. Moreover, Borowicz et al. (2007), measured vascular development in the maternal and fetal portions of the placentome during the last two thirds of gestation and reported that capillary area density increased exponentially from day 50 to 140 of gestation in both maternal and fetal portions of placentome.

Capillary area density is often used as a measure of angiogenesis, and based on stereological principles also is related to capillary volume density (capillary volume as a proportion of total tissue volume; Weibel, 1972; Hudlicka, 1984). However, capillary number

density increased (12.3-fold) dramatically only in the cotyledon, and not as much (1.5-fold) in the maternal caruncular portion of the placenta and indicates that maternal caruncular vascular growth is primarily achieved through increased size of the vessels and rather than number of vessels or surface area (Borowicz et al., 2007). Based on these observations, the empirical model for normal angiogenesis in the ovine placentome throughout the last two-thirds of gestation was developed (Figure 1.9).

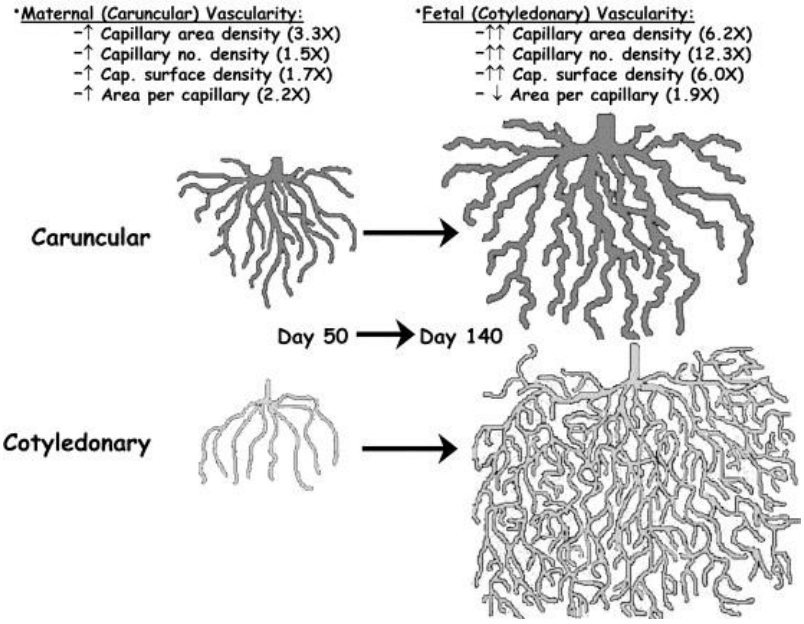

Figure 1.9. Empirical model of angiogenesis in the maternal caruncular and fetal cotyledonary portions of the sheep placenta throughout the last two-thirds of gestation. (Adapted from Borowicz et al., 2007)

Maternal caruncular vascular beds grow by increasing the size of the capillaries with a very slight increase in capillary number or surface densities, resulting in a 3.3-fold increase in capillary area density. In contrast, fetal cotyledonary capillary beds grow by branching, leading to increased capillary area density (6.2-fold), number density (12.3-fold), and surface area density (6-fold), and by decrease in capillary size. The increased demand of the growing fetus from mid to late gestation is met by increasing branching growth of fetal cotyledonary vascular beds, which allows increased umbilical blood flow and efficient transplacental exchange. This is

in agreement with two to four-fold increases of nutrients and oxygen uptake by the gravid uterus from mid- to late gestation (Reynolds, 1986; Ferrell, 1989). Most importantly, Kaufmann et al., (2004) reported that human placental capillary volume density of the fetal villi increases throughout pregnancy and is accompanied by a decrease in capillary diameter. Therefore, exponential increase in fetal cotyledonary vascular bedding tissue during the last half of gestation is crucial for proper fetal growth and development.

Furthermore, from fertilization until the parturition, maternal and fetal physiological changes occur including altered energy balance, osmoregulation, and the metabolism of carbohydrates, amino acids, lipids, nutrients, vitamins and glucocorticoids. Therefore, placenta also plays an important role in maintaining maternal and fetal homeostasis during pregnancy (Ishida et al., 2011).

Angiogenesis refers to the formation of new vascular beds from existing ones through branching and sprouting, and is a critical process for normal tissue growth and development (Aron and Anthony, 2004; Reynolds et al., 1992). The increased vascularity of tissues like the placenta result from the effects of a cadre of many growth factors and receptors that promote angiogeneses, including vascular endothelial growth factor (VEGF), angiopoietins (ANGPT-1 and ANGPT-2), endothelial nitric oxide (eNOS) and other systems. During pregnancy, VEGF, fibroblast growth factor (FGF), placenta growth factor (PGF) and several other factors are critical for normal placental function (Reynolds and Redmer, 2001; Zygmunt et al., 2003). Previous studies demonstrated the role of VEGF and its receptors fms-related tyrosine kinase (FLT1) and kinase insert domain receptor (KDR) as potent angiogenic factors that cause endothelial cell proliferation and migration (Reynolds and Redmer, 2001; Kaufmann et al., 2004; Reynolds et al., 2005b). Additionally, VEGF and FLT-1 were detected in yolk sac mesoderm

and endoderm at the early stage of development (Breier et al., 1995), while KDR was expressed in proliferating endothelial cells of vascular sprouts (Millauer et al., 1993). Moreover, Ferrara et al., (1996) and Carmeliet et al., (1996) demonstrated in a knockout model that the loss of a single allele of VEGF was lethal in the mouse embryo in midgestation. During the late gestation VEGF or FLT1/KDR mRNA are associated with angiogenesis that occurs during development of brain ventricles, kidney glomeruli, and placental tissues in mice (Breier et al., 1997; Dumont et al., 1995). As reviewed by Harry and Paleolog (2003), FLT1 is important for cell and cell-matrix communication, disruption in KDR gene causes embryonic death due to defects in development of hematopoietic and endothelial cells. Therefore, proper expression of VEGF and its receptors play a pivotal role in embryo and placental development.

Interestingly, in sheep placenta, mRNA expression of VEGF and its receptors (FLT and KDR) were differently expressed depending on maternal caruncular and fetal cotyledonary tissues during mid to late pregnancy (Borowicz et al., 2007). For CAR, VEGF expression increased only slightly (about 2-fold) whereas expression of FLT1, and especially KDR, increased dramatically throughout gestation. In contrast, in CAT VEGF and FLT1 mRNA increased only slightly, and KDR mRNA expression did not change throughout gestation (Borowicz et al., 2007). In addition, there was a significant correlation between capillary area density and expression of FLT1 in CAR (Borowicz et al., 2007; as reviewed by Reynolds et al., 2005a). However, in COT, capillary area density (CAD), capillary number density (CND), and capillary surface density (CSD) were correlated with VEGF and ANGPT-2, but not ANGPT-1 (Borowicz et al., 2002, 2007). The vascular-specific growth factors ANGPT-1 and ANGPT-2 are mediated via the tyrosine kinase receptor (Tie2) which is mainly present in endothelial cells (Suri et al., 1996). Thus, the angiopoietin system has been shown to play a role in regulating vascular

growth (Breier et al., 1997; Lindahl et al., 1998). Most importantly it has been demonstrated that ANGPT-2 promotes vascular remodeling in the presence of VEGF and is associated with vascular density in both maternal and fetal portions of the placenta in sheep (Kohl et al., 2002; Borowicz et al., 2007). In addition, ANGPT1 appears to be produced primarily by peri-endothelial cells and thus may act as a "partner" to VEGF in the angiogenic process (Holash et al., 1999).

Activity of the VEGF and ANGPT systems depend on NO synthesis for new blood vessel formation (Bussolati et al., 2001). Kroll et al., (1998) reported that VEGF induces eNOS dependent NO synthesis via activation of FLT1 and KDR (Kroll et al., 1998; Fukumura et al., 2001). Nitric oxide induces cell migration in angiogenesis by increasing the expression of adhesion molecules and extracellular matrix metalloproteases leading to invasion of endothelial cells and new blood vessel formation (Lee et al., 2000; Lopez-Rivera et al., 2005; Genís et al., 2007; Lee et al., 2009; Chen et al., 2004). Angiopoietins (ANGPT1 and ANGPT2) not only activate eNOS to increase cell proliferation and angiogenesis, but also the ANGPT/eNOS system aids in vessels maturation and stabilization (Gavard, 2009; Gonzalez et al., 2003).

In conclusion, balanced expression of angiogenic factors and their receptors is essential for proper embryogenesis, placental vascularization and angiogenesis, and umbilical and uterine blood flow. Specifically for placenta, branching and sprouting of new blood vessels from existing ones is critical for nutrient and metabolite exchange between the fetal and maternal circulation.

Compromised placental growth and development

Failure of the maternal supply of nutrients to match fetal requirements leads to fetal IUGR, which further affects offspring postnatal health (Godfrey et al., 2002). Many factors can

hinder normal placental development and growth, including maternal genetics, age, nutrition, stress, and other environmental factors such as eating disorders, smoking or alcohol abuse. Placental nutrient supply is one of the major determinants of intrauterine growth. In turn, placental nutrient supply is dependent on placental size, morphology, blood supply, abundance of transporters in placenta, and on synthesis and metabolism of nutrients and hormones by uteroplacental tissue. Imprinted genes Igf2 and H19 also play important roles in feto-placental development and may affect growth, morphology and nutrient transfer capacity of the placenta (Fowden et al., 2006). Size of placenta can affect efficient transport of nutrients, as reported by Heasman et al., (1999), depending on the severity, duration and gestational age, both under-nutrition and over-nutrition affects placental size. Jensen et al., (2002) reported that glucocorticoid administration during gestation in sheep decreased placental size but increased placental glucose transport efficiency. It seems that poor/excess nutrition, and glucocorticoid exposure during the crucial stages of gestation increases placental efficiency by transferring nutrients to fetus as a compensating mechanism (Fowden et al., 2006). Placental morphology is also affected by maternal nutritional status and hormonal imbalances.

Moreover, Huang et al., (2012) reported that excessive caffeine consumption along with tobacco use resulted in placental injury leading to morphological changes including a reduction in the villous vasculature, luminal stenosis, trophoblastic karyopycnosis in rodents. Besides these placental morphological pathologies, placenta is largely dependent on proper development of vascular beds. Additionally, caffeine consumption along with smoking can cause vascular pathology in placenta affecting the renin-angiotensin pathway both in maternal circulation and local placental circulation in rodents (Huang et al., 2012). Increased vascularization during gestation is accompanied with increased blood flow to meet nutrient demands of a growing fetus.

Therefore, proper placental blood vessel morphology is crucial for optimal blood flow. Clinically, increased uterine vascular resistance and reduced uterine blood flow are good predictors of high-risk pregnancies associated with fetal growth retardation (Reynolds and Redmer, 1995, 2001). Consequently, factors affecting placental vascular development will have a dramatic impact on fetal growth and development. These vascularization processes are dependent on a wide variety of angiogenic molecules that contribute to the development of the placental circulatory system.

As discussed earlier, VEGF, FGF, and PGF are essential factors for proper placental development and therefore, fetal growth and development. However, in cases of overexpression of soluble FLT-1 (sFLT-1), endogenous angiogenesis inhibitor expression can lead to endothelial cell dysfunction by blocking VEGF and PGF's normal physiological activity (Kendall et al., 1993; Stepan et al., 2006). In addition, sFLT-1 has been identified as an important marker for pregnancy-induced hypertension (PIH) and is released in excess from placenta into the maternal circulation (Maynard et al., 2003; Cudmore et al., 2007). It has also been suggested that the human maternal immune system plays an important role in pathogenesis of pre-eclampsia. For instance, the natural killer (NK) cells normally interact with trophoblast and assist in invasion of the maternal endometrium, however, the number of NK cells decreases towards the end of the normal pregnancy. However, in the case of pre-eclampsia NK cells remain active throughout pregnancy (Berg et al., 1983). Ishida et al., (2011) suggested that increased NK cells can cause systemic inflammation with endothelial damage.

In addition, Belkacemi et al., (2011) measured the levels of glucocorticoids (GC), their receptor (NR3C1), and 11-hydroxysteroid dehydrogenase ß (HSD11ß) in undernourished dams, and reported increased plasma corticostrone levels in undernourshied rat dams compared with

control. Moreover, HSD11B1-2 was decreased, while HSD11B-1 was increased which led to decreased GCs catabolism, and interestingly it was accompanied with down regulation of the nutrient transporter for glucose (SLC2A1, SLC2A3) and amino acids (SLC38A1, 2, and 4) in the mid- and proximal-horns. Therefore, decreased nutrient supply due to down regulation of some of the amino acid system A transporters may affect fetal growth in IUGR pregnancies.

Moreover, Börzsönyi et al., (2011) demonstrated that maternal gestational weight gain and increased BMI is associated with development of IUGR in mice. Fetuses form IUGR pregnancies have reduced insulin and carbohydrate metabolism capacity compared to non-IUGR pregnancies in pigs (He et al., 2011). This could be due to *in utero* decreased number of ß-cells as well as reduced IGF-I circulating during organogenesis in growth restricted fetuses that altered fetal metabolism.

Therefore, abnormal placental morphology, vascularization, changes in circulating hormones, and altered placental transport capacity can affect normal fetal growth and development resulting in IUGR fetuses. One of the ways of rescuing IUGR fetuses is to target those developmental windows with epigenetic, angiogenic, and nutritional factors including proper diet that promotes 1-C metabolism, supplementation of E2, VEGF, and other factors that promote angiogenesis targeting placental growth.

Fetal intestinal biology and growth

We have already discussed the importance of proper placental development for optimal fetal growth. Both placental development and fetal growth are highly dependent on maternal provision of life supporting elements such as nutrients, oxygen, amino acids and transporters. Moreover, placenta and gut are primarily derived from the embryonic endoderm and have very similar biological function in delivering nutrients for survival. Unfortunately, IUGR pregnancies

can lead to complications related to improper fetal gut development. For example, gut tissue at birth is normally adequately matured and supported to provide nutrients to the entire body by adapting to changes in the extrauterine environment and resisting certain diseases. However, IUGR fetuses are at risk of slow GI tract maturation and therefore might potentially cause postnatal complications and complications that can be carried on into adulthood.

Fetal and neonatal gastrointestinal (GI) tract development and growth

Fetal and neonatal GI tract development is highly dynamic and includes formation of gut, appearance of villi and digestive enzymes, development of swallowing, and development of mature motility patterns (Montgomery et al., 1999; Trahair et al., 1997; Trahair and Sangild, 1997). In addition to perinatal GI tract growth, small intestine continues to develop into maturity responding to various physiological and environmental changes. Meyer et al., (2012) has demonstrated in Figure 1.10 the critical windows of small intestinal development and its effects on developmental programming of the small intestine.

During early embryogenesis, the gut tube is formed from the intraembryonic portion of the yolk sac and predominantly consists of endoderm (small intestinal mucosal and submucosal layers of intestine) and some mesoderm (muscularis and connective tissue) and ectoderm (Ross, 2004; Trahair and Sanglid, 2002). In sheep, the gut tube dramatically increases in length and moves out into the vitelline stalk from d 25 to 50 of gestation. By d 50 the intestines move back into the fetal cavity where external muscle grow in size and differentiate, and villi start forming along the entire small intestine. As reported by Trahair and Sangild (2002; Fig. 1.11), the gut tube first forms a simple endodermal tube, which becomes stratified very rapidly. Ridges form and then villi appear from the tips of the ridges. Crypts develop in the stratified regions. Eventually the entire epithelium is comprised of a single thickness of enterocytes.

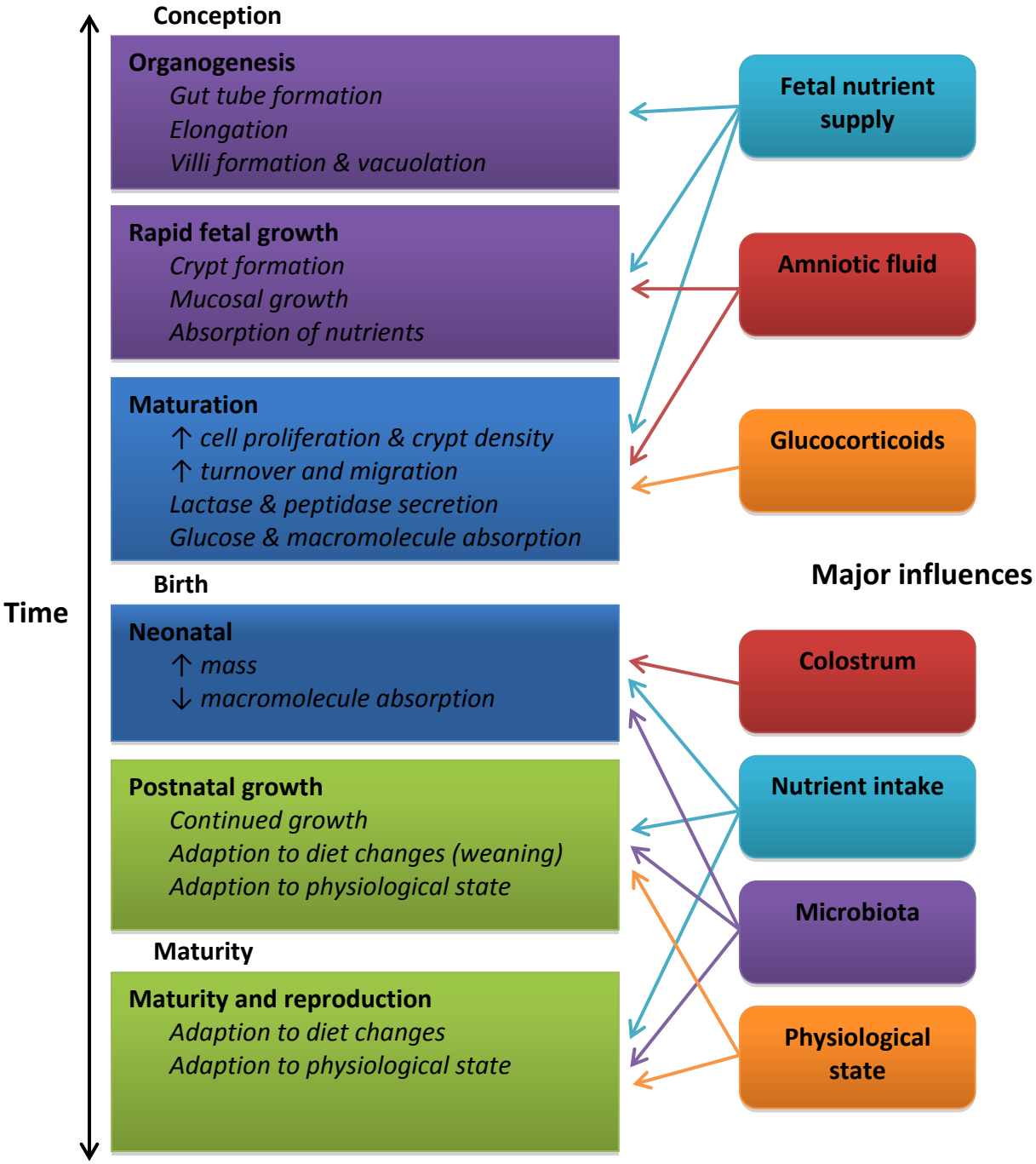

Figure 1.10. Windows of small intestinal growth and development and their influences. (Adapted from Meyer et al., 2012)

Along with villi formation, the first endocrine, goblet, and basal cells start forming. The majority of epithelial cells are still immature at this stage. However, epithelial cell proliferation

is the greatest by d 60 of gestation, which greatly enhances villi formation. Most importantly, villi become longer and morphologically mature, and capillaries in the core of the villi become organized (Figure 1.12).

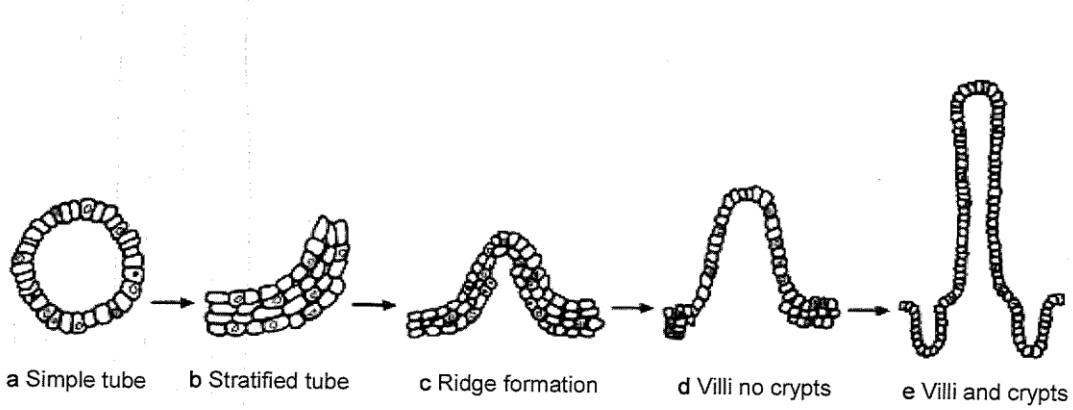

Figure 1.11. Formation of intestinal crypt and villi. (Adapted from Trahair and Sangild, 2002)

Another critical window for GI tract development occurs approximately at d 75 of gestation when the lumen is open and filled with fluid. This is when larger blood vessels start to form in the deeper layer of submucosal mesenchyme possibly enhancing angiogenesis in the GI tract (Trahair and Sangild, 2002). Fetal vacuolated enterocytes are a unique intestinal developmental cell type that supports essential physiological processes for the transition from gestational to adult life (Trahair and Sangild, 2002). Interestingly, these cells can digest material intracellularly and transport intact proteins from the lumen across epithelium into circulation (d 75 to 125 of gestation), but these cells dramatically decrease by late gestation and disappear completely in adulthood. From d 95 to 125 intestinal crypt cells develop between the immature villi, and villi density reaches its maximum (Trahair and Sangild, 2002). However, villi density decreases during late gestation and postnatally, while crypt density increases up until birth (Sangild et al., 2000). These crypt cells are the source of continually renewed enterocytes and are essential to lifelong function of the intestine (Trahair and Sangild, 2002). This continual cell proliferation, migration, and loss of epithelial cells along the mucosal surface involve four cell

lineages that are derived from one pluripotent stem cell located in the crypt (Bjerknes and Cheng, 1999). These are absorptive enterocytes, goblet, paneth, and endocrine cells in small intestine, and epithelial, mucous, and enteroendocrine cells in large intestine (Blum and Baumrucker, 2002).

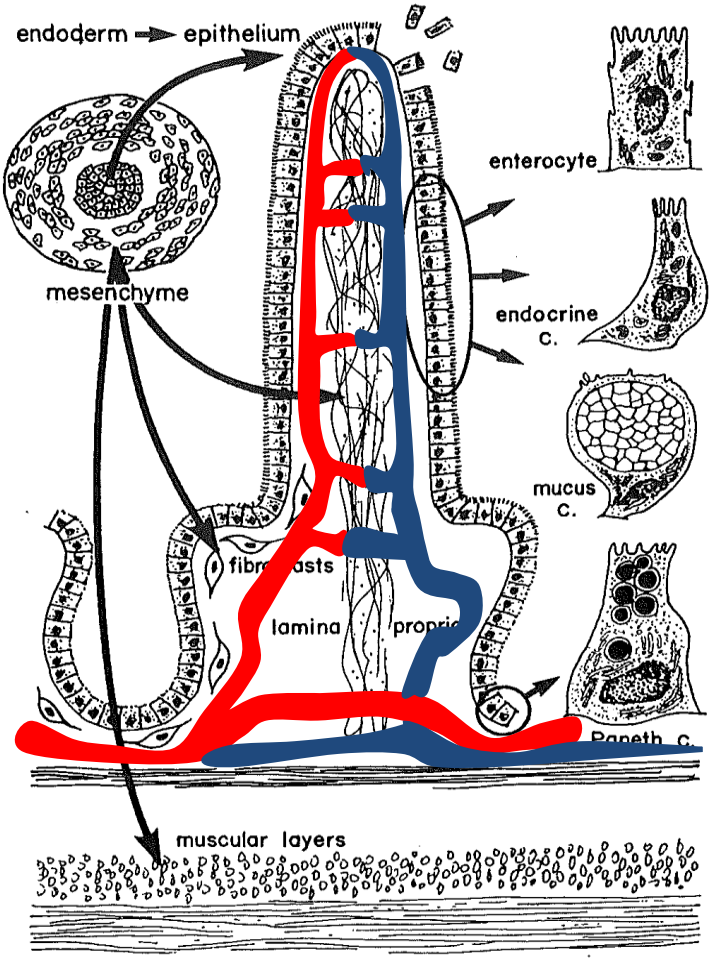

Figure 1.12. Schematic representation of mature intestinal villi with some of the key substrates and nutrients supplied, and blood vessels formed. (Adapted partially from Haffen et al., 1989 as reported in Burrin, 2004)

Nutrients from swallowed amniotic fluid by mid gestation in sheep provides growth factors that affect crypt formation, mucosal growth, and other absorptive functions of the gut epithelium (Trahair and Sangild, 2002; Buddington, 2002). Trahair and Sangild (2000)

demonstrated that fetal esophageal ligation in sheep resulted in suppressed intestinal growth. Moreover, as reported by Kimble et al., (1999), amniotic IGF-I enhances growth and development of sheep fetal gastrointestinal tract. Another key element for proper fetal intestinal maturation is an increase in the maternal cortisol level two weeks before partition, which facilitates increased cell proliferation, crypt density, cell turnover and migration. Moreover, injecting hydrocortisone postnatally accelerates normal tissue development where 3-day old calves injected with hydrocortisone has shown increased pancreas weight, amylase and chymotrypsin activity, as well as protein, RNA, and DNA concentrations (Pelletier and Dunnigan, 1983)

Furthermore, colostrum and mature milk aids growth and development of the GI tract postnatally. Many studies have demonstrated that via mammary gland suckling neonatal GI tract develops mucosal barrier and immune function (as reviewed by Burrin, 2004). Via suckling, fetal GI tract achieves bacterial colonization, and receives immunoprotective factors (IgG, IgA, lactoferrin, and oligosaccharides), all of which help to build immune function. For instance in humans, breast milk feeding limits the incidence of fetal sepsis, infection, and development of necrotizing enterocolitis (Claud and Walker, 2001; Kunz et al., 2000; Rodriguez-Palmero et al., 1999). Moreover, as reported in porcine and bovine studies, colostrum and mature milk causes major changes in the GI tract of neonates (Blum and Baumrucker, 2002; Xu et al., 2002).

Postnatal factors affecting fetal GI tract development

In addition to luminal factors during fetal swallowing of amniotic fluid, colostrum and mature milk enhance the immune system and aids in GI maturation of the neonate. Colostrum and milk-born bioactive compounds are essential in the transition from parenteral nutrition to enteral nutrition. These bioactive compounds include immunoglobulins, lactoferrins, hydrolytic

enzymes, proteins, fatty acids, vitamins and minerals, hormones and growth factors (Blum and Hammon, 2000a,b; Blum and Baumrucker, 2002; Xu et al., 2002).

Bioactive compounds in colostrum and mature milk. Colostrum is essential for newborn survival especially during the first 7 d post-partum. The main compounds are immunoglobulins (IgG and IgA), hydrolytic enzymes, various hormones and growth factors. Milk-born immunoglobulins protect newborns from intestinal infection, while hydrolytic enzymes facilitate nutrient digestion in GI tract of neonates (Cranwell and Moughan, 1989). In addition, various hormones and growth factors regulate and modify intestinal growth and development (Xu et al., 2000). For example in porcine colostrum, there is an increased concentration of IgG on the first day after parturition. This IgG directly enters the blood stream and protects neonates against bacterial and viral infection. Interestingly, as IgG declines during the 1st day of lactation, IgA concentration increases. Immunoglobulin IgA blocks adhesion of microbial pathogens onto intestinal epithelial surface, and also binds to bacterial toxins and forms antibodies against toxins. This important glycoprotein is very stable in the lumen of the GI tract and resistant to proteases, and therefore can remain in the lumen for a longer period of time (Goldman and Goldblum, 1989).

Another glycoprotein, lactoferrin, has high binding capacity to iron and plays an important role in protecting suckling neonatal pigs against GI infection. In addition, lactoferrin, binds to bacterial surfaces, causing cell wall damage and death of the bacteria (Goldman and Goldblum, 1989; Ellison et al., 1988).

Hydrolytic enzymes such as lysozymes in human colostrum and lactoperoxidases found in both human and bovine milk are important enzymes that along with anti-infection agents in the milk provide protection against intestinal bacterial infection in their suckling neonates

(Hernell et al., 1989; Goldman and Goldblum, 1989). Moreover, colostrum ingestion is associated with changes in brush border enzyme activity were lactase is increasing or decreasing depending on species, while maltase and aminopeptidase continually increase (Sanglid et al., 1999; Zhang et al., 1997).

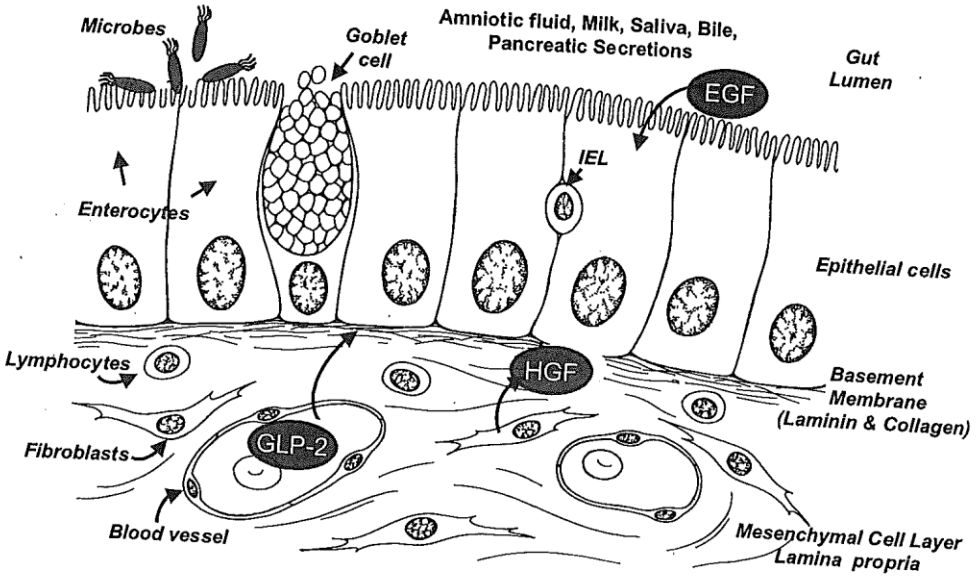

Figure 1.13. Organization of various cell types and structural architecture of intestinal mucosa. Acronyms used are: EGF = epidermal growth factor; GLP-2 = glucagon like peptide 2; HGF = hepatocyte growth factor. Adapted from Burrin, 2004).

In addition to cortisol, other hormones and growth factors further modify the gastrointestinal tract of neonates via milk-borne epidermal growth factors (EGF), insulin, IGF-I, IGF-II, GLP-2, and hepatocyte growth factor (HGF). As reported by Thornburg and Koldovsky (1987), EGF stimulates epithelial cell growth and differentiation, while GLP-2 participates in intestinal adaptation processes (Martin et al., 2006). In addition, in humans HGF is present in sufficient amounts to profoundly affect gastrointestinal maturation in the fetus via swallowed amniotic fluid and neonate via maternal breast milk (Srivastava et al., 1999). Moreover, colostrum increases circulating IGF-I concentration in piglets, which coincides with an increase

in the rate of protein synthesis in liver, spleen, and skeletal muscles (Burrin et al., 1992). Overall, luminal factors derived from amniotic fluids, mammary secretions (milk-borne insulin IGF-I, and EGF), microbes, and local factors secreted via paracrine and autocrine mechanisms from surrounding cells (GLP-2 and HGF) are absorbed into neonatal circulation and exhibit physiological changes in the GI tract of neonates (Figure 1.13).

IUGR effects on GI tract development and functional maturation

Preterm delivery and IUGR can negatively affect fetal GI tract development. As reported by Avila et al., (1989) IUGR fetuses had reduced small intestinal weight and length, accompanied by reduced thickness of the wall, mucosa, and villus height and crypt depth. Moreover, in IUGR and preterm fetuses, protein absorption in GI tract is hindered due to immature/undeveloped protein absorption capacity, which normally develops closer to term (Sanglid et al., 1997). In addition, pancreas in IUGR fetuses has decreased numbers of ß-cells and therefore reduced insulin levels during mid-gestation and continues to be lower compared to control (Limesand et al., 2005). Moreover, IGF-I is reduced in ovine IUGR fetuses (Bloomfield et al., 2001). In fact, Gluckman and Harding (1997) proposed that IUGR is a multihormone relative resistance syndrome referring to fetal insulin and IGF-I resistant syndrome development. Therefore, altered circulating insulin and IGF-I levels during fetal GI tract development can lead to inappropriate development and limited GI tract function. *In utero* infusion of IGF-I into amniotic fluid in sheep restored IUGR gut weight and wall thickness to the control level (Bloomfield et al., 2001). Similarly, supplementing EGF into amniotic fluid in IUGR fetuses in rabbits restored fetal small intestinal villus height (Cellini et al., 2004).

Furthermore, He et al., (2011) reported the significantly distinct metabolic status of IUGR piglets compared with normal weight piglets. IUGR piglets had altered lipogenesis, lipid

oxidation, energy supply and utilization, amino acid and protein metabolism, which can contribute to fetal jejunal impairment.

Preterm neonates and IUGR fetuses are at high risk of developing an immature GI tract and necrotizing enterocolitis. Martin et al., (2006) reported the importance of glucagon like peptide-2 (GLP-2) for intestinal adaptation processes, which exhibited beneficial actions on necrotizing enterocolitis. Within the intestine, GLP-2 has been shown to increase *eNOS* (Guan et al., 2006) and *VEGF* (Bulut et al., 2008) mRNA expression and increase mucosal blood flow (Stephens et al., 2006), which indicates a critical role of the GLP-2 system in intestinal angiogenesis. Therefore, in addition to luminal amniotic, colostrum, and milk nutrient availability, proper angiogenic gene expression is critical for optimal fetal GI tract growth and development.

Estradiol-17ß, its function and role in angiogenesis

Estradiol-17ß and its function

Estrogens are 18 carbon steroids mainly produced by ovaries, adrenals, stroma of the peripheral fat, and placenta during pregnancy (Segner, 2007). Although there are three types of estrogens including estradiol-17β (E2), estrone, and estriol, E2 is considered to be the major estrogen due to the fact that its estrogenic potency is 12 to 80 times higher than estrone and estriol (Guyton, 1986). Moreover, E2 plays a crucial role in embryonic and fetal development by influencing organogenesis, embryogenesis, and maintenance of pregnancy. Furthermore, inadequate levels of E2 during female fetal development results in ovarian pathologies and disruption of metabolic homeostasis (Abbott et al., 2006). For instance, in mammals an excess of E2 affects neuroendocrine pathway of GnRH release leading to diminished ovarian size and function (Wallen and Baum, 2002). In contrast, E2 deficiency results in partial ovarian

masculinization, impaired oocyte and follicular development in baboons (Zachos et al., 2002). E2 is also known to control lipid and cholesterol homeostasis in females, and enhance the survival of neurons (Green and Simpkins, 2000), and therefore, may contribute to neuroprotection (as reviewed by Wilson et al., 2011).

Estradiol-17ß, ERα, and enzyme aromatase (P450) are also critical for masculinization of male brain development and for imprinting male behavior (Beyer, 1999). Actions of estradiol-17β are mediated via two types of estrogen receptors; ERα and ERß. Furthermore, these receptors (ERα, ERβ) are associated with maintenance of fluid absorption in the head of the epididymis and reabsorptive function of efferent ducts that are essential for fertility (Matsuda et al., 2012; Hess et al., 1997, 2000, 2011).

Most importantly, E2 plays critical role for placental and fetal growth and development. During placental development, E2 promotes cell proliferation and growth directly affecting blood vessel walls by inducing the production of vasoactive substances which promotes angiogenesis, blood flow, and overall growth of placenta (Niklaus et al., 2003; Wallace et al., 2000; Reynolds, 2009). Moreover, E2 is important during early placentation, regulation of fetal growth and placental steroidogenesis. For instance, in humans, after the first 9 weeks of gestation maternal E2 levels are 3 to 8 times higher than in non-gestating individuals (Gambino et al., 2010). This increase is due to the unique exchange between mothers and fetuses, where pregnenolone produced by placenta is converted into adrenal dehydroepiandrosterone and dehydroepiandrosterone sulfate by fetus, and subsequently returned into placenta where it further converted into testosterone. Testosterone in the placenta is rapidly converted into E2 and released into maternal circulation (Doria et al., 2006). Moreover, E2 produced from human

placenta in an autocine manner enhances trophoblast differentiation (Cronier et al., 1999; Albrecht et al., 2006).

Interestingly, E2 also upregulates the expression of leptin in the placenta and the leptin expression was detected to be 50-fold higher in first trimester placental villi compared with the term villi (Hassink et al., 1997). Leptin controls the functional integrity of the feto-placenta by maintaining pregnancy and development of the placenta (Gambino et al., 2010) and induces trophoblast cell proliferation, protein synthesis (Magarinos et al., 2007; Perez-Perez et al., 2009), and regulates fetal growth and development (Henson and Castracane, 2000). Moreover, deregulation of autocrine and paracrine action of leptin results in pathogenesis of gestational diabetes and IUGR (Hauguel-de Mouzon, and Lepercq, 2001). But, there is limited research on E2 effects on leptin expression in placental tissues. It has been reported that physiological levels of E2 during pregnancy elevates leptin expression in human placenta, but when E2 is administered in higher doses the increase in leptin expression was not detected (Gambino et al., 2010). This could be due to the fact that high doses of E2 downregulated expression of ERs in placental tissue. Overall, E2 and its receptors play pivotal role regulating and enhancing placental development and growth.

In addition, E2 appears to play important role in intestinal cell proliferation; specifically ERß is present in epithelial cell of digestive tracts (Campbell-Thompson et al., 2001; Konstantinopoulos et al., 2003). As reported by Schleipen et al. (2011), E2 supplementation in ovariectomized rats in the presence of ERß agonist resulted in reduced proliferation rate and an increase in apoptosis in intestinal tissues, whereas in the presence of ERα agonist the cell proliferation and overall turnover was increased. Most importantly, in intestinal tissues E2 binds to both ERα and ERß, however, E2 has a higher affinity towards ERα (Kupier et al., 1997).

Interestingly, the binding of E2 to its receptors is E2 dose dependent. For instance, in the presence of higher levels of E2, ERα and ERß are activated in 2:1 ratio, while in the lower level of E2 there is 1:1 ratio (Kupier et al., 1997). Since E2 dependent activation of ERα stimulates cell proliferation and growth in the intestine and activation of ERß results in effects that are opposite of ERα, it is suggested that ERß acts as a buffer to maintain homeostasis of intestinal cell proliferation (Zhao et al., 2010). In addition, E2 and its receptors (ERα , ERß) have been localized in intestinal macrophages and intestinal neurons (Kawano et al., 2004) and are also critical for proper intestinal vascular bed formation.

Estrogen Receptor α (ERα)

Estrogen receptors belong to the nuclear hormone family of intracellular receptors. Its main function is to bind DNA as a transcription factor that regulates gene expression; however there are other additional functions besides DNA binding (Dahlman-Wright et al., 2006; Prossnitz et al., 2007). As reviewed by Nilsson and Gustaffson (2010), the classical DNA binding effects of nuclear ERs are known as "genomic" and the E2 effects are slower in this instance. However, there are two other rapid response pathways also known as "non-genomic" via cytoplasmic and membrane-localized ERs, which trigger the cytoplasmic signal transduction pathway (mGLUR) or activates the GPR30/GPER membrane-associated receptor that is structurally and genetically completely different from ERs (Figure 1.14).

Receptors ERα and ERß are not isomers of each other, but rather distinct proteins encoded by different genes located on different chromosomes (4 and 16; Couse et al., 1999). Moreover, it was demonstrated that E2 activates ERα but inhibits ERß (Paech et al., 1997; Han et al., 2003). This leads us to believe that these two different receptors can differently regulate DNA transcription.

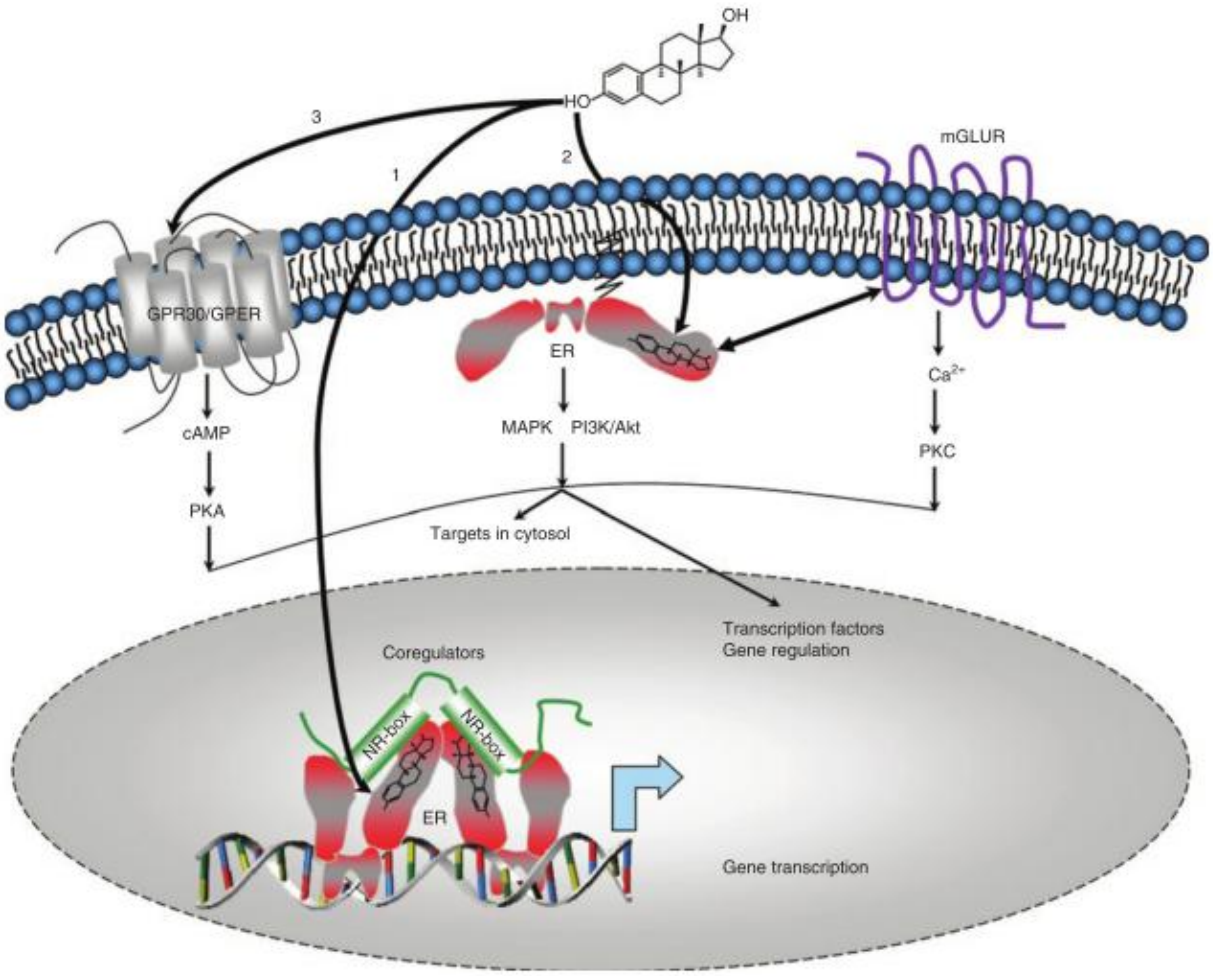

Figure 1.14. Schematic representation of various intracellular pathways of estrogen signaling. Pathway 1 – "genomic" via direct DNA binding; pathway 2 – "non-genomic" cytoplasmic/membrane-localized ERs via mGLUR; and pathway 3 – "non-genomic" membrane associated GpR30/GPER via various cytoplasmic kinases. (Adopted from Nilsson and Gustafsson, 2010)

ERα and ERß are primary mediators of E2 and are important for E2 function in angiogenesis (Harfouchea et al., 2010). In certain types of cells, ERα and ERβ are expressed in similar pattern, but in some they are expressed differently.For instance, ERα is predominantly expressed in uterus, ovaries, testis, bone, breast, liver, kidney, various regions of the brain, and white adipose tissue.Whereas, ERβ is mainly expressed in the colon, epithelium of prostate, ovaries, bone marrow, salivary gland, vascular endothelium, lung, bladder, and certain regions of

the brain as well (Kuiper et al., 1997; Nilsson and Gustafsson, 2010; Ascenzi et al., 2006). Interestingly, ERß appears to regulate blood pressure in rodents (Zhu et al., 2002). However, in this thesis ERα will be given particular attention.

The molecule of ERα consists of 595 amino acids with molecular weight of 66 to 70 kDa and contains six functional domains (Ascenzi et al., 2006; Tora et al., 1989; McKenna and O'Malley, 2002). In addition, ERα functions include DNA binding, ligand binding, dimerization, protein binding and transcriptional activation (Nilsson and Gustafsson, 2010). Bone-sparing effects of E2 are mediated via ERα (Borjesson et al., 2011). Estradiol-17ß effects mediated via ERα are dependent on ERα activation factors (ERα AF-1 and ERα AF-2). For example, ERα AF-1 is essential for uterine function, but its deficiency does not affect vasculoprotective actions of E2 (Borjesson et al., 2011, 2012). ERα is absolutely necessary for the positive actions of E2 on endothelial NO production (Darblade et al., 2002) and re-endothelialization (Brouchet et al., 2001; Toutain et al., 2009). Moreover, ERα is especially sensitive to epigenetic modification due to physiological and environmental changes (Champagne and Curley, 2008). For instance, neonates treated with bisphenol-A (BPA) showed reduced duration of maternal licking/grooming and decreased frequency of nursing their pubs in rodents (Della et al., 2005; Palanza et al., 2002). Therefore, epigenetic modification of ERα affects not only physiological but also behavioral responses. This leads us to believe that epigenetic modifications and genetic mutations due to environmental and other factors can affect the mRNA expression of ERs (transcription), protein expression of ERs (translation), and furthermore affect posttranslational modification resulting in phenotypic expression of ERs gene. Moreover, the expression of ERs are dependent on the type of tissue they are expressed in, which further dictates various

pregnancy outcomes, and in some cases, development of various cancer types in various tissues (Kuiper et al., 1997; Nilsson and Gustafsson, 2010; Ascenzi et al., 2006).

Fortunately, development of selective ER modulators (SERM) based on ERs' slightly different structures in various tissues and understanding E2 mechanisms via its receptors has given better tools in targeting certain diseases including breast cancer, osteoporosis, and other cardiovascular diseases in both pre- and post-menopausal females and males with deficient aromatase (P450) activity (as reviewed by Dutertre and Smith, 2000; Nilsson and Gustafsson, 2010; Zirilli et al., 2009). In a similar way developing SERM for ERs in placental endothelium in IUGR pregnancies and intestinal tissues of IUGR fetuses may have some potential beneficial effects.

Estradiol-17ß role in angiogenesis

Estradiol-17β promotes angiogenesis (Aron and Anthony, 2004) through activating three proangiogenic factors including activation of the NO system (Rubanyi et al., 2002; Arnal et al., 2007; Simoncini T., 2009; Kim and Bender, 2009), FGF-2 (Garmy-Susini et al., 2004; Reynolds and Redmer, 2001; Gospodarowicz, 1991), and VEGF (Losordo et al., 2001; Bussolati et al., 2001; Reynolds and Redmer, 1988).

Estradiol-17β action on the NO system is mainly mediated via ERα in the mouse aorta (Darblade et al., 2002). There are several ER splice variants; therefore, different tissues have slightly different ER structure. As reported by Kim et al., (2008), ER46 isoform of ERα is very critical for eNOS activation leading for rapid endothelial cell NO production. Basically, E2 induces phosphorylation on tyrosine-537 residue of ER46 isoform, which further enhances c-Src (a cytoplasmic protein with tyrosine-specific protein kinase activity) SH2 domain interaction leading to signal transduction via PI3K and Akt and subsequent activation of eNOS and release

of NO from endothelial cells (Kim et al., 2008). This “non-genomic” rapid E2 activation could be critical for maintaining vascular homeostasis, especially for proper placental blood supply for a growing fetus, as well as for optimal nutrient absorption in intestinal tissues.

Furthermore, Fountaine et al. (2006) have demonstrated the importance of FGF-2 in the effect of E2 on re-endothelization and angiogenesis in mice. When comparing control and FGF-2 knockout mice, E2 treatment does not exhibit angiogenic effects (Fountaine et al., 2006). This and several other studies confirmed that FGF-2 is a key partner of E2 and is an important angiogenic factor that stimulates endothelilal cell growth, migration, and re-endothelialization (Kim-Schulze et al., 1998; Garmy-Susini et al., 2004; Lindner et al., 1990). In addition to angiogenic properties of FGF-2, this factor also influences differentiation and other developmental functions (Gospodarowicz, 1991). As reported by Reynolds et al., (2000), FGF-2 also stimulates follicular and luteal cell growth, progesterone production, and enhances cell survival in various types of cells. It is important to note, that FGF-2 stimulates differentiation of embryonic germ layers, particularly acting on mesoderm (Klein and Melton, 1994). Moreover, several studies have demonstrated that placental tissue expresses FGF-2 throughout gestation (Zheng et al., 1998; Reynolds and Redmer, 2001). Therefore, FGF-2 might play an essential role in angiogenesis of endometrium, as well as differentiation of vascular and non-vascular tissues (Reynolds and Redmer, 2001).

Another angiogenic factor that participate in physiological (reproductive cyclicity) and pathophysiological (tumor growth) processes is known as VEGF (Ferrara and David-Smyth, 1997; Fraiser et al., 2000; Breier et al., 1997). Notably, VEGF expression by uterine and vascular tissues increases with increasing E2 concentration in humans (Shifren et al., 1996). In addition, increased VEGF expression by vascular smooth muscles is associated with treatment of

E2 after the arterial injury (Krasinski et al., 1997). For example, in ovariectomized ewes endometrial expression of VEGF mRNA is strongly upregulated within a few hours after treatment with E2, in association with a dramatic increase in uterine vascularization and blood flow (Magness, 1998; Reynolds et al., 1998a, b; Johnson et al., 2006). Moreover, VEGF stimulates endothelial production of NO, a major local vasodilator, which has been shown to mediate estrogen-induced increases in uterine blood flow (Rosenfeld et al., 1996; Zheng et al., 1999). Likewise, NO can also regulate expression of VEGF (Benoit et al., 1999; Frank et al., 1999). Additionally, as reported by Reynolds et al., (1998b, 2000) endometrial VEGF is expressed primarily in arteriolar vascular smooth muscle and capillary pericytes, which is consistent with its localization to peri-endothelial cells in ovarian and other tissues (Redmer et al., 2001). The pathway of E2 via VEGF, FGF, and the NO system pathway can be found in Figure 1.15.

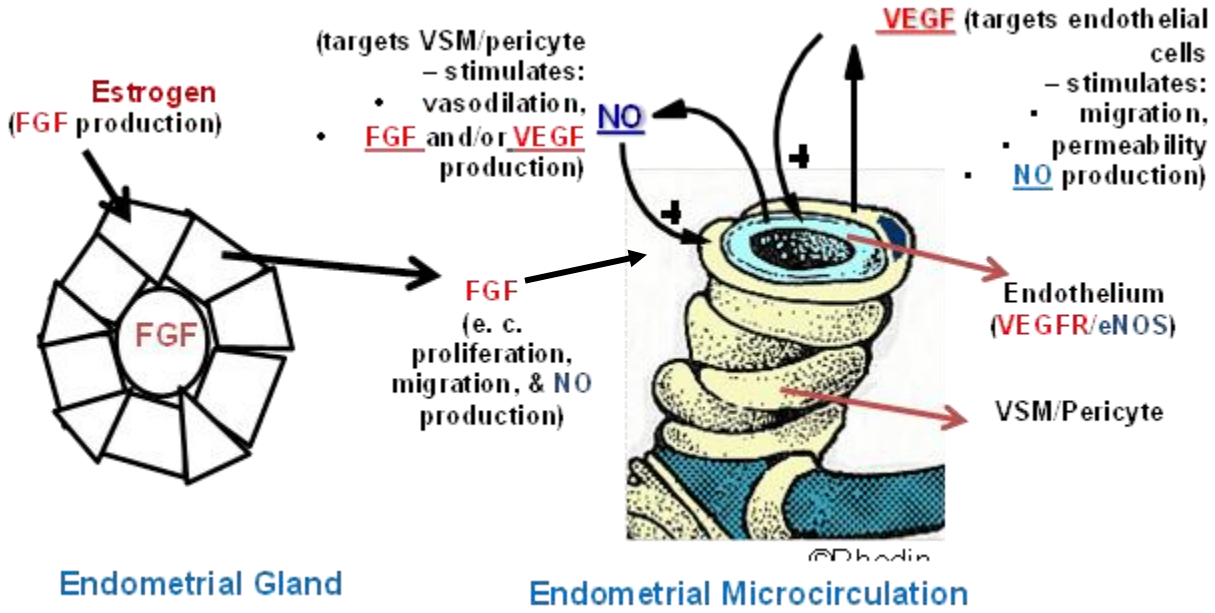

Figure 1.15. Model for E2 pathway via VEGF, FGF, and NO systems. (Adapted from Reynolds et al., 2000).

Furthermore, VEGF stimulates vascular permeability and migration, thus promoting angiogenesis (Ferrara and David-Smyth, 1997; Hanahan, 1997; Neufeld et al., 1999). Most importantly, VEGF and its receptors FLT-1 and KDR are associated with angiogenesis in fetal and placental development. It has been demonstrated that VEGF in sheep placenta is expressed throughout gestation (Zheng et al., 1995; Borowicz et al., 2007; Grazul-Bilska et al., 2010, 2011). For instance, Breier et al. (1997) and Dumont et al. (1995) demonstrated the importance of VEGF and its receptors for fetal brain ventricle, kidney, and placental tissue development during late pregnancy in mice. Numerous studies confirmed the vitality of VEGF and its receptors in fetal and placental angiogenesis. This was demonstrated by VEGF, FLT-1 and KDR knockout models, which resulted in defective blood vessel formation, organization, and morphology (Fong et al., 1995; Carmeliet at al., 1996). Moreover, FLT-1 and KDR have different ways in contributing to angiogenesis (Bussolati et al., 2001). It appears that KDR is important for maturation of endothelial cells, while FLT-1 for organization of vascular structures. Vascular endothelial growth factor receptor, KDR promotes endothelial cell proliferation; however, it seems that FLT-1 negatively affects KDR mediated cell proliferation via NO. It is well known that NO is a potent vasodilator and one of its actions is to inhibit smooth muscle proliferation and migration. Basically, FLT-1 hijacks VEGF from KDR therefore preventing overcrowdings of endothelial cells and allowing normal vascular tubule formation (Bussolati et al., 2001).

Overall, all these three angiogenic factors contribute not only to fetal and placental angiogenesis but also to neuro and cardioprotective effects of E2 (Sheldahl et al., 2007; Iwakura et al., 2006).

Overall Conclusion

Maternal age, genetics, environmental stressors, nutrition including alterations in macro and micronutrients in maternal diet prior and during gestation significantly affect embryonic, placental, and consequently fetal growth and development (Godfrey and Barker, 2000; McMillen and Robinson, 2005; Borowczyk et al., 2006; Grazul-Bilska et al., 2012). The placental insufficiency can negatively affect fetal growth and development resulting in health related complications as well as economic challenges both in humans and livestock production. Majority of IUGR pregnancies, in humans and livestock, are due to insufficient placental development, reduced nutrient transport and uteroplacental blood flow (Reynolds et al., 2010a,b). In humans, IUGR is defined as a fetal birth weight that is below the 10^{th} percentile for gestational age or those fetuses that weigh less than 2.5 kg at birth. However, in livestock, defining IUGR is a little bit complex due to different species and breeds. For instance, in our study IUGR was defined as fetuses with birth weights that are two standard deviation below the CON means (3,591 g). In fact, Sawalha et al. (2006) reported that fetuses with birth weights less than 3,500 g had mortality rates ranging from 5 to 45 %. Although those fetuses that manage to survive still experience increased morbidity, slow postnatal growth, and organ dysfunction (adult onset). Therefore, both in human and livestock, IUGR fetuses have reduced body weights compared to normal gestational age and experience impaired embryo and fetal growth and development. One of the complications of IUGR is immature fetal GI development.

Despite the numerous epidemiological and animal studies on effects of maternal nutrition, IUGR, and fetal development; the fetal intestinal growth and development has not been fully investigated. Fetal intestinal development is as important as placental development because both of these organs provide nutrients and gases for proper fetal survival and growth (Trahair

and Sangild, 2002). This leads us to believe that developmental programming on placenta and intestine is likely to affect fetal overall growth, intestinal development, metabolism, lipid homeostasis, and immune system not only *in utero* and postnatally, but later in adulthood as well (He et al., 2011).

As reported by Stock and Metcalfe (1994), and Wittaker et al. (1996), the amount of circulating blood in mothers increases during pregnancy both in human (40 to 45%) and livestock. Most importantly, increased maternal blood volume is due to altered blood distribution, for instance, in non-pregnant sheep, only 1 to 2 % flows toward uterus, while in pregnant sheep the blood flow to uterus continuously increases up to 16 % (Stock and Metcalfe, 1994) by the third trimester. This increase in uterine blood flow is accompanied with an increase in vascularization of placenta (Reynolds and Redmer, 1995, 2001). Limited vascular growth and development in placenta restricts blood supply to a growing fetus, therefore, resulting in IUGR. Thus, targeting developmental windows during placental development as well as fetal growth *in utero* by manipulating maternal nutrition and therapies may rescue IUGR pregnancies.

For instance, as demonstrated by Wallace (2008), decreased fetal birth weight was correlated with decreased maternal circulating serum E2 levels in overnourished adolescent sheep. The decrease in maternal E2 level might be one of the causes of IUGR, therefore supplementing E2 might be beneficial. This is especially important because E2 promotes angiogenesis via the NO, VEGF and FGF-2 systems (Rubanyi et al., 2012; Arnal et al., 2007; Garmy-Sysini et al., 2004; Losordo et al., 2001; Bussolati et al., 2001) suggesting that regulation and enhancement of E2 in maternal circulation during critical developmental windows in gestation can shed additional light into understanding mechanisms of E2 action via its receptors (ERα and ERß). Enhancement of vascular growth via maternal diet and E2 therapeutic

approaches will most likely optimize placental and fetal growth. Moreover, understanding mechanisms of SERM and determining ways to specifically target placental and fetal small intestinal vascular capacity could be an innovative and more efficient improvement for fetal and neonatal health, and livestock productivity.

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CHAPTER II. EFFECTS OF MATERNAL NUTRITION, INTRAUTERINE GROWTH RESTRICTION (IUGR), AND ESTRADIOL-17β (E2) SUPPLEMENTATION ON PLACENTAL GROWTH, DEVELOPMENT, AND VASCULARITY IN FIRST PARITY EWES

Abstract

Overnourished pregnant adolescent sheep are characterized by feto-placental growth restriction and attenuated peripheral reproductive steroid concentrations. Herein the effects of excess maternal intake, IUGR, and estradiol-17β (E2) treatment during mid-gestation on placental cell proliferation, angiogenic gene expression and vascularity were investigated. Singleton pregnancies (single sire) were established by embryo transfer, and adolescent dams were offered a control diet (CON, n = 12), fed 100% estimated ME and CP requirements or excess diet (HI, n = 26), fed approximately twice the dietary intake of CON treatments. From d 50 to 90, E2 (0.05 mg i.m.) was administered twice daily to 14 HI ewes (HI+E2). At necropsy (d 130), fetal weight in CON was 4435 ± 422 g. IUGR defined as weight at least 2 standard deviations below the CON mean (< 3,591g) appeared in 8 of 14 HI (HI IUGR) and 5 of 12 HI+E2 (HI IUGR+E2) pregnancies. Placental tissues (caruncles [CAR] and cotyledons [COT]) were collected for mRNA or perfusion fixed for vascular evaluation. Data were analyzed for effects of elevated maternal plane of nutrition, IUGR, and E2 supplementation. In HI group fetal: placental weight ratio was greater ($P < 0.001$), but placentome and fetal weights were less ($P < 0.001$) than in CON group. Placentome weight in IUGR was 42% less than in non-IUGR ($P < 0.001$), and not affected by E2 treatment. In HI group, cell proliferation in placentome, and mRNA expression for COT *FLT1* and CAR *ANGPT1* were less ($P < 0.06$ and 0.08) but *ERα* mRNA expression and area per capillary in CAR were greater ($P \leq 0.09$) than in CON group. In

IUGR pregnancies mRNA expression for *ANGPT1*, *NOS3*, and *ERα* in COT and *VEGF*, *FLT1*, *NOS3*, and *ERα* in CAR were greater ($P \leq 0.08$ and 0.09) compared to non-IUGR. This up regulation of angiogenic factors in IUGR may reflect placental adaptation to improve fetal nutrient delivery and is supported by a greater fetal:placental weight ratio in IUGR vs. non-IUGR. Supplementation of E2 had no effect on COT mRNA but in CAR, *NOS3* mRNA expression was greater in HI non-IUGR+E2 and HI IUGR+E2 groups ($P \leq 0.04$). In IUGR+E2 group mRNA expression for *ANGPT2* and *NOS3* in CAR was greater ($P < 0.01$ to 0.09) compared to IUGR group. Overall, placental tissue had limited responsiveness to maternal E2 supplementation, where CAR mRNA expression had greater *NOS3* and *ANGPT2* compared to E2 untreated ewes. This limited responsiveness might change if the dose and timing of E2 is optimized. Moreover, determining protein content and global methylation can shed additional light into understanding molecular mechanisms underlying IUGR and placental development.

Introduction

The placenta, as a main nutrient and gas exchange organ and a powerful hormone producing system affects both maternal and fetal functions. Therefore, when proper vascularization and nutrient availability during placental development are limited, it results in an adverse intrauterine environment leading to preterm and/or low birth weight offspring (Reynolds et al., 2010a,b). These offspring have a higher risk of developing health complications and experience both short and long-term developmental consequences (Wu et al., 2006; Reynolds and Caton, 2012). Due to inappropriate maternal nutrition and age, IUGR is often linked to compromised placental nutrient transport function, vascular development, and uteroplacental blood flows (Reynolds et al., 2010) and therefore, is associated with altered fetal nutrient supply

and poor pregnancy and postnatal outcomes. Factors affecting placental growth and vascular development ultimately affect fetal growth (Reynolds and Redmer, 1995, Mayhew et al., 2004).

It was reported that in an overnourished paradigm an average of 52 % of adolescent ewes produce fetuses that are categorized as markedly growth restricted at term (Wallace et al., 2004b). Because adolescent mothers (both in humans and livestock) are in a stage of rapid growth they therefore compete for nutrients with their growing fetuses (Redmer et al., 2004, 2005, 2009, 2012). Previously in this overnourished adolescent ovine model, alterations in placental angiogenesis and uterine blood flow during mid-gestation were accompanied with development of placental growth restriction later in gestation (Wallace et al., 2003, 2008). In addition, maternal plasma progesterone concentrations were reduced in the overnourished adolescent lambs during pregnancy, and supplementing progesterone during the period of early placental development partially rescued fetal birth weights at term (Wallace et al., 2003). Moreover, maternal circulating estradiol-17β (E2) fail to increase in parallel with control ewes on d 50 and 75 of gestation. This decrease in maternal plasma E2 concentration is also highly correlated with reduced fetal weights at birth (Wallace et al., 2008). There are no known reports on E2 supplementation during pregnancy and its effects on pregnancy outcomes. However, current data indicate that E2 is involved in the regulation of angiogenesis (Niklaus et al., 2003), stimulates cell proliferation, overall growth in placenta, vascular development and blood flow (Wallace et al., 2000; Reynolds, 2009).

Therefore, we hypothesize that supplementing E2 during the period when placenta is at its highest proliferative stage (d 50 to 90) in first parity overfed adolescent ewes, will rescue restricted placental growth and result in greater placental size and nutrient transport efficiency, which will further optimizes overall fetal development and growth. Our objectives were to

investigate E2 effects on 1) maternal organ mass; 2) placental cell proliferation and vascularization; and 3) placental mRNA expression of factors that promote angiogenesis in adolescent ewes fed excess diet.

Materials and Methods

All procedures were licensed under the UK Animals (Scientific Procedures) Act of 1986 and were approved by the Ethical Review Committee of the Rowett Institute of Health and Nutrition, University of Aberdeen. Animal treatment, tissues collection and initial processing, and hormone analysis were performed at the Rowett Institute of Health and Nutrition, and evaluation of vascularization and mRNA expression were performed at the Department of Animal Sciences, North Dakota State University, USA.

Animals and embryo transfer

Embryos were recovered on d 4 after estrus from superovulated adult ewes (Border Leicester × Scottish Blackface) that were inseminated by a single sire, and one embryo per recipient was transferred to synchronized adolescent ewe lambs (Dorset Horn × Greyface), as described previously (Wallace et al., 1997b). Donors (n = 9) had lambed once previously, were 2.5 years old , weighed 70.6 ± 1.03 kg, and had a body condition score (BCS) of 2.25 (on a 5-point scale, with 1 being emaciated and 5 obese, Russel et al. 1969) at the time of embryo recovery. An average of 5.9 early morula (grade1) per donor ewe (range 4 to 13 embryos/donor) was utilized for transfer into 53 adolescent recipients (1 embryo/recipient). This protocol ensured that placental and fetal growth was not influenced by varying fetal number or partial embryo loss. Moreover, the utilization of a single sire and a limited number of embryo donors maximized the homogeneity of the resulting fetuses. Embryo transfer was carried out on 4 separate days during the midbreeding season, and animals were housed in individual pens under natural lighting conditions at the Rowett

(57° N, 2° W). At the time of embryo transfer, adolescent recipients (n = 53) were peripubertal (approximately 8.5 mo of age) and had a mean BW of 42.9 ± 0.37 kg, a BCS of 2.25 ± 0, and an ovulation rate of 2.0 ± 0.13.

Treatments and experimental design

Adolescent recipients (n = 53) were initially allocated to 1 of 2 dietary treatments. Plane of nutritional treatments were control (CON) or high (HI) quantity of the same complete diet. At embryo transfer, adolescent ewes were allocated to either the CON (n = 17) or HI (n = 36) group on the basis of their current BW, BCS, ovulation rate, and, where possible donor source. The dietary amount in the CON group was calculated to maintain normal maternal adiposity throughout gestation and to provide the estimated ME and protein requirement of adolescent ewe lambs carrying a singleton fetus according to the stage of pregnancy (based on Agricultural and Food Research Council; AFRC, 1993). To achieve this objective, the CON group was fed to promote a low maternal weight gain (~ 50 g per day) during the first two-thirds of gestation, followed by step-wise increases in maternal intake during the final third of gestation calculated to meet the increasing demands of the developing fetus. Based on previous studies, it has been demonstrated that this approach in the CON group optimizes placental and fetal growth in this genotype (Wallace et al., 2004a). In contrast, the HI or ad libitum intakes were equivalent to approximately twice the estimated ME requirements and were calculated to promote rapid maternal BW and adiposity gain at the expense of the conceptus. The complete diet supplied 2.9 Mcal of metabolizable energy and 140 g of crude protein per kilogram of body weight and was offered in two equal feeds at 0800 and 1600 h daily. The diet contained 30% (w/w) coarsely milled hay, 42.25% barley, 10% molasses, 16.75% soybean meal, 0.35% salt, 0.5% dicalcium phosphate, and 0.15% vitamin-mineral supplement and had an average dry matter of 86%. The amount of feed

offered in the HI group was gradually increased over a 2-wk period until the amount of daily refusal was approximately 15% of the total offered (equivalent to ad libitum intakes). The amount of feed offered was reviewed 3 times weekly and adjusted, on an individual basis and when appropriate, on the basis of BW change data (recorded weekly) and the amount of feed refusal (recorded daily), as described previously (Wallace et al., 2006b). Maternal body condition was subjectively assessed as detailed above every 2 wk by the same experienced operator.

Conception rate was determined by transabdominal ultrasonography at approximately 45 d of gestation (gestation length = 145 d), when 14 CON and 28 HI recipients were pregnant. At d 50 of gestation, half of the H group (n = 14) were allocated to receive E2 on the basis of maternal live weight, adiposity score, and ovulation rate at embryo transfer, and most importantly maternal live weight gain from day of embryo transfer to d 50 of gestation (hereafter H+E2 group). These latter ewes were injected (i.m. into alternating leg muscles) twice daily (07:30 and 17:30h) with E2 (0.05 mg E2/ml arachis oil on d 50 to 90 of pregnancy inclusive. The detailed outline of experimental design is shown in Figure 2.1.

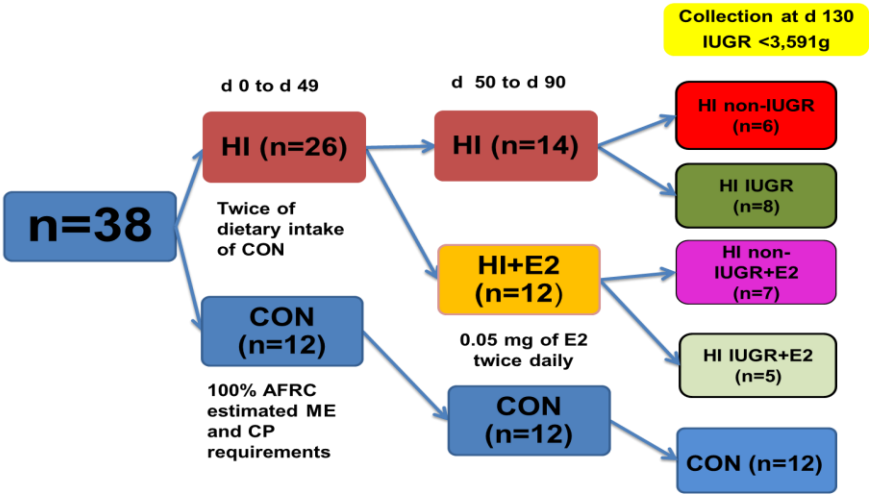

Figure 2.1. Experimental design used in this study.

E2 was from Sigma (E-8875) and arachis oil from JM Loveridge Ltd. purchased via Dunlops Veterinary Supplies, Dumfries, UK. A stock solution of 2mg E2 per ml arachis oil was prepared by heating to 50^{o}C with stirring for 8h until the oil was clear and the E2 completely dissolved. Both stock and using solutions were stored at room temperature and in the dark. The concentration of E2 per injection was determined empirically based on earlier trials in non-pregnant nutritionally manipulated adolescents studied during the anestrus period. The aim was to achieve peripheral E2 concentrations similar to those measured previously in control fed pregnant adolescents. Ewes in the CON and HI groups were temporarily restrained twice daily (but not sham injected) between d 50 and 90 of pregnancy. Blood samples were collected from all ewes at d 25 and 50, and then at 5 d intervals during the period of E2 supplementation followed by ~ 10 d intervals thereafter until necropsy on d 130 of pregnancy. Maternal blood was sampled at ~ 14:00h (i.e. 6.5h after the morning E2 injection where appropriate) from the jugular vein into a lithium heparinised tube for immediate plasma harvest following centrifugation. Plasma was stored at -20^{o}C until E2 concentrations were determined in duplicate by radioimmunoassay as described previously (Johnson et al. 1997). The sensitivity of the assay was 1pg E2/ml and the inter and intra-assay coefficients of variation were 6.2 and 5.5%, respectively.

Necropsy and tissue harvesting procedures

On d 130 of pregnancy, one hour before necropsy, ewes were weighed. Ewes were then euthanized by administration of an overdose of sodium pentobarbitone i.v. (20 mL of Euthesate; 200 mg of pentobarbitone/mL) and were exsanguinated by severing the main blood vessels of the neck. Maternal blood was collected in a plastic container and weighed. The gravid uterus was quickly removed, dissected from the vagina at the cervix, weighed, and opened. Fetuses were euthanized by immediate intracardiac administration of a sodium pentobarbitone overdose (5 mL

of Euthesate, Willows Francis Veterinary). The maternal liver and perirenal fat were dissected and weighed and the empty carcass weight determined.

Placental Tissue Collection and Processing. The reproductive tract was kept at 37^{o}C in warm saline containing procaine (0.5% procaine hydrochloride wt/vol, Sigma P9879) until the catheters were inserted in order to prevent vessel collapse. The circulations of the gravid and non-gravid uterine horns were isolated using multiple bowel clamps and two placentomes representative of the most common grossly observed morphology type were immediately removed and weighed. These placentomes were then separated manually with gentle traction to reveal the individual maternal caruncle and fetal cotyledon components. Individual caruncle and cotyledon components were then snap frozen in liquid nitrogen-cooled isopentane and stored at –80^{o} C for subsequent gene expression studies. Caruncle and cotyledon portions of the placenta were perfused after catheterizing branches of the uterine and umbilical arteries, respectively, as previously described (Borowicz et al. 2007), and with the following modifications. For the carcuncular perfusion this involved catheterization of 3 arteries (2 x cervix and 1 x ovary) in order to perfuse the lower arcade of placentomes in the gravid horn. For the fetal cotyledon perfusion the target placentomes were in the lower arcade of the non-gravid horn. For the caruncular perfusion, the 3 arteries were simultaneously perfused with 30 mL warm (37^{0}C) PBS plus procaine (0.5%wt/vol), followed by 30 ml cold (4^{o}C) PBS plus procaine, then 10 mL cold 70% alcohol and finally 20 ml cold Carnoys fixative. A second operator simultaneously performed the single fetal cotyledon perfusion using the same regimen. Perfused placentomes from each aspect (typically, n = 4 per pregnancy) were then removed from the uterus, weighed, sliced into 7-mm cross sections and immersion fixed in Carnoys solution for 6 h followed by 70% ethanol, changed once after 24 h. All

remaining placentomes were dissected and weighed. The total placentome weight is the combined weight of the snap frozen, perfuse- fixed and residual dissected placentomes.

Histology and Immunohistochemistry. Tissues were embedded in paraffin and 4-µm tissue sections were made from the paraffin blocks, mounted on glass slides and prepared for staining procedures (Soto-Navarro et al., 2004). Tissue sections were stained using periodic acid-Schiff's (PAS) to visualize the blood vessels and counterstained with hematoxylin (Borowicz et al., 2007). The following parameters in CAR and COT tissue were determined: mean capillary area, capillary number, and capillary circumference measurements, along with area of tissue analyzed (Redmer et al. 2009) using the Image-Pro Plus 5.0 analysis software (Media-Cybernetics Inc., Silver Spring, MD). Cell proliferation in placentomes was determined based on immunohistochemical detection of proliferating cell nuclear antigen (Ki-67) as reported early by Grazul-Bilska et al., (2009).

Quantitative Real Time – PCR Analysis. In this study, relative mRNA gene expression in CAR and COT was determined for factors and their receptors involved in the regulation of angiogenesis including vascular endothelial growth factor [***VEGF***], fms-related tyrosine kinase 1[***FLT1***], kinase insert domain receptor [***KDR***], angiopoietin 1 [***ANGPT1***], angiopoietin 2 [***ANGPT2***], endothelial tyrosine kinase [***TEK***], endothelial nitric oxide synthase 3 [***NOS3***], soluble guanylate cyclase [***GUCY1B3***], hypoxia-inducible factor 1, alpha subunit [***HIF1A***]), vasoactive intestinal peptide [***VIP***], basic fibroblast growth factor 2 [***FGF2***], fibroblast growth factor receptor 2 [***FGFR2***], neuropilin 1 [***NRP1***], and neuropilin 2 [***NRP2***] and estrogen receptor alpha **[*ER-α*],** along with *18S* using quantitative real-time RT-PCR. . Methods used for extraction and quantification of mRNA and the analysis of major angiogenic factors have been published (Redmer et al., 2005; Vonnahme et al., 2006; Borowicz et al., 2007; Grazul-Bilska et al., 2010, 2011). All quantitative real-time reverse transcription-PCR data were normalized by dividing

quantity of gene of interest expressed by 18S, a reference standard of tcRNA. Modifications used in the current analysis have recently been published (Vonnahme et al., 2008; Neville et al., 2010a). Human *18S* mRNA (predeveloped assay reagent [**PDAR**]; Applied Biosystems, Foster City, CA) was added to serve as an internal control to minimize sample variation. Analyses were conducted using TaqMan reagents and procedures purchased from and recommended by Applied Biosystems (Foster City, CA).

Expression of each angiogenic factor was normalized to expression of 18S in a multiplex reaction using the human 18S PDAR from Applied Biosystems. The PDAR solution, which is primer-limited and contains a VIC-labeled probe (a proprietary reporter dye; Applied Biosystems), was further adjusted by using one-fourth the normal amount so that it would not interfere with amplification of the FAM (6-carboxy-fluorescein)-labeled gene of interest. The multiplex reaction, similar to previous study (Neville et al., 2010a), was also used to prepare standard curves for 18S and the gene of interest based on dilutions of cDNA obtained from reverse transcription of RNA obtained from pooled late-pregnancy sheep placentome tissues.

Calculations

Maternal organ weights obtained at slaughter are presented on a fresh organ mass basis as well as per unit of maternal BW (**MBW**). Capillary area density was determined by dividing the total capillary area (μm^2) by the area of tissue analyzed (μm^2) and multiplying by 100 to express vascularity as a percentage (Caton et al., 2009a; Meyer et al., 2010b). Capillary number density was calculated by dividing the total number of vessels counted by tissue area in μm^2 and then multiplying by 1,000,000 to express the data as capillaries per mm^2. To estimate the capillary surface density (total capillary circumference per unit of tissue area), the mean capillary perimeter (circumference; μm) was divided by tissue area (μm^2). Although capillary surface

density actually represents the circumference of the capillary cross-sections, it is nevertheless proportional to their surface area (Borowicz et al., 2007). Finally, area per capillary was determined by dividing total capillary area by capillary number resulting in area per capillary in μm^2. Total vascularity (mL) was calculated by multiplying the percentage of capillary area density by tissue mass. The percentage of proliferating cells was estimated by dividing the number of Ki-67-stained nuclei by the total number (Ki-67 + hematoxylin-stained) of nuclei present within the area of tissue analyzed.

Table 2.1. The genes of interest and the proposed function of each angiogenic gene[1]

Gene of interest	Proposed function
Vascular endothelial growth factor (VEGF)	Potent angiogenic factor; causes endothelial proliferation and migration, acts via KDR and FLT receptors
fms-related tyrosine kinase 1 (FLT1)	Receptor for VEGF and PGF
Kinase insert domain receptor (KDR)	Receptor for VEGF
Neuropilin 1 (NRP1)	Receptor for VEGF and PGF
Neuropilin 2 (NRP2)	Receptor for VEGF and PGF
Placental growth factor (PGF)	Increases vascular permeability
Angiopoietin 1 (ANGPT1)	Stabilization blood vessels
Angiopoietin 2 (ANGPT2)	Vascular regression in absence of VEGF; vascular sprouting in the presence of VEGF
Tyrosine kinase, endothelial (TEK)	ANGPT1 and ANGPT2 receptor
Basic fibroblast growth factor 2 (FGF2)	Promotes cell growth, differentiation, transformation, and angiogenesis
Fibrobalst growth factor receptor 2 (FGFR2)	Receptor for FGF2
Endothelial nitric oxide synthase 3 (NOS3)	Aids in nitric oxide production, vasodilator
Soluble guanylate cyclase (GUCY1B3)	Binds to and activates NO
Hypoxia-inducible factor 1, alpha subunit (HIF1A)	Regulates adaptive response to hypoxia
Vasoactive Intestinal Peptide (VIP)	Induces smooth muscle relaxation, vasodilator, inhibits gastric acid secretion, stimulates secretion of water into pancreatic juice
Estrogen receptor- α	In response to E2 binds DNA as a transcription factor regulating gene expression of many genes including angiogenic factors

[1]Adapted from Vonnahme et al. (2006)

Statistical analysis

Data were initially analyzed as a completely randomized design with three treatments using the GLM proceedures of SAS (SAS Inst. Inc., Cary, NC). Specific contrasts were used to address the specific questions did HI treatments differ from controls (CON vs. HI + HI+E2) and within HI fed ewes did E2 supplementation make a difference (HI vs. HI+E2). Based on maternal plasma E2 levels throughout gestation (Figure 2.2) and fetal BW (Figure 2.3) on d 130 of pregnancy we suspected that some ewes in HI treatments were carring IUGR offspring and some were not. Therefore, HI and HI+E group was subdivided into four subgroups (HI IUGR, HI non-IUGR, HI IUGR+E2, and HI non-IUGR+E2; Figures 2,4 and 2.5). Maternal E2 levels throughout gestation and especially between d 50 to 90, and from d 99 to 126 demonstrated five different patterns of E2 concetration; irrespective of maternal E2 supplementation IUGR pregnancies had lower levels of E2 between d 99 to 126 and on d 126. Furthermore, close assessment offetal body weight and total placentome weights further revealed IUGR and non-IUGR patterns within HI treatments. Therefore we decided to subdivide HI and HI+E2 into four subgroups. The mean ± sd fetal weight in CON was 4,435 ± 422 g. IUGR was defined as weight at least 2 standard deviations below the CON mean (< 3,591 g), which appeared in 8 of 14 HI and 5 of 12 HI+E2 pregnancies. The resulting treatments for our final analyses consisted on the following groups: include CON (n = 12), HI non-IUGR (n = 6), HI IUGR (n = 8), HI non-IUGR+E2 (n = 7), HI IUGR+E2 (n = 5). Therefore, statistical analysis for this study was a completely randomized design with CON, HI non-IUGR, HI IUGR, HI non-IUGR+E2, and HI IUGR+E2 treatments in the model. The PROC GLM procedure was used for analyses (SAS Inst. Inc., Cary, NC).

Ewes were individually penned with treatment imposed *in utero,* therefore individual animal served as experimental unit. The four following questions were addressed when structuring

contrasts: 1) was there an effect of elevated maternal nutritional plane (CON vs. HI [mean value of HI non-IUGR, HI IUGR, HI non-IUGR+E2, HI IUGR+E2]), 2) was there an effect of maternal E2 supplementation (mean value of HI non-IUGR + HI IUGR vs. mean value of HI non-IUGR+E2 + HI IUGR+E2), 3) was there an effect of IUGR (mean value of HI IUGR and HI IUGR+E2 vs. mean value of HI non-IUGR and HI non-IUGR+E2), and 4) within IUGR groups was there an effect of maternal E2 supplementation (HI IUGR vs. HI IUGR+E2). Least squares means and SEM are presented for all data. Main effects were discussed if $P \leq 0.10$. In addition, Pearson correlation coefficients for maternal plasma E2 levels and maternal, placental, and fetal parameters measured were calculated and are shown in the Appendix, Tables A1 to A40.

Results

Out of 53 recipient ewes, 42 ewes were confirmed pregnant after embryo transfer. However, out of 42 pregnant ewes two recipients from HI+E2 fed ewes had aborted pregnancies, one from CON resulted with autolysed fetus, and another one from CON had abnormal placenta. Therefore, data was collected from 38 remaining pregnant ewes.

Maternal E2 levels are shown in Figure 2.2, where supplementing E2 between d 50 and 90 of gestation significantly ($P < 0.001$) increased plasma E2 levels in HI+E2 group compared with CON and HI. However, as the supplementation of E2 was ceased at d 90, the maternal plasma E2 level dropped ($P = 0.36$) reaching maternal E2 levels in HI group from d 99 till d 126.

Elevated maternal nutrition resulted in decreased ($P \leq 0.04$; Table 2.3 and Figure 2.2) plasma E2 levels throughout gestation and was accompanied by reduced ($P \leq 0.02$) fetal body weight (FBW), placetnome weight, and placental efficiency compared to CON (Table 2.2). However, supplementation of E2 to HI fed ewes did not restore reduced FBW, placentome weight, and placental efficiency observed in HI group.

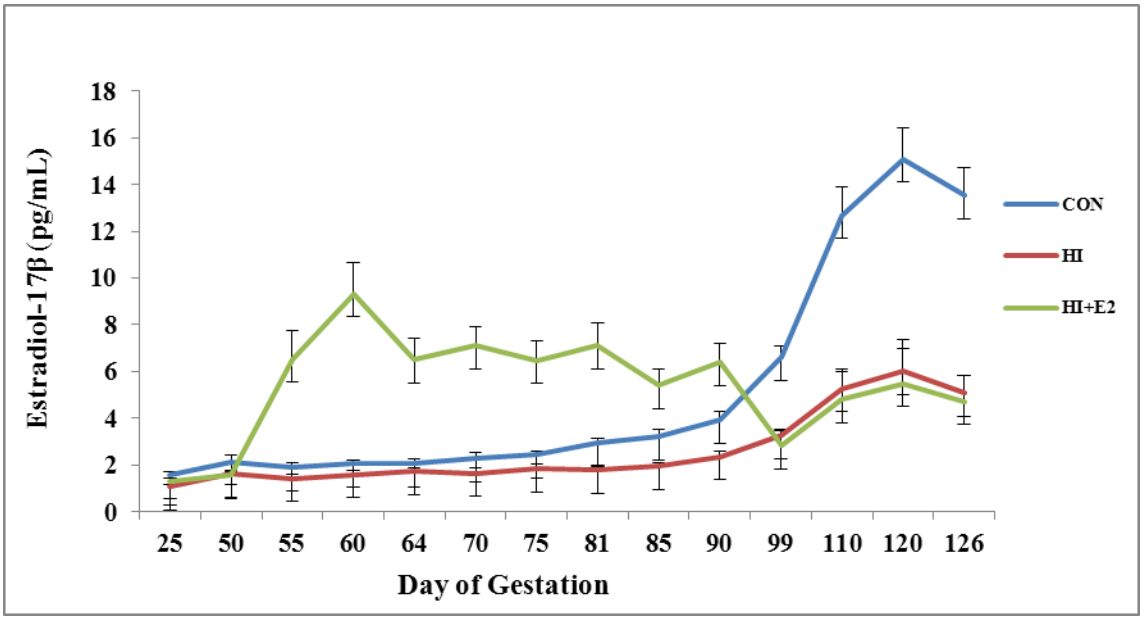

Figure 2.2. Maternal circulating plasma estradiol-17β concentrations throughout gestation in relation to maternal nutrition and E2 supplementation from d 50 to 90 of pregnancy.

Table 2.2. Influence of maternal nutritional plane and estrogen supplementation on fetal and placental main gross parameters at d 130 of pregnancy

	Treatments[1]				Contrasts[2]	
Items	CON	HI	HI+E2	SEM[3]	CON vs. HI	HI vs. HI+E2
FBW, g	4,435	3,611	3,685	223	0.01	0.81
PlacentomeWt, g	474	319	313	30	< 0.001	0.87
Fetal;Plac[4], g/g	9.5	11.8	12.2	0.5	< 0.001	0.48
brain:liver, g/g	0.3	0.4	0.3	0.02	0.14	0.51
Liver, g	142	111	124	10	0.05	0.37
g/kg FBW	32	30.3	33.1	1.2	0.85	0.09
Total small intestine, g	58.9	49.5	48.3	3.8	0.03	0.82
g/kg FBW	13.3	13.7	13.2	0.6	0.85	0.61

[1]Treatments were CON (100% estimated ME and CP requirements; n = 12), HI (approximately twice dietary intake levels as CON; n = 14), HI+E2 (approximately twice dietary intake levels as CON and maternal E2 supplementation between gestational days 50 to 90; n = 12).

[2]*P*-values for contrast that compare CON vs. HI; HI vs. HI+E2.

[3]Standard error of the mean, most conservative used.

[4]Fetal:Plac = ratio of fetal weight to placentome weight

As shown in Figure 2.3 both HI and HI+E2 fed ewes resulted in offspring that weighed significnaly less (P = 0.01) than fetuses from CON, however, there were also fetuses that appeared to have similar body weights as controls.

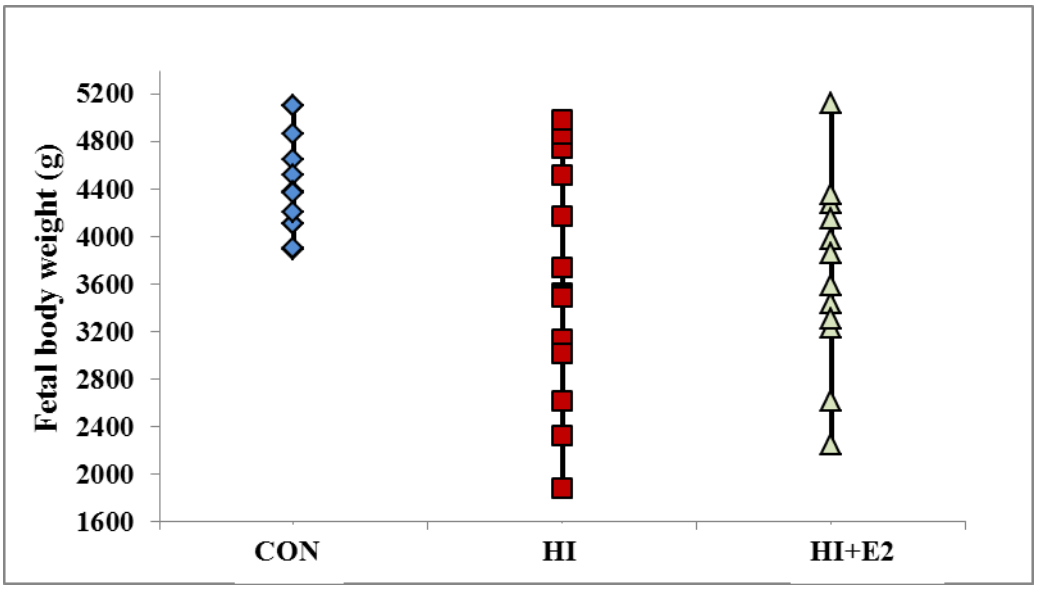

Figure 2.3. Individual fetal body weights at d 130 of gestation in relation to maternal nutrition and estrogen supplementation

From these data it appeared that both HI and HI+E2 treatments contained both IUGR and non-IUGR offspring, which would be consistent with previous results with this model. Therefore, those fetuses in both the HI and HI+E2 treatments that weighed two standard deviation below the means of CON (i.e. < 3591g) were defined as IUGR resulting in five subgroups including CON, HI non-IUGR, HI IUGR, HI non-IUGR+E2, and HI IUGR+E2 (Figure 2.4).

To further validate our decision to subdivide CON, HI, and HI+E groups into CON, HI non-IUGR, HI IUGR, HI non-IUGR+E2, and HI IUGR+E2 we looked at maternal plasma E2 levels across these five subgroups (Figure 2.5). We observed, that mothers whose fetuses were IUGR (HI IUGR, and HI IUGR+E2) had lower (P < 0.001; Table 2.3) plasma E2 levels

irrespective of E2 supplemntation between d 99 to 126 of gestation compared to CON, HI non-IUGR, and HI non-IUGR+E2.

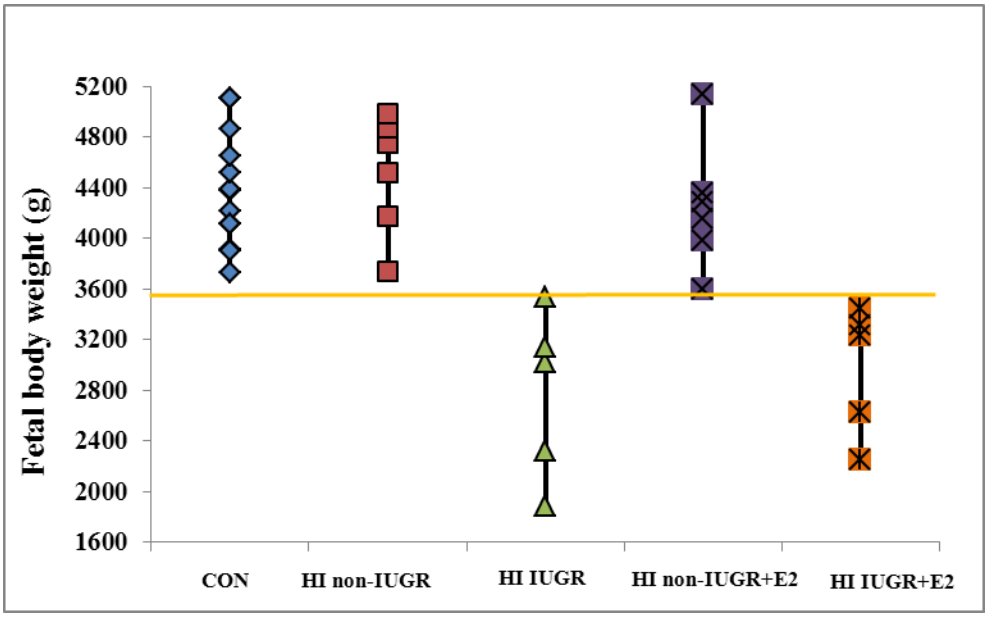

Figure 2.4. Individual fetal body weights at d 130 of gestation in relation to maternal nutrition, estrogen supplementation, and intrauterine growth restriction (IUGR) status. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g) and is indicated by the horizontal yellow line.

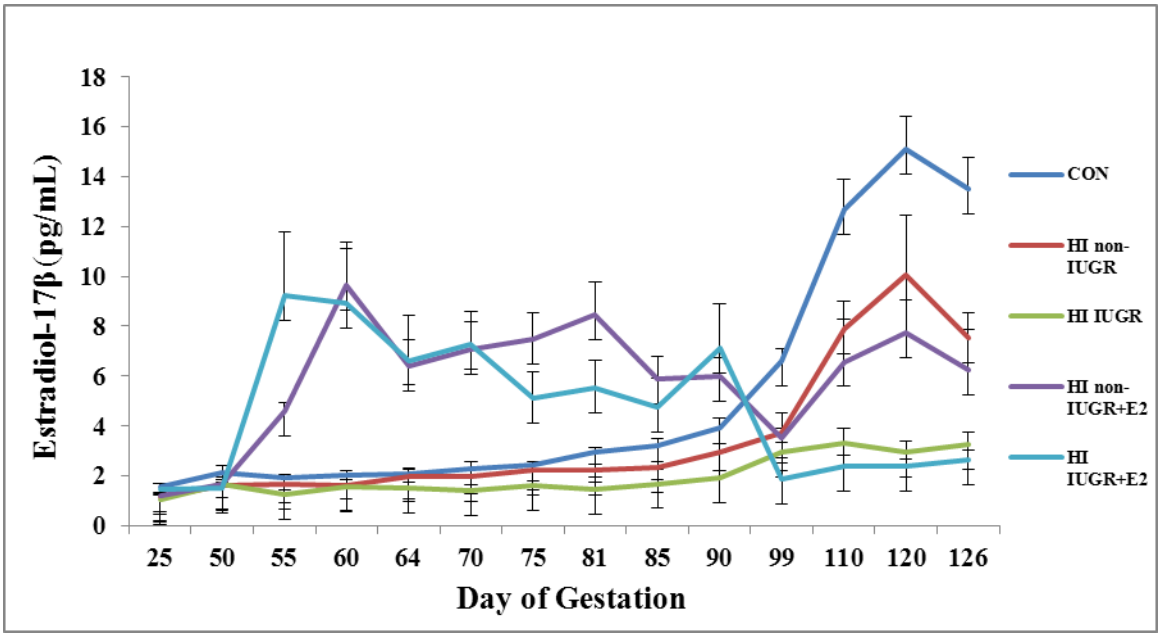

Figure 2.5. Maternal circulating plasma estradiol-17β concentrations throughout gestation in relation to maternal nutrition, E2 supplementation and IUGR status. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g) at necropsy on d 130 of gestation.

As expected the maternal plasma E2 levels were greater ($P < 0.001$) in HI non-IUGR+E2 and HI IUGR+E2 groups on d 55 to 90 compared with HI IUGR and HI non-IUGR groups, however, maternal E2 concetration in E2 supplemented ewes was almost three times greater ($P < 0.0001$) than in CON ewes (Table 2.3). In addition, between d 99 to 126 and on d 126 of gestation, maternal circulating plasma E2 with HI IUGR fetuses (HI IUGR and HI IUGR+E2) was half ($P < 0.0001$) of the E2 concetration in ewes from HI non-IUGR (HI non-IUGR and HI non-IUGR+E2), and aproximately four times less ($P < 0.0001$) than in CON ewes (Table 2.3). Total placentome weights were decreased in HI IUGR and HI IUGR+E2 irrespective of E2 supplementation ($P < 0.0001$) compared to HI non-IUGR and HI non-IUGR+E2 pregnacies (Figure 2.6). This total placentome weights decrease was directly correlated with decrease in fetal body weights at term (Figure 2.7). This relationship is in agreement with previous studies (Wallace et al., 2004). Interestingly, placental efficiency was greater ($P < 0.0001$) in IUGR pregnancies compared to non-IUGR (Figure 2.8). In addition, as shown in Appendix A, Table A9, maternal plasma E2 levels on d 99 to 126 and on d 126 were correlated with FBW and total placentome weights (coefficient of correlation = 0.70; $P < 0.0001$), but negatively correlated with placetal efficiency (coefficient of correlation = - 0.69; $P < 0.0001$) among all animlas (n = 38). Interestingly, total placentome percent proliferation was also positively correlated (Table A41; coefficient of correlation = 0.45; $P = 0.01$) with maternal circulating E2 levels on d 99 to 126 and on d 126 in all animals (n = 38).

Table 2.3. Maternal circulating plasma estradiol-17β concentrations throughout gestation in relation to maternal nutrition, E2 supplementation and IUGR status. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g) at necropsy on d 130 of gestation.

	Treatments[1]						Contrast[2]			
Item	CON	HI non-IUGR	HI IUGR	HI non-IUGR+ E2	HI IUGR+E2	SEM[3]	CON vs. HI	HI IUGR vs. HI non-IUGR	HI vs. HI+E2	HI IUGR vs. HI IUGR + E2
Maternal E2 levels										
d 55 to 70	2.08	1.81	1.45	6.92	7.93	0.73	< 0.001	0.07	< 0.001	< 0.001
d 75 to 90	3.13	2.42	1.67	6.86	5.54	0.58	0.04	0.26	< 0.001	< 0.001
d 55 to 90	2.61	2.12	1.56	6.88	6.74	0.56	< 0.001	0.51	< 0.001	< 0.001
d 99 to 126	11.98	7.29	3.11	6.01	2.30	1.26	< 0.001	< 0.001	0.36	0.62
d 126	13.51	7.51	3.24	6.23	2.62	1.49	< 0.001	< 0.001	0.48	0.74

[1]Treatments were CON (100% estimated ME and CP requirements; n = 12), HI (approximately twice dietary intake levels as CON; n = 6), HI+E2 (approximately twice dietary intake levels as CON and maternal E2 supplementation between gestational days 50 to 90; n = 7), HI IUGR (approximately twice dietary intake levels as CON with fetal BW < 3,591 g at d 130 of gestation; n = 8), and HI IUGR+E2 (approximately twice dietary intake levels as CON and E2 with fetal BW < 3,591 g at d 130 of gestation; n = 5).

[2]P-values for contrast that compare CON vs. all 4 High Treatments; HI (HI non-IUGR + HI IUGR) vs. HI+E2 (HI non-IUGR+E2 + HI IUGR+E2); Non-IUGR (HI non-IUGR + HI non-IUGR+E2) vs. IUGR (HI IUGR + HI IUGR+E2); and HI IUGR vs. HI IUGR+E2.

[3]Standard error of the mean, most conservative used.

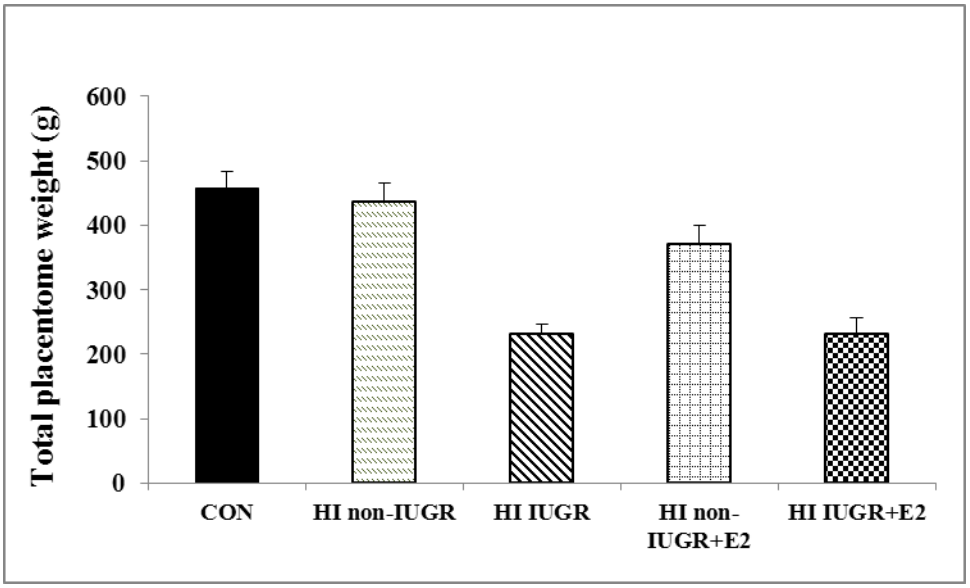

Figure 2.6. Total placentome weight in relation to maternal nutrition, estrogen (E2) supplementation, and intrauterine growth restriction (IUGR) status. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g). Contrasts used CON vs. HI (P = 0.001); IUGR vs. non-IUGR (P = 0.0001); HI non IUGR vs. HI non-IUGR+E2 (P = 0.25); HI IUGR vs. HI IUGR+E2 (P = 0.99).

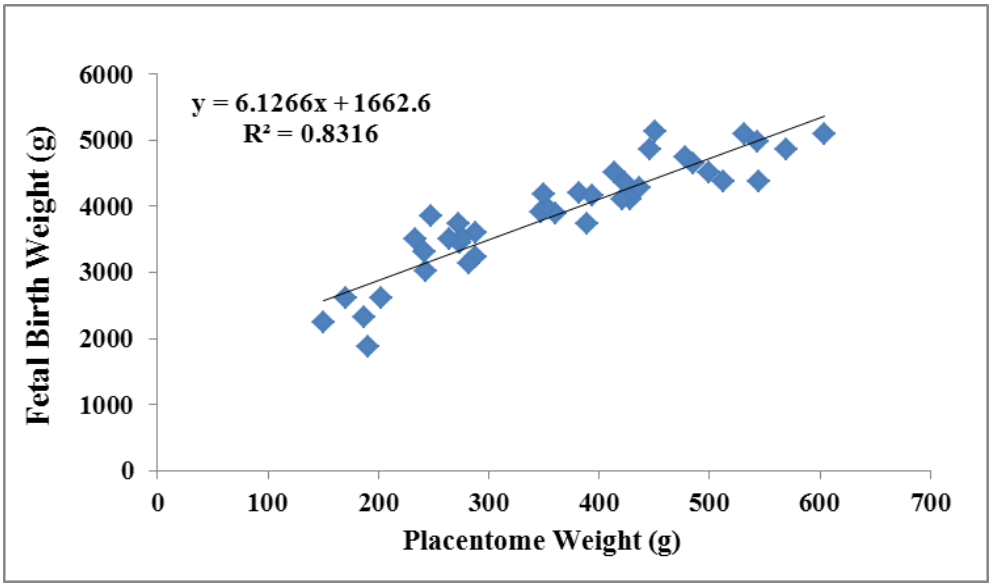

Figure 2.7. Relationship between total placentome weight and fetal weight at d 130 of gestation.

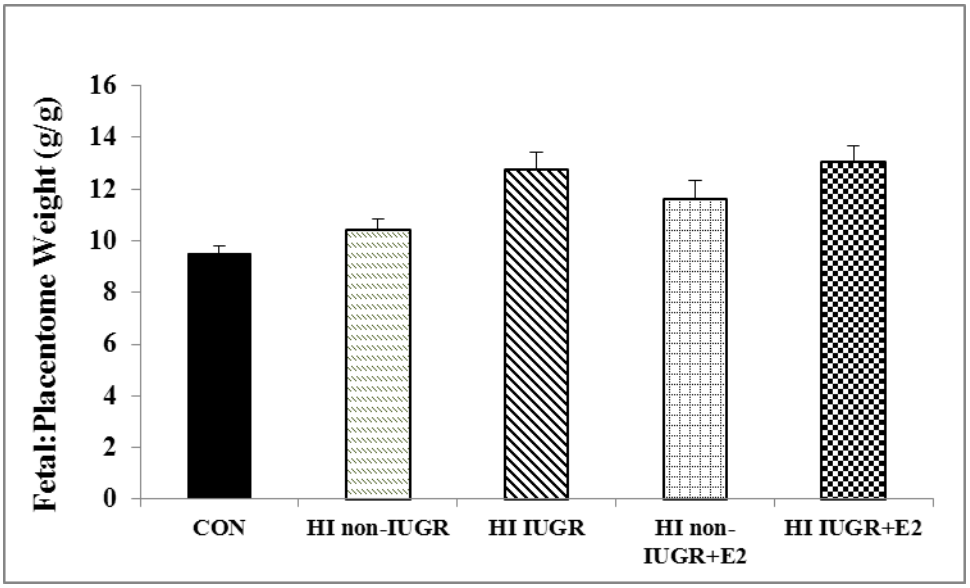

Figure 2.8. Placental efficiency (as inferred from fetal: placentome weight ratio) in relation to maternal nutrition, estrogen (E2) supplementation, and intrauterine growth restriction (IUGR) status. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g). Contrasts used CON vs. HI (P = 0.001); IUGR vs. non-IUGR (P = 0.0001); HI non IUGR vs. HI non-IUGR+E2 (P = 0.2); HI IUGR vs. HI IUGR+E2 (P = 0.7).

Maternal growth, body composition, and internal organ weights were altered in HI compared to CON. Specifically maternal BW (MBW), live weight gain (LWG), body condition score (BCS), BSC change, and maternal average daily gain (ADG), were greater ($P \leq 0.001$) in HI than in CON ewes on d 50, d 90 and d 130 of pregnancy (Table 2.4). The weights of gravid uterus, liver, perirenal fat, and blood were greater ($P \leq 0.001$) in HI than in CON on d 130 of pregnancy. Similarly, maternal LWG from d 4 to 50 and from d 50 to d 90, ADG, and weights of the gravid uterus were greater in ewes carrying IUGR vs. non-IUGR fetuses (Table 2.4). Notable exceptions were maternal perirenal fat and blood absolute and proportional weights were similar ($P \geq 0.39$) between ewes carrying IUGR and non-IUGR offspring. In the HI group, supplementing E2 increased (P = 0.05) BW at d 90 (68.5 vs. 70.6 ± 1.6 kg), maternal LWG (P < 0.001) between d 50 and d 90 (408.5 vs. 321 ± 23.0 g/d, respectively) and ADG (P = 0.09; 255.5 vs. 227 ± 12.6 g/d, respectively). Similarly, HI IUGR+E2 group had increased (P = 0.07) BW at

d 90, ADG, and LWG from d 4 to d 130, but decreased ($P \leq 0.06$) BCS compared with HI IUGR with no E2 supplementation. There were no effects ($P \geq 0.36$) of HI IUGR + E2 on other maternal parameters including MBW, gravid uterus, liver, perirenal fat, and blood weights on d 130 of gestation (Table 2.4). In the HI non-IUGR+E2 group, maternal BCS, gravid uterine, maternal BW, and liver weights were similar to HI non-IUGR (Table 2.4). Gravid uterine weight, maternal BW, liver, perirenal fat, and blood weights were similar in the HI IUGR and HI IUGR+E2 treatments (Table 2.4).

In HI groups, fetal:placentome weight ratio ($P < 0.001$; Fig. 2.9) were greater than in CON ewes. Placentome weight in HI IUGR was 42% lower than in HI non-IUGR ($P < 0.001$) but independent of E2 treatment (Table 2.4).

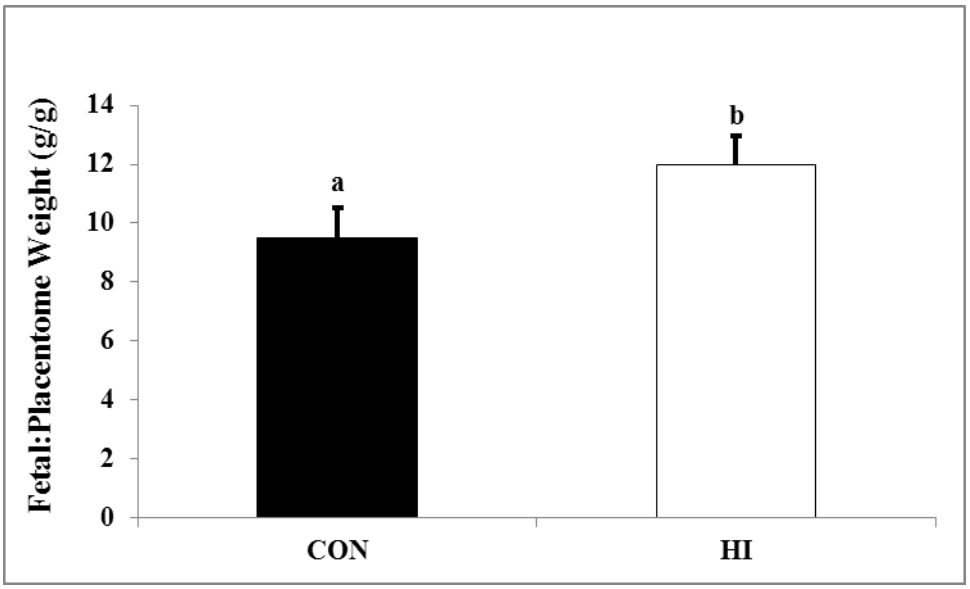

Figure 2.9. FBW:Total placentome weight ratio in CON and HI (HI IUGR + HI non-IUGR + HI IUGR+E2 + HI non-IUGR+E2) groups on d 130 of pregnancy. [a,b] $P < 0.0001$.

Table 2.4. Maternal BW, live weight gain, body condition score, and specific internal organ masses in relation to maternal nutrition, estrogen (E2) supplementation, and intrauterine growth restriction (IUGR) status on d 130 of gestation. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g).

	Treatments[1]						Contrast[2]			
Items	CON	HI non-IUGR	HI IUGR	HI non-IUGR + E2	HI IUGR + E2	SEM[3]	CON vs. HI	IUGR vs. non-IUGR	HI vs. HI+E2	HI IUGR vs. HI IUGR + E2
BW at ET[4], kg	44.91	43.79	42.71	43.11	40.92	1.18	0.02	0.13	0.25	0.24
d 50	47.97	56.08	56.3	55.64	55.49	1.34	0.001	0.98	0.61	0.65
d 90	53.64	68.53	69.49	70.57	73.23	1.58	0.001	0.21	0.05	0.07
d of necropsy	62.71	81.43	79.69	81.34	82.16	1.85	0.001	0.78	0.47	0.3
LWG[5] d 4 to d 50, g/d	66.6	267	294.7	272.2	316.7	21.66	0.001	0.07	0.49	0.43
d 50 to 90	141.8	311.3	330.6	373.4	443.5	22.99	0.001	0.04	0.001	0.001
d 4 to 130	141.3	298.7	293.5	303.4	327.3	13.48	0.001	0.44	0.12	0.06
BCS[6] d 130	2.25	2.79	2.84	2.79	2.75	0.04	0.001	0.80	0.14	0.05
BCS change	0	0.54	0.59	0.54	0.5	0.04	0.001	0.80	0.14	0.05
Fetal BW, g	4,435	4,506	2,940	4,196	2,971	203.2	< 0.01	< 0.001	0.49	0.91
PlacentomeWt, g	474	437	231	371	231	31.6	0.001	0.00	0.25	0.99
Fetal:Plac[7], Wt	9.52	10.4	12.8	11.6	13.1	0.66	0.001	0.00	0.2	0.72
MBW[8], kg	54.77	73.29	74.52	73.89	76.58	1.76	0.001	0.22	0.4	0.36
Maternal ADG[9]	75.8	226.9	244.7	236.7	274.3	12.64	0.001	0.02	0.09	0.08
Gravid uterus, kg	7.95	8.14	5.17	7.46	5.58	0.43	0.001	0.00	0.73	0.47
Liver, g	818	1,318	1,341	1,306	1,339	62.21	0.001	0.61	0.9	0.97
g/kg MBW	14.9	17.9	18	17.7	17.5	0.66	0.001	0.88	0.49	0.53
Perirenal fat, g	616	1,285	1,465	1,598	1,380	126.2	0.001	0.87	0.32	0.6
g/kg MBW	11.3	17.6	19.7	21.5	18	1.71	0.001	0.64	0.47	0.45

Table 2.4. (Continued). Maternal BW, live weight gain, body condition score, and specific internal organ masses in relation to maternal nutrition, estrogen (E2) supplementation, and intrauterine growth restriction (IUGR) status on d 130 of gestation. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g).

	Treatments[1]						Contrast[2]			
Items	CON	HI non-IUGR	HI IUGR	HI non-IUGR + E2	HI IUGR + E2	SEM[3]	CON vs. HI	IUGR vs. non-IUGR	HI vs. HI+E2	HI IUGR vs. HI IUGR + E2
Blood, g										
g/kg MBW	34.2	31.3	31.9	30.9	31	1.4	0.001	0.76	0.59	0.59

[1]Treatments were CON (100% estimated ME and CP requirements; n = 12), HI (approximately twice dietary intake levels as CON; n = 6), HI+E2 (approximately twice dietary intake levels as CON and maternal E2 supplementation between gestational days 50 to 90; n = 7), HI IUGR (approximately twice dietary intake levels as CON with fetal BW < 3,591 g at d 130 of gestation; n = 8), and HI IUGR+E2 (approximately twice dietary intake levels as CON and E2 with fetal BW < 3,591 g at d 130 of gestation; n = 5).

[2]P-values for contrast that compare CON vs. all 4 High Treatments (HI non-IUGR, HI IUGR, HI non-IUGR+E2, and HI IUGR+E2); HI (HI non IUGR + HI IUGR) vs. HI+E2 (HI non-IUGR+E2 + HI IUGR+E2); non-IUGR (HI non-IUGR + HI non-IUGR+E2) vs. IUGR (HI IUGR + HI IUGR+E2); and HI IUGR vs. HI IUGR+E2.

[3]Standard error of the mean, most conservative used.

[4]BW at ET = maternal BW at embryo transfer.

[5]LWG = Total live weight gain (including gravid uterus), g/d.

[6]BCS = body condition score.

[7]Fetal:Plac = ratio of fetal weight to placentome weight.

[8]MBW = maternal live BW at necropsy – gravid uterus wt.

[9]Maternal ADG = maternal average daily gain (with gravid uterus wt removed), g/d.

Table 2.5. Total placentome proliferating nuclei and vascularity at 130 d of pregnancy in relation to maternal nutrition, estrogen (E2) supplementation, and intrauterine growth restriction (IUGR) status on d 130 of gestation. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g).

	Treatments[1]						Contrast[2]			
Items	CON	HI non-IUGR	HI IUGR	HI non-IUGR + E2	HI IUGR + E2	SEM[3]	CON vs. HI	IUGR vs. non-IUGR	HI vs. HI+E2	HI IUGR vs. HI IUGR + E2
Placentome Proliferation										
Proliferating nuclei, %	4.04	2.75	2.5	3.05	3.22	0.81	0.08	0.96	0.5	0.5
Caruncular Vascularity										
Capillary area density, %	32.4	43.4	34	33.6	29.9	7.7	0.65	0.35	0.33	0.69
Capillary number density, mm^2	1,800	1,616	1,282	1,293	1,309	331	0.12	0.60	0.62	0.95
Capillary surface density,($\mu m/\mu m^2$) x 10	11.2	12.4	10	10	10	0.21	0.79	0.55	0.65	0.89
Area per capillary, μm^2	215	252	276	276	245	33.2	0.09	0.90	0.91	0.49
Cotyledonary Vascularity										
Capillary area density[4], %	84.9	20.6	69.1	62.3	63.9	42.1	0.35	0.50	0.62	0.92
Capillary number density[5], mm^2	22,873	5,013	9,618	9,977	13,091	9,841	0.08	0.65	0.62	0.76
Capillary surface density[6], ($\mu m/\mu m^2$) x10	60.9	14.7	36.4	31.2	38.6	138	0.15	0.53	0.69	0.94
Area per capillary[7], μm^2	49.3	43.7	58.2	58.6	74.6	13.8	0.38	0.21	0.2	0.31

[1]Treatments were CON (100% estimated ME and CP requirements; n = 12), HI (approximately twice dietary intake levels as CON; n = 6), HI+E2 (approximately twice dietary intake levels as CON and maternal E2 supplementation between gestational days 50 to 90; n = 7), HI IUGR (approximately twice dietary intake levels as CON with fetal BW < 3,591 g at d 130 of gestation; n = 8), and HI IUGR+E2 (approximately twice dietary intake levels as CON and E2 with fetal BW < 3,591 g at d 130 of gestation; n = 5).

Table 2.5. (Continued). Total placentome proliferating nuclei and vascularity at 130 d of pregnancy in relation to maternal nutrition, estrogen (E2) supplementation, and intrauterine growth restriction (IUGR) status on d 130 of gestation. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g).

[2]P-values for contrast that compare CON vs. all 4 High Treatments (HI non-IUGR, HI IUGR, HI non-IUGR+E2, and HI IUGR+E2); HI (HI non IUGR + HI IUGR) vs. HI+E2 (HI non-IUGR+E2 + HI IUGR+E2); non-IUGR (HI non-IUGR + HI non-IUGR+E2) vs. IUGR (HI IUGR + HI IUGR+E2); and HI IUGR vs. HI IUGR+E2.

[3]Standard error of the means, most conservative used.

[4]Capillary area density = (capillary area/tissue area evaluated) x 100.

[5]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[6]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[7]Area per capillary = capillary area/capillary number per sample area.

In the HI fed ewes, cell proliferation across the whole placentome was less than in CON ewes ($P < 0.08$; Table 2.5). Furthermore, in ewes fed HI, capillary size (APC) in CAR and COT were greater ($P = 0.09$ and $P = 0.04$), respectively than in CON ewes. There were no effects ($P \geq 0.28$) of E2 treatment on cell proliferation, percent of tissue occupied by blood vessels (CAD), surface area of blood vessels available for nutrient exchange (CSD), and the number of capillaries per mm^2 of tissue area analyzed (CND), as reported in Table 2.5

Supplementation of E2 to HI and IUGR groups increased ($P \leq 0.04$) *NOS3* mRNA expression in CAR (Table 2.6). In HI IUGR+E2 group, mRNA expression for *ANGPT2* was less ($P < 0.09$) but for *NOS3* was ~2.5 fold greater ($P < 0.01$) in CAR than in HI IUGR group (Table 2.6). In addition, in HI fed ewes, mRNA expression for *ANGPT1* was less ($P = 0.06$) than in CON group. In IUGR group, *VEGF* ($P < 0.09$), *FLT1* ($P < 0.08$) and *NOS3* ($P < 0.02$) mRNA expression in CAR was greater compared with non-IUGR (Table 2.6). Moreover, in HI group, *ERα* mRNA expression in CAR was greater ($P < 0.06$) compared to CON group (3.78 vs. 2.39 ± 0.91, respectively; Figure 2.10).

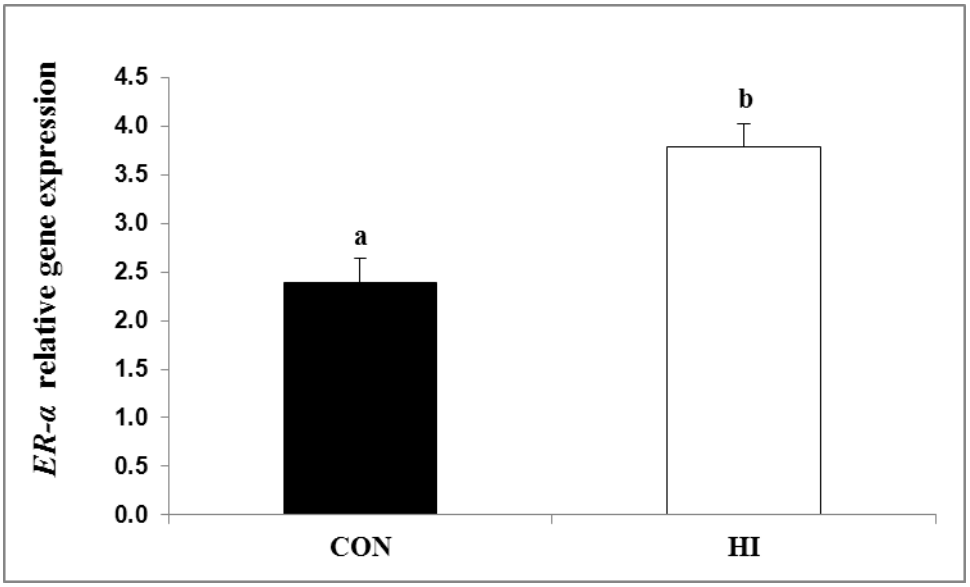

Figure 2.10. *ERα* mRNA expression in maternal placenta (CAR) in CON and HI (HI IUGR + HI non-IUGR + HI IUGR+E2 + HI non-IUGR+E2) groups on d 130 of pregnancy. [a,b] $P < 0.06$.

Table 2.6. Expression of mRNA in caruncle tissue in relation to maternal nutrition, estrogen (E2) supplementation, and intrauterine growth restriction (IUGR) status on d 130 of gestation. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g).

	Treatments[1]						Contrast[2]			
Factors	CON	HI non-IUGR	HI IUGR	HI non-IUGR+E2	HI IUGR+E2	SEM[3]	CON vs. HI	IUGR vs. non-IUGR	HI vs. HI+E2	HI IUGR vs. HI IUGR+E2
VEGF	0.28	0.26	0.35	0.2	0.35	0.08	0.83	0.09	0.66	0.97
FLT1	0.25	0.29	0.39	0.18	0.37	0.09	0.47	0.10	0.43	0.87
KDR	0.33	0.34	0.43	0.14	0.29	0.18	0.82	0.46	0.31	0.54
NRP1	0.24	0.25	0.26	0.14	0.21	0.08	0.68	0.64	0.26	0.63
NRP2	2.21	2.13	1.85	2.44	1.72	0.44	0.62	0.22	0.83	0.81
ANGPT1	0.2	0.15	0.12	0.04	0.11	0.06	0.06	0.75	0.24	0.85
ANGPT2	0.21	0.15	0.33	0.07	0.14	0.1	0.67	0.13	0.13	0.09
TEK	2.15	2.21	2.07	1.67	1.94	0.65	0.67	0.90	0.51	0.86
FGF2	0.74	0.49	0.51	0.46	0.67	0.16	0.13	0.44	0.66	0.46
FGFR2	0.12	0.04	0.07	0.04	0.09	0.06	0.14	0.43	0.92	0.88
NOS3	0.8	0.55	0.75	0.66	1.85	0.29	0.53	0.02	0.04	0.01
GUCY1B3	2.45	1.94	2.65	2.04	1.95	0.96	0.7	0.73	0.74	0.6
HIF1A	0.49	0.56	0.66	0.5	0.57	0.11	0.32	0.34	0.42	0.5
PGF	0.26	0.19	0.26	0.09	0.16	0.07	0.11	0.25	0.11	0.29
ER-α	2.39	3.84	2.5	4.17	4.62	0.91	0.06	0.12	0.59	0.7

[1]Treatments were CON (100% estimated ME and CP requirements; n = 12), HI (approximately twice dietary intake levels as CON; n = 6), HI+E2 (approximately twice dietary intake levels as CON and maternal E2 supplementation between gestational days 50 to 90; n = 7), HI IUGR (approximately twice dietary intake levels as CON with fetal BW < 3,591 g at d 130 of gestation; n = 8), and HI IUGR+E2 (approximately twice dietary intake levels as CON and E2 with fetal BW < 3,591 g at d 130 of gestation; n = 5).

[2]P-values for contrast that compare CON vs. all 4 High Treatments (HI non-IUGR, HI IUGR, HI non-IUGR+E2, and HI IUGR+E2); HI (HI non IUGR + HI IUGR) vs. HI+E2 (HI non-IUGR+E2 + HI IUGR+E2); non-IUGR (HI non-IUGR + HI non-IUGR+E2) vs. IUGR (HI IUGR + HI IUGR+E2); and HI IUGR vs. HI IUGR+E2.

[3]Standard error of the means, most conservative used.

Table 2.7. Expression of mRNA in cotyledon tissue in relation to maternal nutrition, estrogen (E2) supplementation, and intrauterine growth restriction (IUGR) status on d 130 of gestation. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g).

	Treatments[1]						Contrast[2]			
Factors	CON	HI non-IUGR	HI IUGR	HI non-IUGR + E2	HI IUGR + E2	SEM[3]	CON vs. HI	IUGR vs. non-IUGR	HI vs. HI+E2	HI IUGR vs. HI IUGR + E2
VEGF	0.7	0.57	0.49	0.62	0.61	0.1	0.13	0.61	0.33	0.36
FLT1	1.02	0.72	0.65	0.7	0.61	0.23	0.06	0.71	0.88	0.89
KDR	0.46	0.44	0.41	0.51	0.58	0.11	0.81	0.82	0.2	0.2
NRP1	0.19	0.23	0.16	0.25	0.18	0.06	0.72	0.22	0.73	0.82
NRP2	1.11	1.41	0.76	0.62	0.92	0.33	0.5	0.56	0.29	0.7
ANGPT1	0.92	0.87	1.16	0.75	1.17	0.18	0.64	0.03	0.74	0.94
ANGPT2	0.38	0.28	0.33	0.22	0.44	0.1	0.43	0.14	0.77	0.4
TEK	2.44	2.83	2.91	3.06	3.27	0.42	0.11	0.71	0.45	0.52
FGF2	0.29	0.4	0.34	0.37	0.38	0.09	0.26	0.76	0.95	0.74
FGFR2	0.14	0.12	0.12	0.15	0.2	0.04	0.78	0.52	0.18	0.18
NOS3	0.76	0.47	0.77	0.58	1.02	0.2	0.76	0.05	0.33	0.34
GUCY1B3	0.1	0.05	0.16	0.19	0.47	0.16	0.38	0.19	0.15	0.15
HIF1A	0.42	0.34	0.28	0.37	0.38	0.08	0.23	0.72	0.33	0.3
PGF	0.58	0.43	0.41	0.6	0.43	0.12	0.3	0.38	0.4	0.9
ER-α	0.86	0.7	0.91	1.15	1.4	0.24	0.33	0.03	0.29	0.43

[1]Treatments were CON (100% estimated ME and CP requirements; n = 12), HI (approximately twice dietary intake levels as CON; n = 6), HI+E2 (approximately twice dietary intake levels as CON and maternal E2 supplementation between gestational days 50 to 90; n = 7), HI IUGR (approximately twice dietary intake levels as CON with fetal BW < 3,591 g at d 130 of gestation; n = 8), and HI IUGR+E2 (approximately twice dietary intake levels as CON and E2 with fetal BW < 3,591 g at d 130 of gestation; n = 5).

Table 2.7. (Continued). Expression of mRNA in cotyledon tissue in relation to maternal nutrition, estrogen (E2) supplementation, and intrauterine growth restriction (IUGR) status on d 130 of gestation. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g).

[2]P-values for contrast that compare CON vs. all 4 High Treatments (HI non-IUGR, HI IUGR, HI non-IUGR+E2, and HI IUGR+E2); HI (HI non IUGR + HI IUGR) vs. HI+E2 (HI non-IUGR+E2 + HI IUGR+E2); non-IUGR (HI non-IUGR + HI non-IUGR+E2) vs. IUGR (HI IUGR + HI IUGR+E2); and HI IUGR vs. HI IUGR+E2.

[3]Standard error of the means, most conservative used.

In COT, *FLT1* mRNA expression was less ($P = 0.06$) in HI group compared to CON. In COT of IUGR group, expression of mRNA for *ANGPT1* ($P < 0.03$) and *NOS3* ($P < 0.05$) was greater compared to non-IUGR (Table 2.7). Supplementation of E2 had no effect on COT mRNA but in CAR, *NOS3* mRNA expression was greater in HI non-IUGR+E2 and HI IUGR+E2 groups ($P < 0.04$ and $P < 0.01$, respectively). In IUGR group, COT *ERα* mRNA expression in was greater ($P = 0.03$) comparing to non-IUGR groups (1.16 vs. 0.93 ± 0.24, respectively; Figure 2.11).

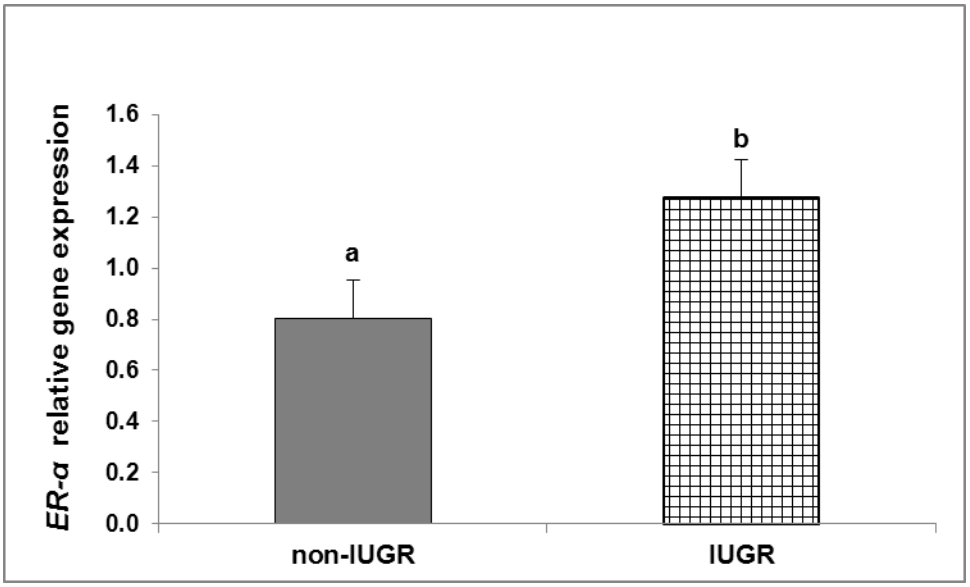

Figure 2.11. *ERα* mRNA expression in fetal (COT) placenta in non-IUGR (HI non-IUGR + HI non-IUGR+E2) and IUGR (HI IUGR + HI IUGR+E2) groups on d 130 of pregnancy. $^{a,b}P = 0.03$.

Discussion

Historically, overnourishing adolescent ewes during pregnancy results in 30 to 40 % placental growth restriction and premature delivery of low birth weight lambs (Wallace et al., 1996, 1999a,b, 2001). Our current data is supportive of these previous studies; therefore, the ovine paradigm used in this study was performing as predicted. Thus, rapidly growing pregnant adolescent lambs used in this study were likely competing for nutrients with the growing gravid

uterus and resulting in placental insufficiency as previously reported (Wallace et al., 2001, 2004). In addition, Wallace et al. (2006b) demonstrated high correlation between decreased maternal plasma E2 levels and IUGR fetuses which were confirmed in our study. As discussed earlier, our study included three treatment groups which are CON, HI, and HI+E2. However, based on fetal body weights, HI and HI+E2 groups resulted in fetuses that weigh as small as 2,251 g to as large as 4,985 g. Therefore, those fetuses that weighed two standard deviation below the CON means (< 3,591 g) were designated as IUGR resulting in CON, HI IUGR, HI non-IUGR, HI IUGR+E2, and HI non-IUGR+E2 subgroups. Furthermore, maternal circulating plasma E2 in all five subgroups demonstrated the significant difference in E2 levels in ewes carrying IUGR fetuses vs. non-IUGR fetuses irrespective of E2 supplementation. In agreement with previous studies (Wallace et al., 2006b), decreased maternal E2 levels were correlated with decreased fetal body weights (IUGR) observed in our study. This subdivision provides a clear separation between IUGR and non-IUGR fetuses, thus resulting in better testing of the maternal E2 supplementation effects in IUGR pregnancies. As shown in Figure 2.5 ewes carrying IUGR fetuses had lower levels of E2 on d 99 to 126 compared to non-IUGR and CON. Perhaps, supplementing E2 in lower doses from d 50 to 90 and gradually increasing the dose similar to CON E2 levels may have a better outcome in rescuing IUGR pregnancies. Unquestionably, increasing the dose of E2 supplementation on d 90 to 126 can also increase the risk of abortion, therefore, further work in optimizing dose and timing of E2 is needed. Further discussions in this paper will be based on these five subgroups (CON, HI IUGR, HI non-IUGR, HI IUGR+E2, and HI non-IUGR+E2).

Maternal Weight and Organ Mass. In this study, HI fed adolescent ewes had increased maternal tissue weights including BW, BCS, maternal average daily gain (ADG), liver, perirenal

fat, blood, and gravid uterus. Moreover, within HI fed ewes, those carrying IUGR fetuses had dramatically increased maternal BW, LWG, BCS, maternal ADG, and perirenal fat. These observations are consistent with other studies suggesting that adolescent mothers are growing at the expense of a growing gravid uterus (Wallace et al., 1996, 1999b, 2001; Luther et al., 2005; Caton et al., 2009a). In addition, maternal liver was also increased in ewes from HI and IUGR groups compared to control group, which is also consistent with previous finding (Wester et al., 1995; Fluharty and McClure, 1997; Swanson et al., 2000, Caton et al., 2009a).

As discussed earlier, Wallace et al., (2006b) demonstrated high correlation between decreased maternal circulating E2 levels and IUGR fetuses, therefore, we hypothesized that supplementing E2 during the mid-gestation might alter maternal parameters and rescue IUGR pregnancies. Specifically, targeting placental growth and development and increasing blood flow via supplementing mothers with E2 during d 50 to 90 which is a crucial developmental window for placental growth. In our study, based on Pearson correlation test, maternal circulating plasma E2 concentration was correlated with fetal and total placentome weights, as well as total placentome proliferation. As shown in Table 2.4 between d 55 to 90 (which is the period of rapid placental proliferation) maternal E2 levels were significantly lower in HI group compared with CON, this could partially explain the reduced placental proliferation in overnourished group. Therefore, our goal was to restore the decreased plasma E2 concentrations. Surprisingly, maternal E2 supplementation to HI fed mothers increased maternal BW in both ewes carrying IUGR and non-IUGR groups without anticipated rescuing effect of E2 on fetal BW. It is possible, that reduced placental weight in IUGR pregnancies resulted in decreased steroid secretion. On the other hand, HI fed ewes may result in altered metabolism including increased

metabolic clearance of E2 through liver compared to CON therefore resulting in decreased E2 levels.

In addition, because E2 was not supplemented to mothers until mid-gestation, the developmental programming during organogenesis and embryogenesis "pre-determined" the offspring outcome by epigenetically altering expression of receptors and factors that promote fetal growth or diminishing expression of estrogen receptors, therefore limiting the effects of E2. It is also possible that the dose and the length of E2 supplementation along with altered nutrient supply to mothers had profound effects on various metabolically important tissues in both mothers and their offspring; therefore interrupting the possible E2 effects.

Placental Weight, Proliferation, and Vascularity. As expected, HI fed ewes had decreased placental and fetal weights, but increased fetal:placentome ratio. This is an indicative of increased placental efficiency in growth restricted pregnancies possibly in an attempt to meet fetal growing metabolic needs. Similar results were seen in other studies (Wallace et al.., 2006b). Reduced placental weight is associated with reduced uterine and umbilical blood flow that further limit the oxygen, glucose, amino acids transport capacity (Reynolds and Redmer, 1995; Reynolds et al., 2010b). Moreover, rapidly growing dams tend to develop decreased glucose uptake capacity; however, the major limiting factor for fetal growth is the size of placenta rather the nutrient metabolism or transport capacity (Wallace et al., 2004b). Placental structural abnormalities including decreased villous number, lumen size and branching also contribute to developing IUGR pregnancies (Lee and Yeh, 1986; Macara et al., 1996). While E2 promotes cell proliferation and growth directly affecting blood vessel walls via release and stimulation of vasoactive substances that promote angiogenesis and blood flow, particularly during placental development and overall growth (Niklaus et al., 2003; Wallace et al., 2000; Reynolds, 2009), in

our study maternal E2 supplementation did not restore a 42% decrease in placentome weight from ewes carrying IUGR fetuses. Determining placental structural morphology in addition to vascularity and mRNA gene expression performed in our study could shed additional light into limited responsiveness of placental development to E2 in our study.

Similarly, the decrease in placentome cell proliferation and vascularization due to elevated maternal nutrition was not offset by maternal E2 supplementation. The mechanism of E2 action in placental tissues in terms of cell proliferation and vascularization is not well studied; therefore it is hard to determine the possible reasons of E2 supplementation being ineffective. However, it is well known that E2 action is mediated via ERα and ERß. Indeed, ERα is absolutely necessary for endothelial NO production (Darblade et al., 2002). Since NO is a potent vasodilator and can also regulate VEGF expression (Benoit et al., 1999), it has been shown to mediate estrogen-induced increase in uterine blood flow (Rosendfeld et al., 1996; Zheng et al., 1998). Therefore, possible explanation of E2 supplementation being ineffective could be due to inadequate levels of ERα. Moreover, ERα is especially sensitive to epigenetic modification due to physiological and environmental changes (Champagne and Curley, 2008). Therefore, it is also possible that maternal gynecological immaturity and elevated nutrition altered ERα responsiveness to E2 via epigenetic modification either at the transcriptional, translational, or at posttranslational levels.

Placental mRNA Expression of Angiogenic Factors. Despite limited response of maternal organ mass, cell proliferation, and vascularization to E2 supplementation, mRNA gene expression of *NOS3* expression was greater in CAR tissues of IUGR pregnant ewes that received E2 supplementation. Nitric oxide synthase (NOS3) is a potent angiogenic factor that aids in NO synthesis. As previously described, NO increases blood flow, vascular permeability, and

stimulates VEGF production. Moreover, NO induces cell migration in angiogenesis by increasing adhesion molecules and extracellular matrix metalloproteases and invades endothelial cells forming new blood vessels (Lee et al., 2000; Lopez-Rivera et al., 2005). Expression of mRNA for *NOS3*, *ANGPT2*, and *VEGF* has been previously confirmed in ovine placental tissues (Luther et al., 2007; Grazul-Bilska et al., 2010, 2011). Interestingly, maternal E2 also decreased CAR *ANGPT2* mRNA expression. In the presence of VEGF, ANGPT2 stimulates vascular sprouting, however, in the absence of VEGF, ANGPT2 regresses vascular sprouting as reported by Vonnahme et al., (2006). Surprisingly, IUGR pregnancies had increased *VEGF*, *FLT1*, and *NOS3* mRNA expression in CAR tissues. This increase in major angiogenic factor expression in IUGR pregnancies can be due to the compensating mechanism of the placenta. However, we may speculate that mRNA expression of these potent angiogenic factors were "hijacked" by epigenetic modification at the translational or posttranslational level therefore limiting expected cell proliferation and angiogenesis. Therefore, additional work in supplementing E2 in a smaller dose but for a longer period starting from early gestation till the term coupled with supplementing methyl donors might have beneficial effect. Methyl donors such as Vitamin B12, choline, and folate can reverse epigenetic alterations due to inappropriate maternal diet and prevent adverse long-term epigenetic changes in genes regulating vascularization of key nutrient exchange tissues via one-carbon metabolism (Dolinoy et al., 2007; Sinclair et al., 2007; McNeil et al., 2009).

In fetal portion of placenta (COT), mRNA expression of *ERα*, *ANGPT1* and *NOS3* were increased in IUGR pregnancies compare to non-IUGR group. The increase in these angiogenic factors observed both in CAR and COT tissues may indicate the compensating mechanism of placenta in IUGR pregnancies. Together, the ANGPT/NO systems not only aid in cell

proliferation and angiogenesis, but also are important participants of vessels maturation and stability (Gavard, 2009; Gonzalez et al., 2003). As expected, elevated maternal nutrition decreased *FLT1 (VEGFR-1)* mRNA expression. Normally, VEGF induces eNOS dependent NO synthesis via activation of FLT1 and KDR (Kroll et al., 1998; Fukumura et al., 2001). Therefore, reduced *FLT1* mRNA expression in COT can partially contribute to decreased cell proliferation and vascularization observed in our study.

In conclusion, HI fed ewes resulted in increased maternal organ masses at the expense of the growing gravid uterus and supplementing mothers with E2 during gestation didn't alter increased maternal organ masses. Placental weight, vascularity, and proliferation also were not restored in the presence of maternal E2 supplementation. However, E2 supplementation within the IUGR pregnant ewes increased *NOS3* mRNA expression. The effects of maternal E2 supplementation on placental growth and development have not been reported previously. In our study, very high E2 supplementation (d 50 to 90) did not restore placental growth and fetal parameters suggesting that E2 is not the central driving link between nutrition and fetal growth restriction between d 50 and 90 of gestation in our model. However, since the E2 dose was three times higher in HI+E2 group compared to CON, on a molecular level, plasma E2 levels that are three times above physiological E2 levels could have "desensitized" ERs response via negative feedback mechanism. This could partially explain the limited responsiveness of placental growth in our model.

Interestingly, by the final third of pregnancy (d 99 to 126) shown in Figure 2.4, ewes with IUGR fetuses had E2 levels which were ~ 50 % lower than non-IUGR and this was completely unaffected by the previous period of E2 supplementation. As discussed earlier, metabolic clearance of E2 via liver and intestines during d 50 and 90 could be greater in HI+E2 compared

with CON due to unusually high levels of E2 concentration. Thus, when the E2 supplementation ceased on d 90, it is possible that maternal metabolic clearance rate of E2 remained the same, which further depleted the maternal E2 levels in HI IUGR+E2 group. The reduced placental size and along with increased E2 metabolic clearance rate in HI IUGR+E2 group could explain the rapid decreased in maternal plasma E2 concentration compared with HI non-IUGR+E2 group. Suggesting, that placental small size is the driving force for IUGR pregnancies, and possibly supplementing E2 during the third trimester can possible enhance fetal growth but its effect on placental growth and efficiency is not fully understood.

Therefore, optimizing timing, dose, and length of E2 supplementation along with determining global methylation status of angiogenic factors and protein content might give us a better understanding of mechanism of IUGR pregnancies and placental development.

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CHAPTER III. EFFECTS OF MATERNAL NUTRITION, INTRAUTERINE GROWTH RESTRICTION (IUGR), AND ESTRADIOL-17β (E2) SUPPLEMENTATION IN FIRST PARITY EWES; IMPACTS ON FETAL GASTROINTESTINAL BIOLOGY

Abstract

Objectives were to investigate the effects of maternal nutritional plane, IUGR, and estradiol-17ß (E2) treatment during mid-gestation on fetal growth, visceral tissues, and intestinal biology. Singleton pregnancies were established by embryo transfer, and then adolescent dams were offered a control diet (CON, n = 12), fed 100% estimated ME and CP requirements or a high nutritional plane diet (HI, n = 26), fed approximately twice the dietary intake of CON group. From d 50 to 90, i.m. injections of E2 (0.05 mg) were given twice daily to 12 ewes fed HI diets (HI+E2). Ewes were fed twice daily and housed individually indoors. On d 130 of pregnancy, fetal organ masses were measured and intestinal tissues harvested. Ewes in HI and HI+E2 treatments having fetuses ≤ 3,591 g were designated as HI IUGR (n = 8) and HI+E2 IUGR (n = 5) groups, respectively. Data were analyzed for effects of maternal plane of nutrition, E2 treatment, and IUGR. In HI group, fetal BW and several organ masses were less ($P < 0.001$) than in CON group. However, within IUGR groups the presence of E2 restored ($P = 0.02$) fetal total small intestinal length. Small intestinal tissues were perfusion fixed with Carnoy's solution, paraffin embedded, and vascular structures were visualized microscopically. Total small intestinal vascularity was decreased ($P = 0.005$) due to IUGR. Fetuses from the HI IUGR+E2 group had less ($P = 0.08$) small intestinal capillary area density (30.9 vs. 24.6 ± 2.76%, respectively) and less ($P = 0.09$) capillary surface density (1.15 vs. 0.98 ± 0.08 µm/µm2 × 10, respectively) compared to HI IUGR. Furthermore, in both offspring from HI treated ewes and those that were IUGR, maternal E2 supplementation increased [($P = 0.05$) and ($P = 0.004$),

respectively] intestinal *GUCY1B3* mRNA expression. In addition, in HI group, small intestinal estrogen receptor-α (*ERα*) mRNA expression was less ($P = 0.02$) than in CON group. In conclusion, maternal high nutritional plane and E2 treatment during mid-gestation resulted in changes in offspring intestinal vascularity, selected gene mRNA expression, and small intestinal length. However, additional work in determining protein content and global methylation status along with optimization of maternal E2 dose and E2 supplementation length might present additional insight into underlying molecular mechanisms occurring in growth restricted fetal intestinal tissues.

Key words: intestine, maternal nutrition, 17ß-estradiol

Introduction

In ruminants, adult liver and gut consume approximately 40% of maintenance energy demands (Huntington, 1990; Reynolds et al., 1991). Nutrient utilization by the gastrointestinal tissues is dynamic in terms of blood flow and nutrient flux, and is responsive to diet quality (Reynolds et al., 1991; Seal and Reynolds, 1993; Goetsch, 1998), quantity (Huntington et al., 1990), and physiological changes associated with compensatory growth (Burrin et al., 1989). Similarly, fetal and neonatal gastrointestinal tract is highly dynamic in terms of villi formation, appearance of digestive enzymes, and development of mature motility patterns (Mongomery et al., 1999; Trahair et al., 1997; Trahair and Sangild, 1997). In addition to perinatal gastrointestinal (GI) tract growth, small intestine continues to develop even into maturity responding to various physiological and environmental changes. Recent data indicate that maternal intestinal vascular networks (Reed et al., 2007) and angiogenic factor profiles (Neville et al., 2007) are altered by diet. However, little is known about fetal intestinal vascularization and angiogenic factor profiles in response to alteration in maternal nutrition.

Previous studies have demonstrated that overnourishing adolescent ewes result in pregnancies where rapid maternal growth takes place at the expense of developing placenta and fetus resulting in intrauterine growth restriction (IUGR) (Wallace et al., 1996, 1999b, 2002, 2006a, b). Additionally, in this overnourished model, maternal plasma E2 concentration was reduced from d 50 to 90 of gestation compared with ewes fed maintenance diet (Wallace at al., 2008). In fact, the reduced maternal plasma E2 concentration during pregnancy was associated with reduced fetal birth weights (IUGR) at term in adolescent sheep model (Wallace et al., 2008). Unfortunately, IUGR pregnancies can lead to complications related to improper fetal gut development. For instance, placental growth restriction during the important fetal developmental windows including organogenesis and rapid fetal growth (Chapter I, Figure 1.10) may hinder fetal gut tube formation, elongation, villi and crypt formation, mucosal growth, and absorption capacity, and therefore might potentially cause postnatal complications and complications that can be carried on into adulthood. The impact of IUGR coupled with E2 supplementation on fetal intestinal growth and vascularity and implications on developmental programming effects in this particular model has not been characterized. Although, there is no known reports regarding the direct effects of maternal plasma E2 concentration on fetal plasma E2 concentration, current data indicate that E2 is involved in the regulation of angiogenesis (Niklaus et al., 2003), stimulates cell proliferation and overall growth in placenta and other tissues, and vascular development and blood flow (Wallace et al., 2000; Reynolds, 2009). Therefore, by attempting to increase placental cell proliferation and vascularization during mid-gestation via maternal E2 supplementation in IUGR pregnancies, we hypothesize that alterations in fetal intestinal biology due to IUGR may be reversed by maternal E2 supplementation administered between d 50 and 90 of pregnancy. Thus, the objectives of this study are to investigate E2 effects on 1) fetal gastrointestinal organ

mass; 2) small intestinal cell proliferation and vascularization; and 3) small intestinal mRNA expression of genes related to angiogenesis in adolescent ewes fed maintenance and high energy diet.

Materials and Methods

All procedures were licensed under the UK Animals (Scientific Procedures) Act of 1986 and were approved by the Ethical Review Committee of the Rowett Institute of Health and Nutrition, University of Aberdeen. Animal treatment, tissues collection and initial processing, and hormone analysis were performed at the Rowett Institute of Health and Nutrition, and evaluation of vascularization and mRNA expression were performed at the Department of Animal Sciences, North Dakota State University, USA.

Animals and embryo transfer

Embryos were recovered on d 4 after estrus from superovulated adult ewes (Border Leicester × Scottish Blackface) that were inseminated by a single sire, and one embryo per recipient was transferred to synchronized adolescent ewe lambs (Dorset Horn × Greyface), as described previously (Wallace et al., 1997). Donors (n = 9) had lambed once previously, were 2.5 years old, weighed 70.6 ± 1.03 kg, and had a body condition score (BCS) of 2.25 (on a 5-point scale, with 1 being emaciated and 5 obese, Russel et al. 1969) at the time of embryo recovery. An average of 5.9 early morula (grade1) per donor ewe (range 4 to 13 embryos) was utilized for transfer into 53 adolescent recipients. This protocol ensured that placental and fetal growth was not influenced by varying fetal number or partial embryo loss. Moreover, the utilization of a single sire and a limited number of embryo donors maximized the homogeneity of the resulting fetuses. Embryo transfer was carried out on 4 separate days during the midbreeding season, and animals were housed in individual pens under natural lighting conditions at the Rowett (57° N, 2° W). At

the time of embryo transfer, adolescent recipients (n = 53) were peripubertal (approximately 8.5 mo of age) and had a mean BW of 42.9 ± 0.37 kg, a BCS of 2.25 ± 0, and an ovulation rate of 2.0 ± 0.13.

Treatments and experimental design

Adolescent recipients (n = 53) were initially allocated to 1 of 2 dietary treatments. Plane of nutrition treatments were control (CON) or high (HI) quantity of the same complete diet. At embryo transfer, adolescent ewes were allocated to either the CON (n = 17) or HI (n = 36) group on the basis of their current BW, BCS, ovulation rate, and, where possible donor source. The dietary amount in the CON group was calculated to maintain normal maternal adiposity throughout gestation and to provide the estimated ME and protein requirement of adolescent ewe lambs carrying a singleton fetus according to the stage of pregnancy (based on Agricultural and Food Research Council; AFRC, 1993). To achieve this objective, the CON group was fed to promote a low maternal weight gain (~ 50 g per day) during the first two-thirds of gestation, followed by step-wise increases in maternal intake during the final third of gestation calculated to meet the increasing demands of the developing fetus. Based on previous studies, it has been demonstrated that this approach in the CON group optimizes placental and fetal growth in this genotype (Wallace et al., 2004a). In contrast, the HI or ad libitum intakes were equivalent to approximately twice the estimated ME requirements and were calculated to promote rapid maternal BW and adiposity gain at the expense of the conceptus. The complete diet supplied 2.9 Mcal of metabolizable energy and 140 g of crude protein per kilogram of body weight and was offered in two equal feeds at 0800 and 1600 h daily. The diet contained 30% (w/w) coarsely milled hay, 42.25% barley, 10% molasses, 16.75% soybean meal, 0.35% salt, 0.5% dicalcium phosphate, and 0.15% vitamin-mineral supplement and had an average dry matter of 86%. The amount of feed

offered in the HI group was gradually increased over a 2-wk period until the amount of daily refusal was approximately 15% of the total offered (equivalent to ad libitum intakes). The amount of feed offered was reviewed 3 times weekly and adjusted, on an individual basis and when appropriate, on the basis of BW change data (recorded weekly) and the amount of feed refusal (recorded daily), as described previously (Wallace et al., 2006b). Maternal body condition was subjectively assessed as detailed above every 2 wk by the same experienced operator.

Conception rate was determined by transabdominal ultrasonography at approximately 45 d of gestation (gestation length = 145 d), when 14 CON and 28 HI recipients were pregnant. At d 50 of gestation, half of the H group (n = 14) were allocated to receive E2 on the basis of maternal live weight, adiposity score, and ovulation rate at embryo transfer, and most importantly maternal live weight gain from embryo transfer to d 50 of gestation (hereafter H+E2 group). These latter ewes were injected (i.m. and into alternating leg muscles) twice daily (07:30 and 17:30h) with E2 (0.05 mg estradiol- 17β/ml arachis oil on d 50 to 90 of pregnancy inclusive. Estradiol-17ß was from Sigma (E-8875) and arachis oil from JM Loveridge Ltd. purchased via Dunlops Veterinary Supplies, Dumfries, UK. A stock solution of 2 mg E2 per/mL arachis oil was prepared by heating to 50º C with stirring for 8h until the oil was clear and the E2 completely dissolved. Both stock and using solutions were stored at room temperature and in the dark. The concentration of E2 per injection was determined empirically based on earlier trials in non-pregnant nutritionally manipulated adolescents studied during the anestrus period. The aim was to achieve peripheral E2 concentrations similar to those measured previously in control fed pregnant adolescents. Ewes in the CON and HI groups were temporarily restrained twice daily (but not sham injected) between d 50 and 90 of pregnancy. Blood samples were collected from all ewes at d 25 and 50, and then at 5 d intervals during the period of E2 supplementation followed by ~ 10 d intervals thereafter until

necropsy on d 130 of pregnancy. Maternal blood was sampled at ~ 14:00h (i.e. 6.5h after the morning E2 injection where appropriate) from the jugular vein into a lithium heparinised tube for immediate plasma harvest following centrifugation. Plasma was stored at -20oC until estradiol-17β concentrations were determined in duplicate by radioimmunoassay as described previously (Johnson et al. 1997). The sensitivity of the assay was 1pg estradiol-17β/ml and the inter and intra-assay coefficients of variation were 6.2 and 5.5%, respectively.

Necropsy and tissue harvesting procedures

On d 130 of pregnancy, one hour before necropsy, ewes were weighed. Ewes were then euthanized by administration of an overdose of sodium pentobarbitone i.v. (20 mL of Euthesate; 200 mg of pentobarbitone/mL) and were exsanguinated by severing the main blood vessels of the neck. Maternal blood was collected in a plastic container and weighed. The gravid uterus was quickly removed, dissected from the vagina at the cervix, weighed, and opened. Fetuses were euthanized by immediate intracardiac administration of a sodium pentobarbitone overdose (5 mL of Euthesate, Willows Francis Veterinary).

Fetal Intestinal Tissue Collection. The fetal liver, spleen, and pancreas were dissected out of the visceral tissues and weighed. The stomach complex was separated from the esophagus at the cardia and from the intestine at the pyloric valve. At a site 3 mesenteric vein branches distal from the mesenteric-ileocecal vein junction, a 100-cm measurement was made caudally down the small intestine, where at both the cranial and caudal demarcations the intestine was excised by following up the mesenteric arcade to the point of intestinal intersection. A 10-cm sample was collected from the cranial end of the fetal jejunum and then fixed for quantification of cellular proliferation. The remaining portion was immediately used for vascular perfusion as described by Neville et al., (2010) minus the inclusion of a casting resin. The stomach complex, remaining small intestine, and

large intestine were dissected, gently stripped of fat and digesta, and weighed. Subsamples of fetal small intestinal tissues (8 to 10 cm) were collected to be either frozen or immersion fixed. Frozen samples were wrapped in foil, snap-frozen in supercooled isopentane (submerged in liquid N), and stored at −80°C, as described previously (Caton et al., 2009a) and were analyzed for relative mRNA expression of several factors involved in the regulation of angiogenesis and tissue growth. Small intestinal tissue was fixed by vascular perfusion followed by immersion with Carnoy's fixative (60% ethanol, 30% chloroform, and 10% glacial acetic acid), embedded in paraffin, and then used for immunohistochemistry followed by image analysis to determine cellular proliferation and vascularization (Neville et al., 2010).

Histology and Immunohistochemistry. Tissues were embedded in paraffin and 4-μm tissue sections were mounted on glass slides and prepared for staining procedures (Soto-Navarro et al., 2004). Tissue sections were stained using periodic acid-Schiff's (PAS) to visualize the blood vessels and counterstained with hematoxylin (Borowicz et al., 2007). The following parameters in fetal small intestinal tissue were determined: mean capillary area, capillary number, and capillary circumference measurements, along with area of tissue analyzed (Reed et al., 2007; Caton et al., 2009a) using the Image-Pro Plus 5.0 analysis software (Media-Cybernetics Inc., Silver Spring, MD).

To determine small intestinal cellular proliferation, proliferating cell nuclear antigen (Ki-67) was used. Tissue sections were treated with blocking buffer consisting of PBS and 1.5% (vol/vol) normal horse serum (Vector Laboratories, Burlingame, CA) for 20 min followed by incubation with ki-67 mouse monoclonal antibody (1:100; Clone MM1; Vector Laboratories, CA). Primary antibody was detected using a secondary antibody (ImmPress Kit) and DAB substrate. Tissue sections were counterstained with hematoxylin. Then five images/tissue

sections were taken for image analysis using Image Pro Plus 5.0 analysis software (MediaCybernetics, Inc., Silver Spring, MD). Labeling index was determined as a proportion (%) of proliferating cells out of total cells/tissue area.

Quantitative Real Time – PCR Analysis. Expression of mRNA for several factors involved in the regulation of angiogenesis and tissue growth including vascular endothelial growth factor [***VEGF***], fms-related tyrosine kinase 1[***FLT1***], kinase insert domain receptor [***KDR***], angiopoietin 1 [***ANGPT1***], angiopoietin 2 [***ANGPT2***], endothelial tyrosine kinase [***TEK***], endothelial nitric oxide synthase 3 [***NOS3***], soluble guanylate cyclase [***GUCY1B3***]), estrogen receptor alpha **[*ER-α*],** hypoxia-inducible factor 1, alpha subunit [***HIF1A***]), vasoactive intestinal peptide [***VIP***], basic fibroblast growth factor 2 [***FGF2***], fibroblast growth factor receptor 2 [***FGFR2***], neuropilin 1 [***NRP1***], and neuropilin 2 [***NRP2***] and as well as, *18S* was determined in fetal small intestinal tissues (Redmer et al., 2005; Vonnahme et al., 2006; Borowicz et al., 2007;Vonnahme et al., 2008; Neville et al., 2010). Human *18S* mRNA (predeveloped assay reagent [**PDAR**]; Applied Biosystems, Foster City, CA) was added to serve as an internal control to minimize sample variation. Analyses were conducted using TaqMan reagents and procedures purchased from and recommended by Applied Biosystems (Foster City, CA).

Expression of each factor was normalized to expression of 18S in a multiplex reaction using the human 18S PDAR from Applied Biosystems. The PDAR solution, which is primer-limited and contains a VIC-labeled probe (a proprietary reporter dye; Applied Biosystems), was further adjusted by using one-fourth the normal amount so that it would not interfere with amplification of the FAM (6-carboxy-fluorescein)-labeled gene of interest. The multiplex reaction, similar to previous study (Neville et al., 2010), was also used to prepare standard curves

for 18S and the gene of interest based on dilutions of cDNA obtained from reverse transcription of RNA obtained from pooled late-pregnancy sheep placentome tissues.

Calculations

Fetal organ weights obtained at slaughter are presented on a fresh organ mass basis as well as per unit of fetal BW (**FBW**). Capillary area density was determined by dividing the total capillary area (μm^2) by the area of tissue analyzed (μm^2) and multiplying by 100 to express vascularity as a percentage total tissue area (Caton et al., 2009a; Meyer et al., 2010b). Capillary number density was calculated by dividing the total number of vessels counted by tissue area in μm^2 and then multiplying by 1,000,000 to express the data as capillaries per mm^2. To estimate the capillary surface density (total capillary circumference per unit of tissue area), the mean capillary perimeter (circumference; μm) was divided by tissue area (μm^2). Although capillary surface density actually represents the circumference of the capillary cross-sections, it is nevertheless proportional to their surface area (Borowicz et al., 2007). Finally, area per capillary was determined by dividing total capillary area by capillary number resulting in area per capillary in μm^2. Total vascularity (mL) was calculated by multiplying the percentage of capillary area density by tissue mass. The percentage proliferating cells was estimated by dividing the number of Ki-67-stained nuclei by the total number (Ki-67 + hematoxylin-stained) of nuclei present within the area of tissue analyzed.

Statistical analysis

Data were initially analyzed as a completely randomized design with three treatments using the GLM proceedures of SAS (SAS Inst. Inc., Cary, NC). Specific contrasts were used to address the specific questions did HI treatments differ from controls (CON vs. HI + HI+E2) and within HI fed ewes did E2 supplementation make a difference (HI vs. HI+E2). Based on

maternal plasma E2 levels throughout gestation (Figure 2.2) and fetal BW (Figure 2.3) on d 130 of pregnancy we suspected that some ewes in HI treatments were carring IUGR offspring and some were not. Therefore, HI and HI+E group was subdivided into four subgroups (HI IUGR, HI non-IUGR, HI IUGR+E2, and HI non-IUGR+E2; Figures 2,4 and 2.5). Maternal E2 levels throughout gestation and especially between d 50 to 90, and from d 99 to 126 demonstrated five different patterns of E2 concetration; irrespective of maternal E2 supplementation IUGR pregnancies had lower levels of E2 between d 99 to 126 and on d 126. Furthermore, close assessment offetal body weight and total placentome weights further revealed IUGR and non-IUGR patterns within HI treatments. Therefore we decided to subdivide HI and HI+E2 into four subgroups. The mean ± sd fetal weight in CON was 4,435 ± 422 g. IUGR was defined as weight at least 2 standard deviations below the CON mean (< 3,591 g), which appeared in 8 of 14 HI and 5 of 12 HI+E2 pregnancies. The resulting treatments for our final analyses consisted on the following groups: include CON (n = 12), HI non-IUGR (n = 6), HI IUGR (n = 8), HI non-IUGR+E2 (n = 7), HI IUGR+E2 (n = 5). The Figure 3.1 represents the experimental design used in this study.

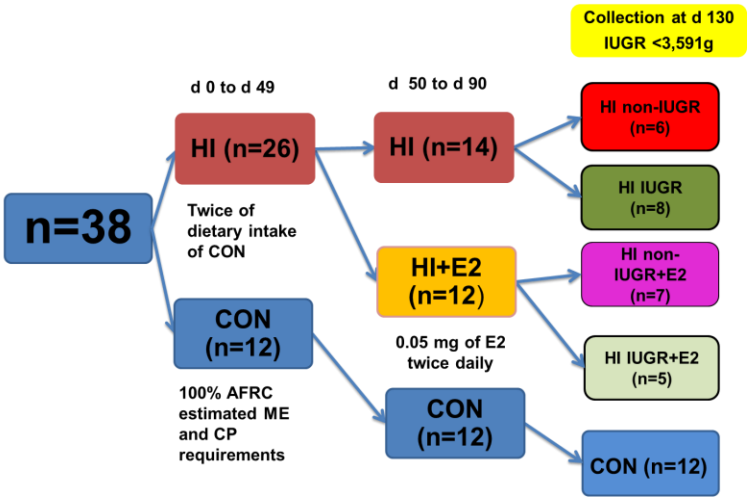

Figure 3.1. Experimental design used in this study.

The statistical analysis for this study was a completely randomized design with CON, HI non-IUGR, HI IUGR, HI non-IUGR+E2, and HI IUGR+E2 treatments in the model. The PROC GLM procedure was used for analyses (SAS Inst. Inc., Cary, NC). Ewes were individually penned with treatment imposed *in utero,* therefore individual animal served as experimental unit. The four following questions were addressed when structuring contrasts: 1) was there an effect of elevated maternal nutritional plane (CON vs. HI [mean value of HI non-IUGR, HI IUGR, HI non-IUGR+E2, HI IUGR+E2]), 2) was there an effect of maternal E2 supplementation (mean value of HI non-IUGR + HI IUGR vs. mean value of HI non-IUGR+E2 + HI IUGR+E2), 3) was there an effect of IUGR (mean value of HI IUGR and HI IUGR+E2 vs. mean value of HI non-IUGR and HI non-IUGR+E2), and 4) within IUGR groups was there an effect of maternal E2 least squares means (LSM) and standard errors (SEM) are presented for all data. Main effects were discussed if $P \leq 0.10$.

Results

Out of 53 recipient ewes, 42 ewes were confirmed pregnant after embryo transfer. However, out of 42 pregnant ewes two recipients from HI+E2 fed ewes had aborted pregnancies, one from CON resulted with autolysed fetus, and another one from CON had abnormal placenta. Therefore, data was collected from 38 remaining pregnant ewes.

As reported in Chapter II, elevated maternal nutrition resulted in decreased ($P < 0.001$) plasma E2 levels (Figure 3.2) and was accompanied with reduced ($P \leq 0.02$) fetal body weight (FBW), placetnome weight, and placental efficiency compared to CON (Chapter II; Table 2.2). Our attempt to increase maternal plasma E2 in HI group did not restore reduced FBW, placentome weight, and placental efficiency observed in HI group. Perhaps, because E2 supplementation on d 50 to 90 of gestation resulted in concentration of plasma E2 that was three

times greater ($P < 0.001$) in HI+E2 than in HI and which was also significantly above physiological levels of plasma E2 concentration in CON pregnancies (Chapter II; Table 2.3).

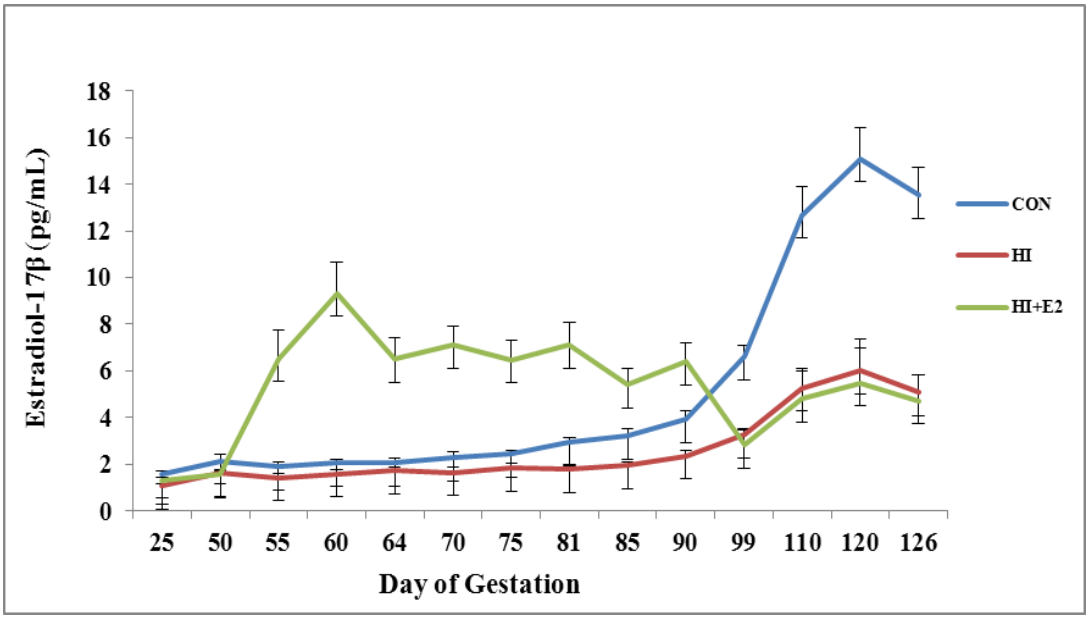

Figure 3.2. Maternal circulating plasma estradiol-17β concentrations throughout gestation in relation to maternal nutrition and E2 supplementation from d 50 to 90 of pregnancy.

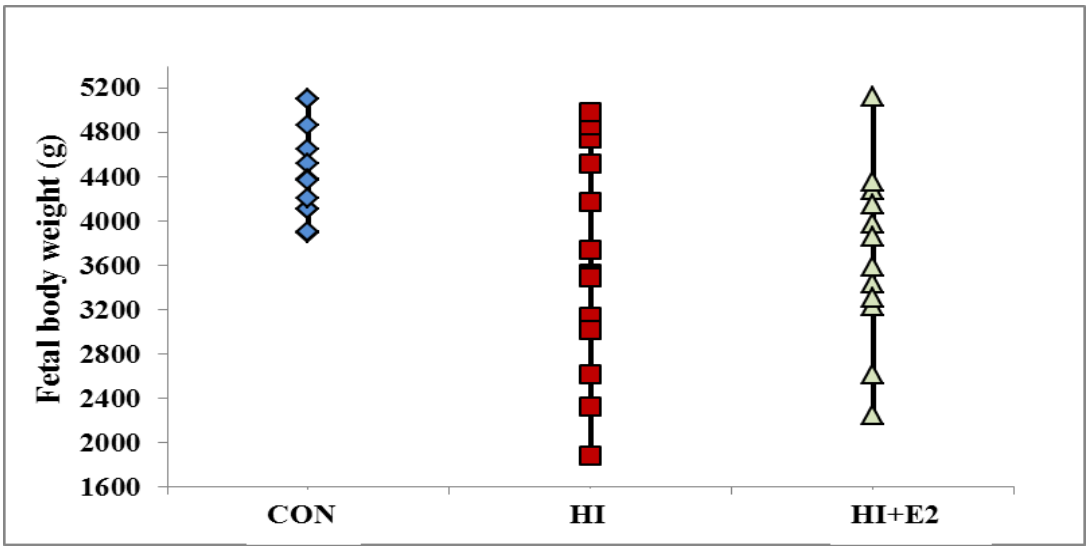

Figure 3.3. Individual fetal body weights at d 130 of gestation in relation to maternal nutrition and estrogen supplementation.

As shown in Figure 3.3, both HI and HI+E2 fed ewes resulted in offspring that weighed significnaly less ($P = 0.01$) than fetuses from CON, however, there were also fetuses that

appeared to have similar body weights as CON fetuses. Therefore, those fetuses that weighed two standard deviation below the means of CON (i.e. < 3591g) were defined as IUGR resulting in five subgroups including CON, HI non-IUGR, HI IUGR, HI non-IUGR+E2, and HI IUGR+E2 (Figure 3.4).

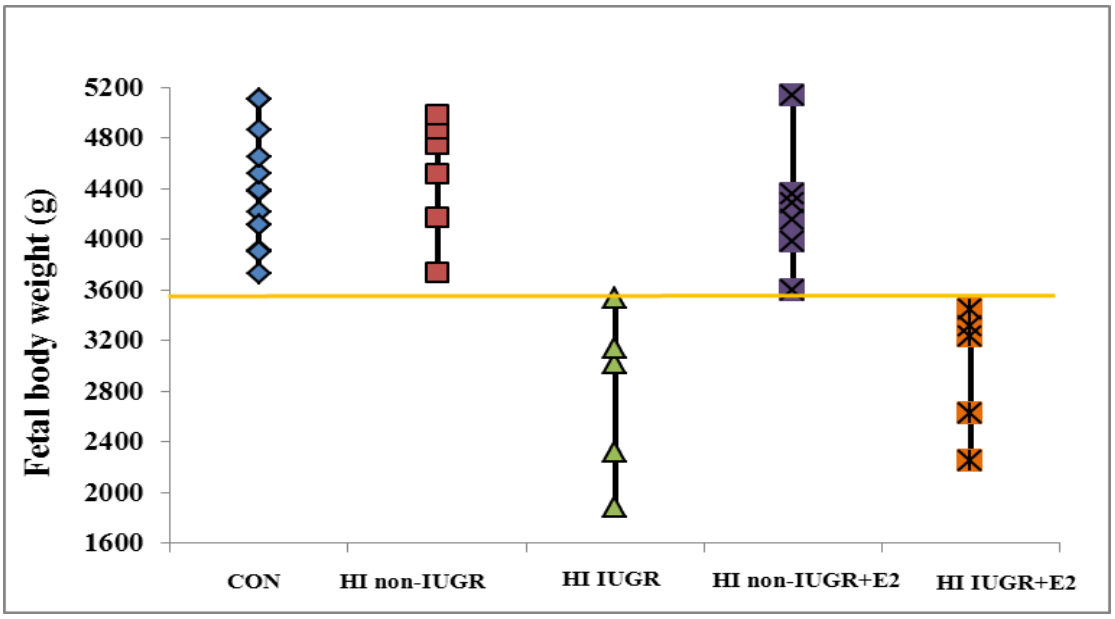

Figure 3.4. Individual fetal body weights at d 130 of gestation in relation to maternal nutrition, estrogen supplementation, and intrauterine growth restriction (IUGR) status. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g) and is indicated by the horizontal yellow line.

In adition, maternal plasma E2 concetration when observed across the five groups (CON, HI non-IUGR, HI IUGR, HI non-IUGR+E2, and HI IUGR+E2) reflect that ewes whose fetuses were IUGR (HI IUGR, and HI IUGR+E2) had lower ($P < 0.001$) plasma E2 levels irrespective of maternal E2 supplementation between d 99 to 126 compared with CON and HI non-IUGR, and HI non-IUGR+E2 (Figure 3.5). In fact, maternal circulating plasma E2 with HI IUGR fetuses (HI IUGR and HI IUGR+E2) was half ($P < 0.0001$) of the E2 concetration in ewes from HI non-IUGR (HI non-IUGR and HI non-IUGR+E2), and aproximately four times less ($P < 0.0001$) than in CON ewes on d 99 to 126 of gestation (Chapter II; Table 2.3).These results

further validated our decision to subdivide CON, HI, and HI+E groups into CON, HI non-IUGR, HI IUGR, HI non-IUGR+E2, and HI IUGR+E2.

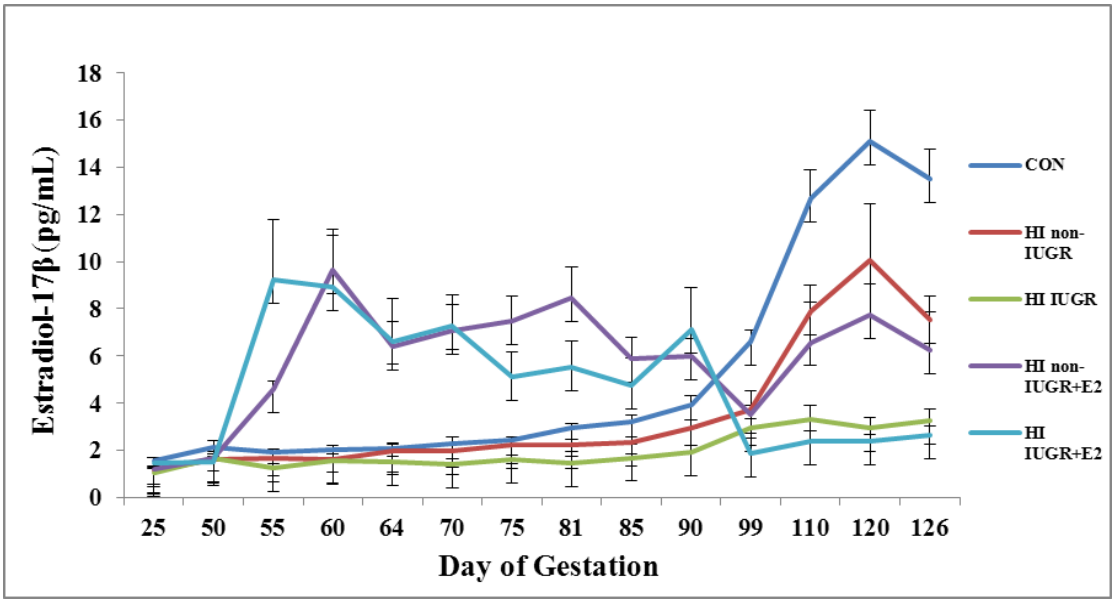

Figure 3.5. Maternal circulating plasma estradiol-17β concentrations throughout gestation in relation to maternal nutrition, E2 supplementation and IUGR status. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g) at necropsy on d 130 of gestation.

In fetuses from HI groups (which includes HI non-IUGR, HI IUGR, HI non-IUGR+E2, and HI IUGR+E2), fetal BW (FBW), empty BW (FBW), and girth (4,435 vs. 3,653 ± 203.2 g; 3,025 vs. 2,474 ± 157 g; and 35.1 vs. 32.5 ± 1.25 g, respectively) were less than CON. In addition, total fetal gastrointestinal (GI) tract, stomach complex, small intestine, large intestine, and liver weights (g) were less ($P \leq 0.03$; Table 3.1) in fetuses from HI compared with CON group. Fetuses from IUGR had reduced ($P \leq 0.01$) internal organ weights including stomach complex, total small intestinal mass, total large intestine, pancreas, and liver weights expressed in (g) compared to non-IUGR. Interestingly, when expressed in g/kg of FBW only total large intestine and liver were reduced ($P \leq 0.10$) due to IUGR on d 130 of gestation.

Table 3.1. Fetal body weight (FBW) and gastrointestinal (GI) organ mass in relation to maternal nutrition, E2 supplementation and IUGR status on d 130 of gestation. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g) at necropsy on d 130 of gestation.

Item	Treatments[1]						Contrast[2]			
	CON	HI non-IUGR	HI IUGR	HI non-IUGR + E2	HI IUGR + E2	SEM[3]	CON vs. HI	IUGR vs. non-IUGR	HI vs. HI+E2	HI IUGR vs. HI IUGR + E2
Fetal BW, g	4,435	4,506	2,940	4,196	2,971	203.2	< 0.01	<.0001	0.49	0.91
Fetal EBW[4], g	3,025	3,073	2,015	2,826	1,979	157.4	<0.01	<.0001	0.32	0.86
Girth, cm	35.1	35	29.8	34.7	30.4	1.25	0.01	0.00	0.87	0.7
GIT[5]	261.5	256.6	180.1	238.9	171.2	23.4	0.01	0.00	0.53	0.77
Empty GIT, g	96.6	103.5	69.4	92.4	71	6.47	0.02	<.0001	0.42	0.85
g/kg FBW	21.8	22.9	23.6	22	24.1	1.09	0.12	0.15	0.79	0.75
Stomach complex[6], g	35.3	38.1	26.1	34.6	26.7	2.19	0.03	<.0001	0.47	0.83
g/kg FBW	7.96	8.47	8.91	8.25	8.96	0.39	0.03	0.11	0.8	0.92
Total small intestine, g	58.9	60.3	41.4	53.7	40.7	5.04	0.02	0.00	0.43	0.93
g/kg FBW	13.3	13.3	13.9	12.7	13.9	0.97	0.83	0.30	0.74	0.99
Total large intestine, g	12	13.9	9.75	12.4	9.7	0.72	0.37	<.0001	0.23	0.96
g/kg FBW	2.71	3.08	3.48	2.95	3.33	0.26	0.02	0.10	0.56	0.66
Pancreas, g	3.94	4.19	3.29	3.98	2.92	0.5	0.38	<.0001	0.52	0.57
g/kg FBW	0.9	0.94	1.16	0.94	0.95	0.13	0.34	0.33	0.36	0.21
Liver, g	142	148	83.54	146.5	91.7	10.7	0.01	<.0001	0.74	0.56
g/kg FBW	32	32.9	28.4	34.8	30.8	1.72	0.84	0.01	0.16	0.28

[1]Treatments were CON (100% estimated ME and CP requirements; n = 12), HI (approximately twice dietary intake levels as CON; n = 6), HI+E2 (approximately twice dietary intake levels as CON and maternal E2 supplementation between gestational days 50 to 90; n = 7), HI IUGR (approximately twice dietary intake levels as CON with fetal BW < 3,591 g at d 130 of gestation; n = 8), and HI IUGR+E2 (approximately twice dietary intake levels as CON and E2 with fetal BW < 3,591 g at d 130 of gestation; n = 5).

Table 3.1. (Continued). Fetal body weight (FBW) and gastrointestinal (GI) organ mass in relation to maternal nutrition, E2 supplementation and IUGR status on d 130 of gestation. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g) at necropsy on d 130 of gestation.

[2]P-values for contrast that compare CON vs. all 4 High Treatments; HI (HI non-IUGR + HI IUGR) vs. HI+E2 (HI non-IUGR+E2 + HI IUGR+E2); non-IUGR (HI Non-IUGR + HI Non-IUGR+E2) vs. IUGR (HI IUGR + HI IUGR+E2); and HI IUGR vs. HI IUGR+E2.

[3]Standard error of the mean, most conservative (highest) used.

[4] Empty BW was defined as eviscerated fetal BW.

[5] GIT (gastrointestinal tract) = large intestine + small intestine + stomach, including digesta and fluids.

[6] Stomach complex = reticulum + rumen + omasum + abomasum, minus digesta.

Table 3.2. Fetal internal organ mass in relation to maternal nutrition, E2 supplementation and IUGR status on d 130 of gestation. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g) at necropsy on d 130 of gestation.

Item	Treatments[1]					SEM[3]	Contrast[2]			
	CON	HI non-IUGR	HI IUGR	HI non-IUGR + E2	HI IUGR + E2		CON vs. HI	IUGR vs. non-IUGR	HI vs. HI+E2	HI IUGR vs. HI IUGR + E2
Brain, g	41.9	42.35	34.6	40.3	37.2	2.23	0.07	0.01	0.89	0.37
g/kg FBW	9.52	9.5	11.6	9.66	12.8	0.67	0.01	0.00	0.26	0.17
Perirenal fat, g	25.1	22.4	18.1	24.4	15.9	1.76	0.001	0.00	0.93	0.32
g/kg FBW	5.68	4.93	6.2	5.79	5.53	0.41	0.84	0.17	0.8	0.21
Kidney, g	23.3	25.7	17.9	25.8	16.7	1.64	0.17	0.00	0.69	0.57
g/kg FBW	5.26	5.73	6.42	6.11	5.65	0.58	0.12	0.82	0.71	0.31
Spleen, g	9.11	9.68	5.54	9.21	5.43	0.84	0.02	0.00	0.7	0.92
g/kg FBW	2.07	2.16	1.87	2.21	1.79	0.2	0.69	0.05	0.93	0.75
Lung, g	138	149	105	135	101	12.3	0.116	0.00	0.43	0.81
g/kg FBW	31.2	33.1	35	32.1	33.8	2.51	0.26	0.43	0.64	0.71
Heart, g	44	46.5	27.5	40.2	29.6	3.99	0.02	0.00	0.55	0.69
g/kg FBW	10	10.5	9.49	9.57	10	1.05	0.88	0.78	0.85	0.69

[1]Treatments were CON (100% estimated ME and CP requirements; n = 12), HI (approximately twice dietary intake levels as CON; n = 6), HI+E2 (approximately twice dietary intake levels as CON and maternal E2 supplementation between gestational days 50 to 90; n=7), HI IUGR (approximately twice dietary intake levels as CON with fetal BW < 3,591 g at d 130 of gestation; n = 8), and HI IUGR+E2 (approximately twice dietary intake levels as CON and E2 with fetal BW < 3,591 g at d 130 of gestation; n = 5).

[2]P-values for contrast that compare CON vs. all 4 High Treatments; HI (HI non-IUGR + HI IUGR) vs. HI+E2 (HI non-IUGR+E2 + HI IUGR+E2); non-IUGR (HI Non-IUGR + HI Non-IUGR+E2) vs. IUGR (HI IUGR + HI IUGR+E2); and HI IUGR vs. HI IUGR+E2.

[3]Standard error of the mean, most conservative used.

Additionally, fetal large intestinal proportional mass (g/kg FBW) was greater ($P = 0.02$) in offspring from HI compared with CON (3.08 vs. 2.71 ± 0.26 g/kg, respectively). In the IUGR fetuses, large intestinal proportional mass was greater compared with non-IUGR (3.40 vs. 3.01 ± 0.26 g/kg, respectively). However, the proportional masses of remaining fetal visceral organs (Table 3.1) were similar between CON and HI treatments. Fetal BW and visceral tissue organ masses were less ($P \leq 0.06$) in IUGR than in non-IUGR fetuses (Tables 3.1 and 3.2), except for proportional masses (g/kg FBW) of the small intestine and the pancreas. Fetal BW and visceral tissue weights were not altered by maternal supplementation of E2 in either IUGR or non-IUGR treatments.

In fetuses from ewes fed HI treatments, brain, perirenal fat, spleen, and heart weights (g) were less, although perirenal fat, spleen, and heart proportional masses (g/kg FBW) were similar ($P \geq 0.7$) to CON. Only fetal brain proportional mass (g/kg FBW) was greater ($P \leq 0.01$) in HI than CON (Table 3.2). Fetuses from IUGR pregnancies demonstrated a more pronounced divergence from normal than those from non-IUGR pregnancies ($P \leq 0.05$). In IUGR fetuses, brain, perirenal fat, kidney, spleen, lung, and heart absolute weights were less ($P \leq 0.01$) compared with non-IUGR. In addition, proportional mass of spleen (g/kg FBW) was lower ($P \leq 0.05$) in IUGR compared with non-IUGR fetuses. Interestingly, the proportional mass (g/kg FBW) of IUGR fetal brain was greater ($P \leq 0.001$) than in non-IUGR fetuses (Table 3.2). There were no E2 effects ($P \geq 0.17$) on internal organ masses in HI or IUGR fetuses compared with CON (Table 3.2).

However, E2 supplementation in ewes carrying IUGR fetuses restored ($P = 0.02$) fetal total small intestinal length (Figure 3.6).

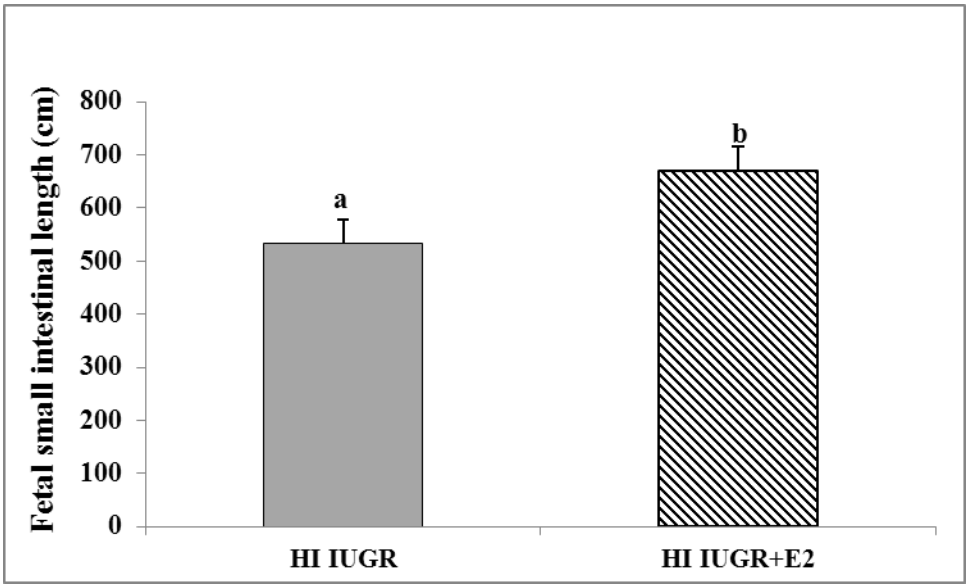

Figure 3.6. Fetal small intestnial length in HI IUGR vs. HI IUGR+E2 on d 130 of pregnancy. [a,b]$P < 0.02$

Small intestinal crypt cell proliferation was similar among all treatments (Table 3.3). Total fetal small intestinal vascular measurements were not altered ($P \geq 0.13$) by plane of maternal nutrition. However, in the IUGR fetuses total small intestinal vascularity (11.5 vs. 16.8 ± 2.24 mL) was less ($P = 0.01$) than in non-IUGR group (Table 3.3). In addition, small intestinal APC was less ($P = 0.09$) in IUGR fetuses compared with non-IUGR (235 vs. 287 ± 34, respectively; Table 3.3). However, within IUGR groups, E2 supplementation resulted in decreased ($P = 0.09$) capillary surface density (Figure 3.7) and reduced ($P = 0.08$) capillary area density (Figure 3.8) in the intestinal villi. There were no other treatment effects ($P \geq 0.11$) on fetal intestinal vascularity (Table 3.3).

Table 3.3. Fetal small intestinal vascularity in relation to maternal nutrition, E2 supplementation and IUGR status on d 130 of gestation. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g) at necropsy on d 130 of gestation.

Item	Treatments[1]						Contrast[2]			
	CON	HI non-IUGR	HI IUGR	HI non-IUGR + E2	HI IUGR + E2	SEM[3]	CON vs. HI	IUGR vs. non-IUGR	HI vs. HI+ E2	HI IUGR vs. HI IUGR + E2
Jejunal Cell Proliferation										
Proliferating nuclei, %	50.1	44.7	46.5	52.5	47.5	6.72	0.67	0.79	0.47	0.91
Jejunal Vascularity										
Capillary area density, %	28.3	29.7	30.9	28.9	24.6	2.76	0.93	0.52	0.16	0.08
Capillary number density, mm^2	1,278	1,187	1,321	1,196	1,205	111.9	0.56	0.48	0.6	0.42
Capillary surface density, ($\mu m/\mu m^2$) x 10	1.11	1.13	1.15	1.08	0.98	0.08	0.77	0.60	0.11	0.09
Area per capillary, μm^2	248.7	300.7	247.3	273.8	222.6	34.06	0.65	0.09	0.4	0.57
Total jejunal vascularity, mL	16.8	17.6	13.1	15.9	9.82	2.237	0.13	0.01	0.23	0.27

[1]Treatments were CON (100% estimated ME and CP requirements; n = 12), HI (approximately twice dietary intake levels as CON; n = 6), HI+E2 (approximately twice dietary intake levels as CON and maternal E2 supplementation between gestational days 50 to 90; n = 7), HI IUGR (approximately twice dietary intake levels as CON with fetal BW < 3,591 g at d 130 of gestation; n = 8), and HI IUGR+E2 (approximately twice dietary intake levels as CON and E2 with fetal BW < 3,591 g at d 130 of gestation; n = 5).

[2]P-values for contrast that compare CON vs. all 4 High Treatments; HI (HI non-IUGR + HI IUGR) vs. HI+E2 (HI non-IUGR+E2 + HI IUGR+E2); non-IUGR (HI Non-IUGR + HI Non-IUGR+E2) vs. IUGR (HI IUGR + HI IUGR+E2); and HI IUGR vs. HI IUGR+E2.

[3]Standard error of the means, most conservative used.

[4]Capillary area density = (capillary area/tissue area evaluated) x 100.

[5]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[6]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[7]Area per capillary = capillary area/capillary number per sample area.

[8]Total jejunal vascularity = capillary area density (%) x jejunal mass (g).

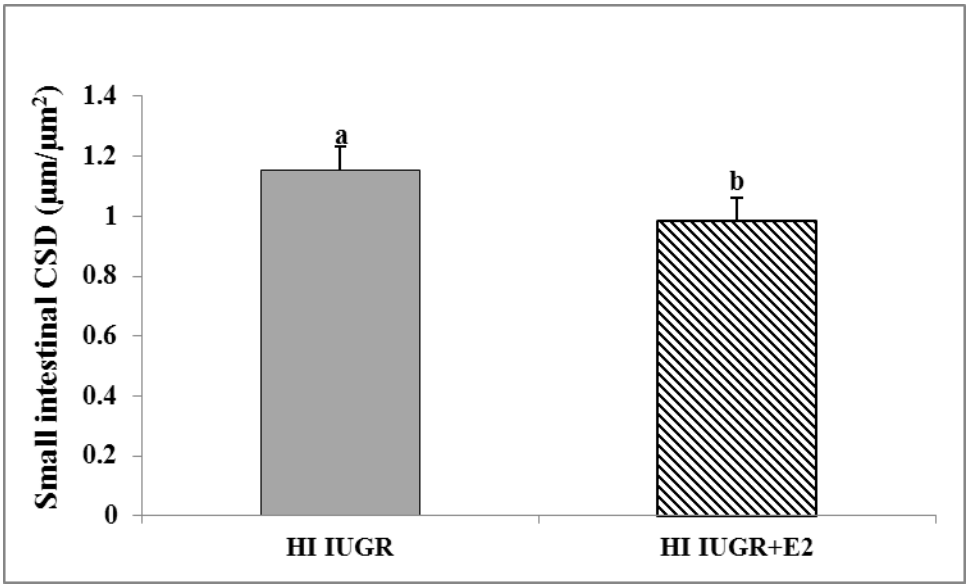

Figure 3.7. Fetal small intestinal CSD in HI IUGR vs. HI IUGR+E2 on d 130 of pregnancy. [a,b]*P* < 0.09

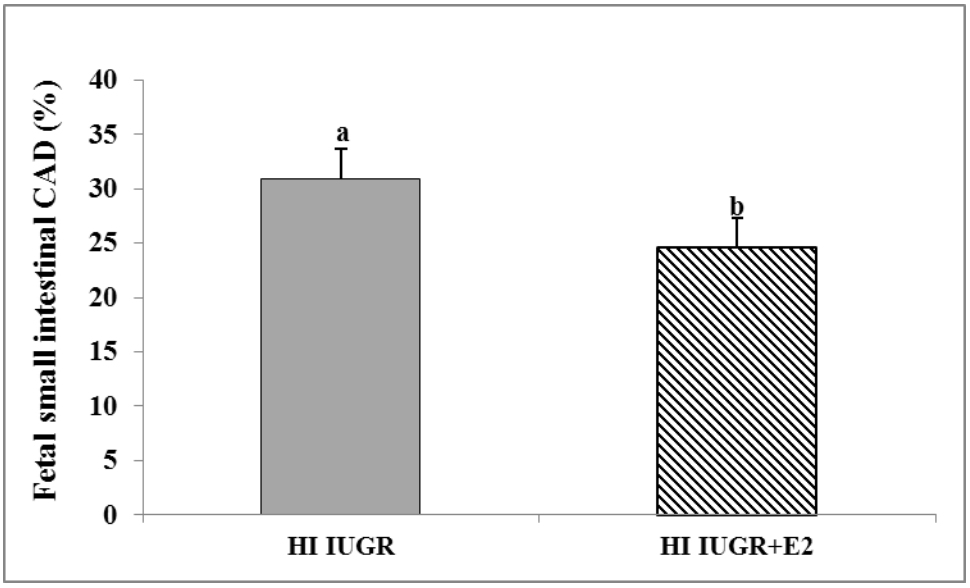

Figure 3.8. Fetal small intestnial CAD in HI IUGR vs. HI IUGR+E2 on d 130 of pregnancy. [a,b]*P* < 0.08.

Table 3.4. Fetal small intestinal mRNA gene expression in relation to maternal nutrition, E2 supplementation and IUGR status on d 130 of gestation. The latter was defined on the basis of a minus two standard deviation cutoff below the control group mean fetal weight (i.e. < 3591g) at necropsy on d 130 of gestation.

Item	Treatments[1]					SEM[3]	Contrast[2]			
	CON	HI non-IUGR	HI IUGR	HI non-IUGR + E2	HI IUGR + E2		CON vs. HI	IUGR vs. non-IUGR	HI vs. HI+E2	HI IUGR vs. HI IUGR + E2
VEGF	0.266	0.223	0.268	0.254	0.273	0.02	0.49	0.08	0.32	0.85
FLT1	0.033	0.036	0.036	0.031	0.037	0.003	0.42	0.31	0.61	0.74
KDR	0.073	0.089	0.084	0.087	0.08	0.011	0.18	0.55	0.74	0.73
NRP1	0.112	0.148	0.103	0.124	0.116	0.036	0.7	0.41	0.86	0.78
NRP2	2.871	2.729	2.554	2.973	3.128	0.369	0.93	0.98	0.22	0.23
ANGPT1	0.011	0.009	0.008	0.01	0.008	0.002	0.11	0.55	0.84	0.99
ANGPT2	0.086	0.121	0.095	0.109	0.117	0.024	0.21	0.67	0.81	0.47
TEK	1.181	1.234	1.226	1.111	1.337	0.129	0.65	0.35	0.96	0.51
FGF2	0.166	0.194	0.19	0.165	0.213	0.031	0.32	0.42	0.93	0.56
FGFR2	0.124	0.123	0.126	0.108	0.13	0.016	0.83	0.40	0.71	0.84
NOS3	0.697	0.522	0.585	0.547	0.582	0.109	0.12	0.62	0.91	0.99
HIF1A	0.184	0.174	0.181	0.161	0.167	0.016	0.31	0.63	0.35	0.49
VIP	0.102	0.105	0.093	0.077	0.121	0.016	0.8	0.29	0.99	0.19
GUCY1B3	0.202	0.156	0.128	0.145	0.275	0.038	0.38	0.14	0.05	0.004
ER-α	0.01	0.008	0.007	0.008	0.006	0.001	0.02	0.68	0.18	0.24

[1]Treatments were CON (100% estimated ME and CP requirements; n = 12), HI (approximately twice dietary intake levels as CON; n = 6), HI+E2 (approximately twice dietary intake levels as CON and maternal E2 supplementation between gestational days 50 to 90; n = 7), HI IUGR (approximately twice dietary intake levels as CON with fetal BW < 3,591 g at d 130 of gestation; n = 8), and HI IUGR+E2 (approximately twice dietary intake levels as CON and E2 with fetal BW < 3,591 g at d 130 of gestation; n = 5).

[2]P-values for contrast that compare CON vs. all 4 High Treatments; HI (HI non-IUGR + HI IUGR) vs. HI+E2 (HI non-IUGR+E2 + HI IUGR+E2); non-IUGR (HI Non-IUGR + HI Non-IUGR+E2) vs. IUGR (HI IUGR + HI IUGR+E2); and HI IUGR vs. HI IUGR+E2.

[3]Standard error of the mean, most conservative used.

Fetal small intestinal mRNA expression of factors involved in the regulation of angiogenesis and tissue growth including *FLT1*, *KDR*, *ANGPT1*, *ANGPT2*, *TEK*, *NOS3*, *HIF1A*, *VIP*, *FGF2*, *FGFR2*, *NRP1*, and *NRP2* were not altered ($P \geq 0.16$) by treatments (Table 3.4). Although, according to Pearson correlation test, *KDR* in CON and *HIF1A* both in all HI treated groups and HI IUGR were correlated (correlation coefficient = 0.77 and 0.95; $P = 0.003$ and 0.001, respectively) with small intestinal crypt proliferation (Appendix A, Tables A58, A59, and A61).

Small intestinal *VEGF* mRNA expression in the IUGR fetuses was greater ($P = 0.08$) compared with non-IUGR group (0.27 vs. 0.12 ± 0.02, respectively). In addition, based on Pearson correlation test, *VEGF* mRNA expression was negatively correlated (R = -0.92; $P = 0.009$) to small intestinal crypt proliferation in HI non-IUGR group (Appendix A, Table A63). E2 supplementation within HI IUGR groups increased ($P = 0.04$) small intestinal *GUCY1B3* relative mRNA expression (Figure 3.9). Interestingly, within HI non-IUGR E2 supplementation decreased ($P = 0.05$) small intestinal *GUCY1B3* mRNA expression.

Moreover, in fetuses from HI group, small intestinal *ERα* mRNA expression was less ($P = 0.02$) compared with CON (Figure 3.10). In HI non-IUGR group, fetal small intestinal *ERα* mRNA expression was correlated (R = 0.85; $P = 0.03$) with total small intestinal vascularity (Appendix, Table A63).

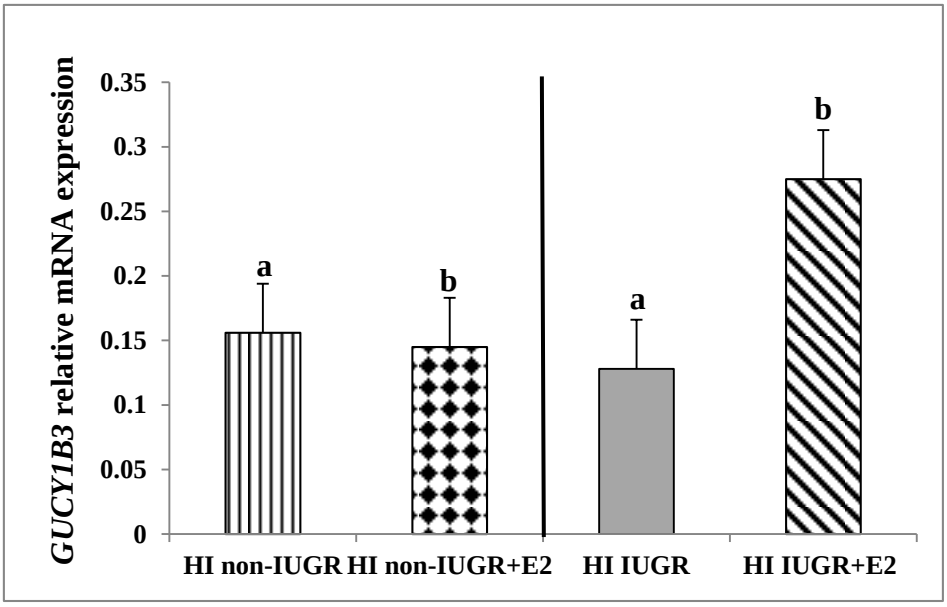

Figure 3.9. Fetal small intestnial *GUCY1B3* mRNA expression in HI non-IUGR vs. HI non-IUGR+E2 ($P = 0.05$), HI IUGR vs. HI IUGR+E2 ($P = 0.004$) on d 130 of pregnancy. $^{a,b}P \leq 0.05$.

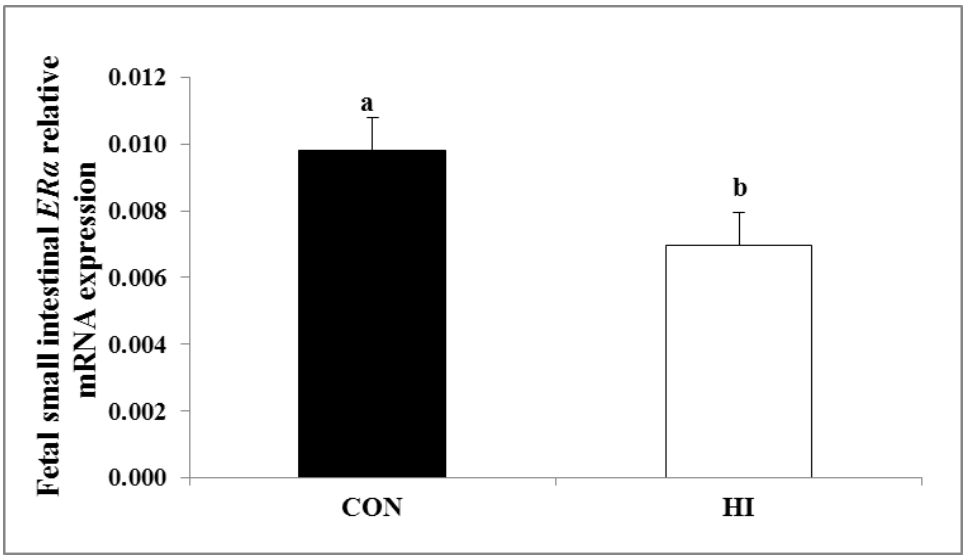

Figure 3.10. Fetal small intestinal *ERα* mRNA expression in CON and HI (HI IUGR + HI non-IUGR + HI IUGR+E2 + HI non-IUGR+E2) on 130 d of pregnancy. $^{a,b}P < 0.02$.

Discussion

Insufficient placental development results in low birth weight offspring. These IUGR offspring are at high risk of developing an array of health related complications not only at birth but also into adulthood (Arnon et al., 2001; Adams-Chapman, 2006; Scholtz and Philips, 2009;

Reynolds et al. 2005a,b, 2006; Wu et al., 2006). One of the IUGR pregnancy complications is an improper and immature gut development. As reported by Trahair (1997) interruptions in normal fetal gastrointestinal tract growth occurs in compromised offspring before any other fetal visceral organs develop. In addition, IUGR in piglets precipitates the development of necrotizing enterocolitis (Thornbury et al., 1993). Moreover, protein absorption capacity is hindered in IUGR fetuses due to immature or undeveloped GI tract which normally develops closer to parturition (Sanglid et al., 1997). These GI tract immaturities and functional impairments prevent fetuses and neonates from getting optimal nutrition for postnatal growth.

In ruminants, the liver and gut consume approximately 40% of maintenance energy (Huntington et al., 1990; Reynolds et al., 1991). The gastrointestinal tract is a primary site of nutrient absorption accompanied with high cellular turnover rate and metabolic activity (Meyer et al., 2012). Several studies confirmed that maternal GI tract changes in response to maternal nutrient intake (Reed et al., 2007; Carlson et al., 2009) and the stage of gestation (Caton et al., 2009a; Meyer et al., 2010b; Schaeffer et al 2003) in sheep and cattle. Moreover, maternal gut tissues are also responsive to changes in diet and physiological state resulting in altered tissue mass and cellularity (Scheaffer et al., 2003, 2004b), vascularization (Reed et al., 2007), and expression of angiogenic factors (Neville et al., 2010). However, fetal GI tract response to maternal diet, age, and other environmental factors is not well understood. Specifically, the developmental programming effects of elevated maternal nutrition and IUGR pregnancies with maternal supplementation of E2 (in attempt to rescue IUGR pregnancies) on fetal GI tract has not been previously well characterized.

Although, initially our study included three treatment groups CON, HI, and HI+E2, HI and HI+E2 groups were further subdivided into four subgroups including HI IUGR, HI non-

IUGR, HI IUGR+E2, and HI non-IUGR+E2. The reason for subdividing three groups into five subgroups is that in original HI and HI+E2 groups there were fetuses that were significantly small (2,251 g) and relatively large (4,985g) similar to CON fetuses. This introduces a wide range of fetal body weights within one subgroup. Therefore, fetuses that weighed less than 3,951 g (which is two standard deviation below the means of CON) were designated as IUGR. In addition, maternal plasma E2 concentration was reduced in those mothers carrying IUGR fetuses compared with non-IUGR irrespective of E2 supplementation as demonstrated in Figure 3.5. Thus, this subdivision provides clear separation between IUGR and non-IUGR fetuses and allows us a better testing of the maternal E2 supplementation effects in IUGR pregnancies.

Further work in determining the status of circulating E2 plasma levels in fetuses will be a better approach in determining if an increase in maternal E2 levels is accompanied with an increase in plasma E2 concentrations in fetuses, and if these changes are affected by IUGR pregnancies. However, based on Figure 3.2, perhaps supplementing E2 in lower doses from d 50 to 90 and gradually increasing the dose by d 90 and 126 similar to CON E2 levels may have a better outcome in rescuing IUGR pregnancies. Unquestionably, increasing the dose of E2 supplementation on d 90 to 126 can also increase the risk of abortion, therefore, further work in optimizing dose and timing of E2 is needed. Further discussions in this paper will be based on these five subgroups (CON, HI IUGR, HI non-IUGR, HI IUGR+E2, and HI non-IUGR+E2).

Fetal Visceral Organ Mass. In this study, as expected, elevated maternal nutrition decreased fetal total GI tract and other visceral organ masses including liver weights. These observations are in agreement with previous studies (Wallace et al., 2006b; Caton et al., 2009a; Yunusova et al., 2012). On the other hand, undernutrition of pregnant ewes resulted in IUGR pregnancies leading to reduced offspring liver and total GI tract masses (Vonnahme et al., 2003;

Trahair et al., 1997). The liver is important for metabolism of energy substrates (Widdowson, 1971); therefore, in our study reduced liver weights in fetuses from HI fed ewes may indicate reduced functional capacity. Moreover, reduced liver weights can potentially cause complications related to glucose and lipid homeostasis. However, additional work is needed to determine if reduced liver weights alter fetal glucose and lipid homeostasis in this particular model. In addition, fetal proportional large intestinal mass from HI fed ewes expressed in g/kg FBW was greater compared to CON.

Fetuses from HI group had decreased brain, perirenal fat, spleen, and heart weights (g). Although, the fetal proportional brain weight was greater in HI group compared to CON. Increased brain weights in fetuses from HI fed ewes could be due to the nutrient distribution in IUGR fetuses where the brain gets a priority in terms of nutrient demands. (Wu et al., 2006; Thorn et al., 2011).

As reported by Avila (1989), IUGR results in reduced fetal small intestinal weights in sheep. This was confirmed in our study, where IUGR fetuses had approximately 30% reduction in total small intestinal mass (g) compared to fetuses from non-IUGR and CON groups. However, fetal small intestinal proportional masses (g/kg) and pancreas weights were not affected by treatments. The reduction in fetal total small intestinal mass (g) can be expected since IUGR fetuses weigh less than non-IUGR fetuses. Moreover, supplementing mothers carrying IUGR fetuses with E2 increased small intestinal length. However, the increase in intestinal length doesn't necessarily characterize intestinal functionality. Therefore, additional work is needed to determine if increased small intestinal length is accompanied with increased formation of villi number and shape providing increased surface area for nutrient exchange. In addition, fetuses from IUGR pregnancies exhibited decreased large intestine (g/kg), spleen, and other

visceral organ masses (g). Intestinal function is highly dependent on appropriate blood flow to support nutrient exchange capacity in intestinal growth, simultaneously supplying nutrients for other visceral tissues (Yunusova et al., 2012). Therefore, we expected that maternal E2 supplementation will increase angiogenesis and vascularization of small intestine optimizing nutrient delivery to other visceral organs. However, there were no other maternal E2 supplementation effects on fetal visceral organ masses.

Fetal Small Intestinal Cell Proliferation and Vascularization. Previously, it was reported that nutrient restrictions during pregnancy resulted in increased small intestinal cell proliferation and vascularization depending on gestational stage in cows (Meyer et al., 2010b). However, in our study fetuses from IUGR had decreased total small intestinal vascularity compared to non-IUGR. It is possible that a decrease in small intestinal vascularity in IUGR fetuses restricted normal fetal small intestinal growth, thus, resulting in decreased total small intestinal mass (g) observed in IUGR fetuses vs. non-IUGR. Interestingly, total small intestinal vascularity was positively correlated with *ERα* mRNA expression in non-IUGR group (Appendix A, Table A63). It is possible that the decrease in ER expression partially caused the reduction in fetal small intestinal vascularity. Although, cell proliferation was not affected by treatments, fetal small intestinal crypt proliferation in HI non-IUGR groups was negatively correlated with *VEGF* and *ANGPT2* mRNA expression. Besides, the attempt to increase cell proliferation and vascularization in both HI and IUGR fetuses by maternal E2 supplementation did not succeed. However, since ERß in the presence of E2 decreases cell proliferation and increases cell apoptosis in intestinal epithelium (Zhao et al., 2010; Schleipen et al., 2011), it is possible that in IUGR pregnancies, especially during crypt and villi formation and maturation, E2 activated ERß pathway therefore leading to decreased proliferation and vascularization. In fact, the decrease in

intestinal cell proliferation and vascularization in IUGR fetuses coincides with decreased total small intestinal mass. Possible explanation for decreased total small intestinal mass is that, at some point during critical developmental windows in fetal gastrointestinal formation and maturation, IUGR and E2 via ERß activation adversely affected the fetal GI outcome. However, by d 130 it is possible that due to various compensating mechanisms and physiological adaptations, cell proliferation and vascularization became similar across all treatments. Interestingly, within IUGR offspring E2 supplementation caused a decrease of CAD and CSD. It is most likely, that the dose of E2 was high compared to physiological E2 levels in maternal serum during gestation, therefore, down regulating the expression of ERs (ERα and ERß).

However, this decrease in CAD and CSD was not accompanied with changes in factors (*FLT-1, KDR, ANGPT1, 2, NOS3, VIP, ERα)* that promote angiogenesis across all treatments on d 130. However, there was a weak negative correlation (R = -0.45 and – 0.38; *P* = 0.004 and 0.02, respectively) between CAD and *ANGPT2* and *FGFR* mRNA expression, and (correlation coefficient = -0.40; *P* = 0.01) between CSD and *ANGPT2* mRNA expression (Appendix A, Table A57). Since the small intestinal cell proliferation was not affected by treatments, determining cell proliferation closer to d 60 of gestation (since the fetal intestinal cell proliferation is the greatest at this stage) and vascularization approximately d 75 of gestation (the phase when lumen is open and filled with amniotic fluid and large blood vessels start forming in the deeper layers of submucosa; Trahair and Sangild, 2002) can shed additional light into developmental programming during fetal gastrointestinal growth.

Even though fetal small intestinal CAD and CSD was decreased in offspring from HI fed ewes, *GUCY1B3* mRNA expression was greater compared with HI IUGR without E2 supplementation. Possibly, because E2 activates NO system via ERα (Darblade et al., 2002),

partial induction of *GUCY1B3* mRNA (receptor for *NOS3*) was an attempt to restore the decreased vascularization in IUGR small intestine; however, additional work in determining protein expression of factors promoting angiogenesis in small intestinal tissues could enhance our understanding in this particular animal model.

Fetal Small Intestinal mRNA expression of factors involved in the regulation of angiogenesis and tissue growths. As stated above, there were no major differences in angiogenic gene expression between treatments. However, maternal E2 supplementation in HI and IUGR groups exhibited increased *GUCY1b3* mRNA expression. This angiogenic factor binds and activates NO as (Vonnahme et al., 2006). Growing evidence shows that NO is a key regulator for angiogenesis and fetal growth (Reynolds and Redmer, 2001; Zheng et al., 2006). Moreover, it was reported that elevated or restricted maternal nutrition in both ewes and pigs result in impaired NO synthesis (Wu et al., 2004a). Elevated maternal nutrition resulted in decreased *ERα* mRNA expression in fetal small intestinal tissue without the effects of maternal E2 supplementation. It is possible that either due to negative feedback mechanism of increased E2 levels in blood that decreased *ERα* mRNA expression or, perhaps, the decrease in *ERα* expression limited expected angiogenic effects of E2. It is also important to note, that different types of tissues have different splice variants of *ERs*, moreover, only specific *ERα* splice variant (ER46 isoform; Kim et al., 2008) is critical for NO system activation. Perhaps, targeting this particular isoform (ER46) mRNA and protein expression in small intestinal tissue could provide additional insight into mechanisms and pathways of E2, ERs, and NO system in promoting angiogenesis. This data is novel and represents one of the first reports on *ERα* mRNA expression in fetal ovine small intestine. While elevated maternal nutrition and IUGR altered fetal growth and development, there was limited response to maternal E2 supplementation. In order to

investigate fully the rescuing effects of maternal E2 supplementation on IUGR fetuses optimization of timing, the dose, and the length of E2 supplementation, as well as determining fetal plasma E2 levels is needed.

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CHAPTER IV. CONCLUSION AND FUTURE DIRECTIONS

Results of this study enhance our understanding of the effects of HI maternal nutrition during gestation (adolescent sheep) and IUGR on placental and fetal gastrointestinal development and growth. Moreover, this study represents one of the first attempts in rescuing IUGR pregnancies via maternal E2 supplementation during mid-gestation.

In this study, total placentome weights was decreased and fetal:placentome ratio increased in ewes fed HI plane of nutrition (Figure 2.1 from Chapter 2). Moreover, elevated maternal nutrition decreased fetal gastrointestinal weights (Table 3.1 from Chapter 3). Therefore, decreased placental growth limits nutrient delivery to a growing fetus that results in IUGR. As confirmed in our studies these IUGR offspring have decreased gastrointestinal organ masses along with altered mRNA expression of factors promoting angiogenesis (Table 3.1 and Table 3.4 from Chapter 3). Placenta and gut are both derived from embryonic endoderm and have very similar biological function in delivering nutrients for fetal growth and survival (Trahair and Sangild, 2002). Supplementing mothers with E2 in an attempt to rescue placental and fetal intestinal growth and development in IUGR offspring demonstrated limited responsiveness. Although, fetal intestinal length was increased due to maternal E2 (Figure 3.1 from Chapter 3), little is known regarding fetal intestinal morphology and efficiency in developmental programming paradigms. Moreover, the effects of specific nutrient deficiencies/excess prior to and during gestation that may alter both placental development and fetal gastrointestinal growth are not well understood. Therefore, additional work is needed to determine morphology and efficiency, protein content, and global methylation status of factors promoting angiogenesis in both placenta and fetal small intestine. In addition, future work in optimizing the dose, timing, and length of maternal E2 supplementation might enhance our understanding of the potential

mechanisms of E2 effects on IUGR pregnancies. Furthermore, supplementing Vitamin B12, choline, and folate along with E2 treatment to mothers could improve our understanding of molecular mechanisms underlying IUGR pregnancies and epigenetic manipulation via one-carbon metabolism (Figure 1.1 from Chapter 1) specifically targeting placental and fetal small intestinal tissues. In fact, high concentration of choline in utero has been shown to be evolutionary favored (Zeisel, 2006) and circulating choline concentrations in offspring at birth from nutritionally perturbed adolescent mothers appears compromised (Caton et al., unpublished data).

In conclusion, investigating developmental windows to rescue placental and gastrointestinal growth and development in utero and postanatally has protential to improve quality of life in humans and increase profitability and productivity in livestock production.

APPENDIX

Pearson Correlation coefficients for maternal circulating E2 concentrations and all other measured variables.

Table A1. Pearson correlation coefficients for maternal E2 levels and maternal organ masses in 38 animals.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
MBW, kg	**0.278**	**0.166**	**0.242**	**-0.738**	**-0.766**
P-value	0.091	0.320	0.144	<.0001	<.0001
MBW on d 50, kg	**0.159**	**0.137**	**0.156**	**-0.565**	**-0.623**
P-value	0.340	0.412	0.349	0.000	<.0001
MBW on d 90, kg	**0.338**	**0.246**	**0.313**	**-0.668**	**-0.704**
P-value	0.038	0.137	0.056	<.0001	<.0001
MBW at necropsy, kg	**0.266**	**0.195**	**0.247**	**-0.668**	**-0.706**
P-value	0.107	0.240	0.135	<.0001	<.0001
LWG on d 50, g/d	**0.307**	**0.217**	**0.281**	**-0.720**	**-0.744**
P-value	0.061	0.191	0.087	<.0001	<.0001
LWG on d 50-90, g/d	**0.451**	**0.308**	**0.410**	**-0.647**	**-0.656**
P-value	0.005	0.060	0.011	<.0001	<.0001
LWG on d 4-130, g/d	**0.327**	**0.227**	**0.298**	**-0.721**	**-0.741**
P-value	0.045	0.172	0.070	<.0001	<.0001
BCS on d 130	**0.276**	**0.152**	**0.234**	**-0.718**	**-0.732**
P-value	0.094	0.363	0.158	<.0001	<.0001
Change BCS	**0.276**	**0.152**	**0.234**	**-0.718**	**-0.732**
P-value	0.094	0.363	0.158	<.0001	<.0001
Gravid uterus, kg	**-0.176**	**0.123**	**-0.053**	**0.689**	**0.637**
P-value	0.290	0.461	0.754	<.0001	<.0001
ADG, g/d	**0.332**	**0.196**	**0.288**	**-0.775**	**-0.787**
P-value	0.041	0.238	0.080	<.0001	<.0001
Liver, g	**0.204**	**0.102**	**0.168**	**-0.739**	**-0.767**
P-value	0.219	0.543	0.313	<.0001	<.0001

Table A1. (Continued). Pearson correlation coefficients for maternal E2 levels and maternal organ masses in 38 animals.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Liver, g/kg MBW	**0.105**	**0.022**	**0.072**	**-0.631**	**-0.662**
P-value	0.532	0.898	0.668	<.0001	<.0001
Perirenal fat, g	**0.328**	**0.286**	**0.326**	**-0.683**	**-0.712**
P-value	0.044	0.082	0.046	<.0001	<.0001
Perirenal fat, g/kg FBW	**0.315**	**0.298**	**0.323**	**-0.606**	**-0.636**
P-value	0.054	0.069	0.048	<.0001	<.0001
Blood, g	**0.101**	**-0.012**	**0.055**	**-0.676**	**-0.705**
P-value	0.547	0.943	0.742	<.0001	<.0001
Blood, g/kg	**-0.263**	**-0.257**	**-0.273**	**0.158**	**0.156**
P-value	0.111	0.120	0.098	0.344	0.350

Table A2. Pearson correlation coefficients for maternal E2 levels and maternal organ masses in CON.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
MBW, kg	**0.217**	**0.365**	**0.314**	**-0.147**	**-0.416**
P-value	0.498	0.243	0.321	0.649	0.178
MBW on d 50, kg	**0.627**	**0.642**	**0.670**	**0.059**	**-0.282**
P-value	0.029	0.024	0.017	0.855	0.375
MBW on d 90, kg	**-0.101**	**0.301**	**0.146**	**-0.414**	**-0.479**
P-value	0.755	0.342	0.650	0.181	0.115
MBW at necropsy, kg	**0.450**	**0.554**	**0.534**	**0.208**	**-0.152**
P-value	0.143	0.062	0.074	0.517	0.638
LWG on d 50, g/d	**-0.253**	**-0.122**	**-0.192**	**0.218**	**0.188**
P-value	0.428	0.705	0.551	0.496	0.559
LWG on d 50-90, g/d	**0.324**	**0.450**	**0.415**	**-0.071**	**-0.358**
P-value	0.305	0.142	0.179	0.827	0.254
LWG on d 4-130, g/d	**-0.281**	**0.098**	**-0.062**	**-0.396**	**-0.432**
P-value	0.376	0.762	0.847	0.203	0.161
Gravid uterus, kg	**0.663**	**0.617**	**0.682**	**0.332**	**0.114**
P-value	0.019	0.033	0.015	0.291	0.724
ADG, g/d	**-0.417**	**-0.017**	**-0.195**	**-0.471**	**-0.467**
P-value	0.178	0.957	0.543	0.122	0.126
Liver, g	**-0.002**	**0.021**	**0.009**	**-0.187**	**-0.391**
P-value	0.994	0.948	0.979	0.560	0.209
Liver, g/kg MBW	**-0.087**	**-0.138**	**-0.124**	**-0.168**	**-0.301**
P-value	0.788	0.669	0.700	0.602	0.342
Perirenal fat, g	**0.312**	**0.060**	**0.174**	**-0.272**	**-0.287**
P-value	0.324	0.852	0.589	0.393	0.365
Perirenal fat, g/kg FBW	**0.281**	**-0.003**	**0.123**	**-0.246**	**-0.220**
P-value	0.376	0.992	0.704	0.442	0.493
Blood, g	**0.305**	**0.220**	**0.266**	**0.030**	**-0.203**
P-value	0.335	0.491	0.404	0.926	0.526

Table A2. (Continued). Pearson correlation coefficients for maternal E2 levels and maternal organ masses in CON.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Blood, g/kg	**0.225**	**0.049**	**0.128**	**0.124**	**0.014**
P-value	0.481	0.880	0.691	0.701	0.965

Table A3. Pearson correlation coefficients for maternal E2 levels and maternal organ masses in HI.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
MBW, kg	**-0.218**	**-0.149**	**-0.191**	**-0.205**	**-0.169**
P-value	0.454	0.612	0.512	0.482	0.563
MBW on d 50, kg	**-0.424**	**-0.204**	**-0.326**	**-0.140**	**-0.137**
P-value	0.131	0.484	0.255	0.634	0.640
MBW on d 90, kg	**-0.315**	**-0.190**	**-0.263**	**-0.165**	**-0.138**
P-value	0.272	0.516	0.363	0.573	0.638
MBW at necropsy, kg	**-0.091**	**0.090**	**0.008**	**0.120**	**0.141**
P-value	0.756	0.760	0.978	0.683	0.631
LWG on d 50, g/d	**-0.191**	**-0.059**	**-0.128**	**-0.118**	**-0.119**
P-value	0.512	0.841	0.663	0.687	0.686
LWG on d 50-90, g/d	**0.123**	**-0.036**	**0.042**	**-0.112**	**-0.051**
P-value	0.674	0.902	0.886	0.702	0.863
LWG on d 4-130, g/d	**0.151**	**0.261**	**0.226**	**0.197**	**0.221**
P-value	0.606	0.367	0.436	0.499	0.449
BCS on d 130	**-0.250**	**-0.235**	**-0.259**	**-0.207**	**-0.204**
P-value	0.389	0.418	0.372	0.477	0.484
Change BCS	**-0.250**	**-0.235**	**-0.259**	**-0.207**	**-0.204**
P-value	0.389	0.418	0.372	0.477	0.484
Gravid uterus, kg	**0.295**	**0.588**	**0.484**	**0.800**	**0.767**
P-value	0.307	0.027	0.080	0.001	0.001
ADG, g/d	**0.007**	**-0.026**	**-0.010**	**-0.195**	**-0.156**
P-value	0.980	0.930	0.974	0.503	0.595
Liver, g	**-0.013**	**0.143**	**0.077**	**-0.123**	**-0.112**
P-value	0.964	0.625	0.794	0.676	0.703
Liver, g/kg MBW	**0.144**	**0.318**	**0.254**	**-0.016**	**-0.029**
P-value	0.623	0.268	0.381	0.956	0.922
Perirenal fat, g	**-0.618**	**-0.221**	**-0.434**	**-0.387**	**-0.475**
P-value	0.019	0.447	0.121	0.172	0.086

Table A3. (Continued). Pearson correlation coefficients for maternal E2 levels and maternal organ masses in HI.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Perirenal fat, g/kg FBW	**-0.548**	**-0.157**	**-0.363**	**-0.314**	**-0.417**
P-value	0.043	0.592	0.202	0.274	0.138
Blood, g	**-0.565**	**-0.475**	**-0.552**	**-0.375**	**-0.374**
P-value	0.035	0.086	0.041	0.187	0.188
Blood, g/kg	**-0.458**	**-0.412**	**-0.465**	**-0.259**	**-0.294**
P-value	0.099	0.144	0.094	0.372	0.308

Table A4. Pearson correlation coefficients for maternal E2 levels and maternal organ masses in HI+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
MBW, kg	**-0.571**	**-0.323**	**-0.563**	**-0.277**	**-0.323**
P-value	0.052	0.306	0.057	0.384	0.306
MBW on d 50, kg	**-0.527**	**0.132**	**-0.295**	**0.332**	**0.358**
P-value	0.078	0.684	0.351	0.292	0.253
MBW on d 90, kg	**-0.600**	**-0.226**	**-0.532**	**0.108**	**0.074**
P-value	0.039	0.480	0.075	0.739	0.818
MBW at necropsy, kg	**-0.754**	**-0.255**	**-0.656**	**-0.037**	**-0.082**
P-value	0.005	0.424	0.021	0.909	0.799
LWG on d 50, g/d	**-0.124**	**0.226**	**0.032**	**-0.023**	**-0.044**
P-value	0.701	0.480	0.922	0.943	0.892
LWG on d 50-90, g/d	**-0.338**	**-0.367**	**-0.424**	**-0.111**	**-0.170**
P-value	0.283	0.241	0.170	0.731	0.597
LWG on d 4-130, g/d	**-0.503**	**-0.203**	**-0.456**	**-0.206**	**-0.270**
P-value	0.096	0.527	0.137	0.520	0.395
BCS on d 130	**0.339**	**0.715**	**0.618**	**-0.001**	**-0.032**
P-value	0.281	0.009	0.032	0.997	0.921
Change BCS	**0.339**	**0.715**	**0.618**	**-0.001**	**-0.032**
P-value	0.281	0.009	0.032	0.997	0.921
Gravid uterus, kg	**-0.475**	**0.208**	**-0.226**	**0.681**	**0.685**
P-value	0.118	0.516	0.481	0.015	0.014
ADG, g/d	**-0.312**	**-0.248**	**-0.345**	**-0.396**	**-0.456**
P-value	0.324	0.437	0.272	0.203	0.137
Liver, g	**-0.569**	**-0.350**	**-0.577**	**-0.538**	**-0.561**
P-value	0.054	0.265	0.049	0.071	0.058
Liver, g/kg MBW	**-0.372**	**-0.248**	**-0.389**	**-0.561**	**-0.558**
P-value	0.233	0.438	0.211	0.058	0.059
Perirenal fat, g	**-0.039**	**0.314**	**0.140**	**-0.084**	**-0.153**
P-value	0.903	0.321	0.664	0.795	0.635

Table A4. (Continued). Pearson correlation coefficients for maternal E2 levels and maternal organ masses in HI+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Perirenal fat, g/kg FBW	**0.072**	**0.431**	**0.279**	**-0.035**	**-0.099**
P-value	0.824	0.162	0.379	0.913	0.759
Blood, g	**-0.347**	**-0.346**	**-0.421**	**-0.562**	**-0.591**
P-value	0.269	0.270	0.173	0.057	0.043
Blood, g/kg	**-0.132**	**-0.269**	**-0.233**	**-0.551**	**-0.561**
P-value	0.682	0.398	0.467	0.063	0.058

Table A5. Pearson correlation coefficients for maternal E2 levels and maternal organ masses in HI IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
MBW, kg	**-0.233**	**-0.014**	**-0.124**	**0.228**	**0.264**
P-value	0.578	0.974	0.770	0.587	0.528
MBW on d 50, kg	**-0.570**	**-0.342**	**-0.464**	**-0.051**	**0.015**
P-value *P*-value	0.140	0.408	0.247	0.904	0.973
MBW on d 90, kg	**-0.297**	**-0.043**	**-0.172**	**0.183**	**0.231**
P-value	0.475	0.920	0.685	0.665	0.582
MBW at necropsy, kg	**-0.206**	**0.030**	**-0.088**	**0.332**	**0.376**
P-value	0.625	0.943	0.837	0.422	0.359
LWG on d 50, g/d	**0.100**	**0.464**	**0.289**	**0.237**	**0.300**
P-value	0.813	0.247	0.488	0.572	0.470
LWG on d 50-90, g/d	**0.452**	**0.618**	**0.549**	**0.579**	**0.563**
P-value	0.261	0.103	0.159	0.132	0.147
LWG on d 4-130, g/d	**0.323**	**0.602**	**0.474**	**0.575**	**0.603**
P-value	0.436	0.115	0.236	0.136	0.113
BCS on d 130	**-0.247**	**-0.262**	**-0.259**	**0.092**	**0.116**
P-value	0.555	0.531	0.536	0.829	0.785
Change BCS	**-0.247**	**-0.262**	**-0.259**	**0.092**	**0.116**
P-value	0.555	0.531	0.536	0.829	0.785
Gravid uterus, kg	**0.067**	**0.185**	**0.129**	**0.488**	**0.530**
P-value	0.875	0.660	0.762	0.220	0.177
ADG, g/d	**0.314**	**0.568**	**0.452**	**0.452**	**0.469**
P-value	0.449	0.142	0.261	0.261	0.241
Liver, g	**-0.133**	**0.151**	**0.010**	**-0.027**	**0.038**
P-value	0.754	0.721	0.981	0.949	0.929
Liver, g/kg MBW	**-0.007**	**0.213**	**0.104**	**-0.242**	**-0.178**
P-value	0.986	0.613	0.806	0.564	0.673
Perirenal fat, g	**-0.807**	**-0.635**	**-0.737**	**-0.678**	**-0.614**
P-value	0.015	0.091	0.037	0.064	0.105

Table A5. (Continued). Pearson correlation coefficients for maternal E2 levels and maternal organ masses in HI IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Perirenal fat, g/kg FBW	**-0.745**	**-0.660**	**-0.719**	**-0.810**	**-0.757**
P-value	0.034	0.075	0.044	0.015	0.030
Blood, g	**-0.700**	**-0.704**	**-0.716**	**-0.104**	**-0.075**
P-value	0.053	0.052	0.046	0.807	0.859
Blood, g/kg	**-0.581**	**-0.781**	**-0.696**	**-0.315**	**-0.315**
P-value	0.131	0.022	0.055	0.447	0.448

Table A6. Pearson correlation coefficients for maternal E2 levels and maternal organ masses in HI IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
MBW, kg	**-0.890**	**0.073**	**-0.712**	**0.182**	**-0.059**
P-value	0.043	0.907	0.178	0.769	0.925
MBW on d 50, kg	**-0.495**	**0.419**	**-0.232**	**0.403**	**0.249**
P-value	0.397	0.483	0.708	0.501	0.686
MBW on d 90, kg	**-0.855**	**0.143**	**-0.651**	**0.294**	**0.058**
P-value	0.065	0.819	0.234	0.631	0.926
MBW at necropsy, kg	**-0.887**	**0.012**	**-0.735**	**0.203**	**-0.026**
P-value	0.045	0.985	0.157	0.743	0.966
LWG on d 50, g/d	**-0.263**	**0.896**	**0.168**	**0.093**	**-0.144**
P-value	0.669	0.040	0.787	0.881	0.818
LWG on d 50-90, g/d	**-0.742**	**-0.194**	**-0.704**	**0.034**	**-0.153**
P-value	0.151	0.755	0.185	0.956	0.806
LWG on d 4-130, g/d	**-0.786**	**0.095**	**-0.616**	**0.038**	**-0.209**
P-value	0.115	0.880	0.269	0.952	0.736
Gravid uterus, kg	**-0.705**	**-0.427**	**-0.774**	**0.316**	**0.213**
P-value	0.184	0.473	0.125	0.605	0.731
ADG, g/d	**-0.753**	**0.166**	**-0.557**	**-0.005**	**-0.259**
P-value	0.141	0.789	0.330	0.993	0.674
Liver, g	**-0.817**	**0.124**	**-0.629**	**0.344**	**0.108**
P-value	0.091	0.843	0.256	0.571	0.863
Liver, g/kg MBW	**-0.599**	**0.246**	**-0.394**	**0.633**	**0.423**
P-value	0.286	0.690	0.512	0.252	0.478
Perirenal fat, g	**-0.166**	**0.844**	**0.226**	**0.552**	**0.363**
P-value	0.789	0.073	0.715	0.334	0.549
Perirenal fat, g/kg FBW	**0.108**	**0.907**	**0.483**	**0.528**	**0.395**
P-value	0.863	0.033	0.410	0.361	0.510
Blood, g	**-0.659**	**-0.021**	**-0.559**	**-0.796**	**-0.905**
P-value	0.227	0.973	0.327	0.108	0.035

Table A5. (Continued). Pearson correlation coefficients for maternal E2 levels and maternal organ masses in HI IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Blood, g/kg	**-0.178**	**-0.066**	**-0.177**	**-0.920**	**-0.896**
P-value	0.775	0.916	0.776	0.027	0.040

Table A7. Pearson correlation coefficients for maternal E2 levels and maternal organ masses in HI non-IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
MBW, kg	**-0.093**	**-0.207**	**-0.175**	**-0.571**	**-0.480**
P-value	0.861	0.694	0.740	0.237	0.335
MBW on d 50, kg	**-0.216**	**-0.048**	**-0.159**	**-0.357**	**-0.391**
P-value	0.681	0.928	0.764	0.488	0.443
MBW on d 90, kg	**-0.300**	**-0.376**	**-0.398**	**-0.481**	**-0.434**
P-value	0.563	0.462	0.435	0.334	0.390
MBW at necropsy, kg	**-0.098**	**-0.159**	**-0.150**	**-0.451**	**-0.394**
P-value	0.854	0.764	0.776	0.370	0.440
LWG on d 50, g/d	**-0.386**	**-0.304**	**-0.408**	**0.057**	**-0.019**
P-value	0.450	0.558	0.422	0.914	0.972
LWG on d 50-90, g/d	**-0.236**	**-0.839**	**-0.624**	**-0.352**	**-0.148**
P-value	0.653	0.037	0.185	0.494	0.780
LWG on d 4-130, g/d	**-0.262**	**-0.438**	**-0.410**	**-0.159**	**-0.121**
P-value	0.617	0.386	0.420	0.764	0.820
BCS on d 130	**-0.085**	**0.133**	**0.024**	**-0.193**	**-0.224**
P-value	0.873	0.802	0.963	0.715	0.670
Change BCS	**-0.085**	**0.133**	**0.024**	**-0.193**	**-0.224**
P-value	0.873	0.802	0.963	0.715	0.670
Gravid uterus, kg	**-0.030**	**0.199**	**0.096**	**0.491**	**0.346**
P-value	0.954	0.705	0.857	0.323	0.502
ADG, g/d	**-0.295**	**-0.576**	**-0.510**	**-0.347**	**-0.255**
P-value	0.570	0.231	0.301	0.501	0.626
Liver, g	**0.216**	**0.380**	**0.349**	**-0.149**	**-0.165**
P-value	0.681	0.457	0.498	0.778	0.755
Liver, g/kg MBW	**0.384**	**0.697**	**0.633**	**0.168**	**0.084**
P-value	0.452	0.124	0.177	0.750	0.875
Perirenal fat, g	**-0.343**	**0.627**	**0.152**	**0.053**	**-0.218**
P-value	0.506	0.182	0.774	0.920	0.679

Table A7. (Continued). Pearson correlation coefficients for maternal E2 levels and maternal organ masses in HI non-IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Perirenal fat, g/kg FBW	**-0.308**	**0.662**	**0.192**	**0.180**	**-0.103**
P-value	0.553	0.153	0.715	0.734	0.846
Blood, g	**-0.162**	**0.228**	**0.033**	**-0.661**	**-0.679**
P-value	0.760	0.664	0.951	0.153	0.138
Blood, g/kg	**-0.106**	**0.555**	**0.254**	**-0.140**	**-0.280**
P-value	0.841	0.253	0.628	0.791	0.591

Table A8. Pearson correlation coefficients for maternal E2 levels and maternal organ masses in HI non-IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
MBW, kg	**-0.262**	**-0.572**	**-0.452**	**-0.245**	**-0.307**
P-value	0.570	0.180	0.309	0.596	0.503
MBW on d 50, kg	**-0.610**	**-0.147**	**-0.394**	**0.551**	**0.614**
P-value	0.146	0.753	0.382	0.200	0.143
MBW on d 90, kg	**-0.543**	**-0.368**	**-0.478**	**0.614**	**0.562**
P-value	0.208	0.416	0.278	0.143	0.189
MBW at necropsy, kg	**-0.536**	**-0.590**	**-0.605**	**-0.005**	**-0.062**
P-value	0.215	0.163	0.150	0.991	0.896
LWG on d 50, g/d	**-0.342**	**0.270**	**-0.032**	**0.521**	**0.494**
P-value	0.453	0.559	0.946	0.230	0.260
LWG on d 50-90, g/d	**-0.166**	**-0.303**	**-0.249**	**0.287**	**0.185**
P-value	0.722	0.509	0.591	0.533	0.691
LWG on d 4-130, g/d	**-0.293**	**-0.293**	**-0.318**	**-0.046**	**-0.138**
P-value	0.524	0.524	0.487	0.922	0.769
BCS on d 130	**0.762**	**0.851**	**0.865**	**-0.159**	**-0.190**
P-value	0.047	0.015	0.012	0.733	0.684
Change BCS	**0.762**	**0.851**	**0.865**	**-0.159**	**-0.190**
P-value	0.047	0.015	0.012	0.733	0.684
Gravid uterus, kg	**-0.492**	**0.124**	**-0.189**	**0.566**	**0.595**
P-value	0.262	0.790	0.686	0.185	0.159
ADG, g/d	**-0.084**	**-0.307**	**-0.217**	**-0.246**	**-0.338**
P-value	0.858	0.503	0.641	0.596	0.459
Liver, g	**-0.455**	**-0.571**	**-0.548**	**-0.731**	**-0.746**
P-value	0.305	0.181	0.203	0.062	0.054
Liver, g/kg MBW	**-0.414**	**-0.430**	**-0.450**	**-0.773**	**-0.761**
P-value	0.356	0.335	0.312	0.042	0.047
Perirenal fat, g	**0.166**	**0.036**	**0.103**	**-0.360**	**-0.441**
P-value	0.722	0.939	0.825	0.427	0.322

Table A8. (Continued). Pearson correlation coefficients for maternal E2 levels and maternal organ masses in HI non-IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Perirenal fat, g/kg FBW	**0.251**	**0.159**	**0.214**	**-0.369**	**-0.449**
P-value	0.588	0.734	0.644	0.415	0.313
Blood, g	**-0.154**	**-0.472**	**-0.340**	**-0.637**	**-0.640**
P-value	0.742	0.285	0.456	0.124	0.122
Blood, g/kg	**-0.105**	**-0.401**	**-0.275**	**-0.720**	**-0.699**
P-value	0.823	0.373	0.550	0.068	0.080

Table A9. Pearson correlation coefficients for maternal E2 and fetal organ masses in all 38 animals.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
FBW, g	**-0.172**	**0.107**	**-0.057**	**0.707**	**0.657**
P-value	-0.303	0.524	0.734	<.0001	<.0001
EFBW, g	**-0.199**	**0.079**	**-0.086**	**0.715**	**0.656**
P-value	0.232	0.638	0.610	<.0001	<.0001
Placentome Wt., g	**-0.236**	**0.022**	**-0.133**	**0.802**	**0.762**
P-value	0.154	0.896	0.428	<.0001	<.0001
Fetal:placentome	**0.283**	**0.080**	**0.206**	**-0.693**	**-0.676**
P-value	0.086	0.634	0.215	<.0001	<.0001
BPHD	**-0.279**	**-0.010**	**-0.174**	**0.569**	**0.549**
P-value	0.090	0.954	0.295	0.000	0.000
Girth	**-0.145**	**0.088**	**-0.050**	**0.579**	**0.511**
P-value	0.384	0.600	0.765	0.000	0.001
Brain, g	**-0.017**	**0.128**	**0.047**	**0.482**	**0.491**
P-value	0.921	0.445	0.779	0.002	0.002
Brain, g/kg FBW	**0.272**	**0.037**	**0.182**	**-0.489**	**-0.426**
P-value	0.099	0.824	0.274	0.002	0.008
Brain:liver	**0.148**	**-0.137**	**0.029**	**-0.434**	**-0.374**
P-value	0.375	0.412	0.862	0.006	0.021
Pancreas, g	**-0.223**	**-0.035**	**-0.152**	**0.363**	**0.288**
P-value	0.179	0.836	0.361	0.025	0.079
Pancreas, g/kg FBW	**-0.209**	**-0.206**	**-0.220**	**-0.165**	**-0.198**
P-value	0.207	0.215	0.184	0.323	0.234
Perirenal fat, g	**-0.161**	**0.099**	**-0.055**	**0.658**	**0.634**
P-value	0.336	0.553	0.745	<.0001	<.0001
Perirenal fat, g/kg FBW	**0.007**	**0.003**	**0.004**	**-0.046**	**-0.021**
P-value	0.966	0.985	0.979	0.786	0.900
Kidney, g	**-0.161**	**0.093**	**-0.058**	**0.456**	**0.364**
P-value	0.333	0.577	0.730	0.004	0.025

Table A9. (Continued). Pearson correlation coefficients for maternal E2 and fetal organ masses in all 38 animals.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Kidney, g/kg FBW	**-0.078**	**-0.112**	**-0.098**	**-0.286**	**-0.324**
P-value	0.643	0.503	0.557	0.082	0.047
Liver, g	**-0.086**	**0.177**	**0.025**	**0.574**	**0.519**
P-value	0.606	0.289	0.880	0.000	0.001
Liver, g/kg FBW	**0.085**	**0.262**	**0.167**	**0.160**	**0.124**
P-value	0.613	0.113	0.316	0.337	0.459
Stomach complex, g	**-0.130**	**0.145**	**-0.014**	**0.632**	**0.602**
P-value	0.437	0.386	0.934	<.0001	<.0001
Small intestine, g	**-0.128**	**0.058**	**-0.052**	**0.490**	**0.442**
P-value	0.442	0.727	0.755	0.002	0.006
Large intestine, g	**-0.089**	**0.062**	**-0.027**	**0.450**	**0.423**
P-value	0.597	0.714	0.872	0.005	0.008
GIT, g	**-0.149**	**0.082**	**-0.054**	**0.592**	**0.553**
P-value	0.373	0.624	0.749	<.0001	0.000
GIT, g/kg FBW	**0.070**	**-0.036**	**0.026**	**-0.245**	**-0.225**
P-value	0.677	0.830	0.875	0.138	0.174
Spleen, g	**-0.124**	**0.135**	**-0.015**	**0.515**	**0.501**
P-value	0.457	0.419	0.928	0.001	0.001
Spleen, g/kg FBW	**-0.073**	**0.094**	**-0.002**	**0.127**	**0.152**
P-value	0.665	0.576	0.990	0.447	0.363
Ling, g	**-0.145**	**0.094**	**-0.046**	**0.536**	**0.496**
P-value	0.386	0.575	0.786	0.001	0.002
Lung, g/kg FBW	**-0.005**	**0.028**	**0.010**	**-0.090**	**-0.084**
P-value	0.974	0.869	0.953	0.592	0.617
Heart, g	**-0.140**	**-0.001**	**-0.086**	**0.469**	**0.418**
P-value	0.401	0.996	0.609	0.003	0.009
Heart, g/kg FBW	**-0.009**	**-0.125**	**-0.061**	**-0.036**	**-0.056**
P-value	0.957	0.455	0.714	0.832	0.737

Table A10. Pearson correlation coefficients for maternal E2 and fetal organ masses in CON.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
FBW, g	**0.658**	**0.663**	**0.713**	**0.500**	**0.273**
P-value	0.020	0.019	0.009	0.098	0.391
EFBW, g	**0.714**	**0.678**	**0.745**	**0.513**	**0.226**
P-value	0.009	0.015	0.005	0.088	0.480
Placentome Wt., g	**0.367**	**0.387**	**0.414**	**0.421**	**0.256**
P-value	0.241	0.214	0.181	0.173	0.421
Fetal:placentome	**-0.043**	**-0.122**	**-0.104**	**-0.325**	**-0.239**
P-value	0.895	0.706	0.747	0.303	0.455
BPHD	**0.501**	**0.196**	**0.343**	**0.071**	**0.097**
P-value	0.097	0.542	0.276	0.826	0.764
Girth	**0.432**	**0.223**	**0.331**	**0.097**	**-0.088**
P-value	0.161	0.486	0.293	0.764	0.786
Brain, g	**0.143**	**-0.283**	**-0.122**	**0.389**	**0.559**
P-value	0.657	0.373	0.706	0.211	0.059
Brain, g/kg FBW	**-0.509**	**-0.719**	**-0.685**	**-0.284**	**-0.001**
P-value	0.091	0.008	0.014	0.372	0.999
Brain:liver	**-0.261**	**-0.425**	**-0.384**	**-0.311**	**-0.064**
P-value	0.413	0.169	0.217	0.325	0.842
Pancreas, g	**0.492**	**0.318**	**0.410**	**0.017**	**-0.173**
P-value	0.105	0.314	0.186	0.958	0.592
Pancreas, g/kg FBW	**0.193**	**0.018**	**0.088**	**-0.184**	**-0.278**
P-value	0.548	0.955	0.786	0.567	0.381
Perirenal fat, g	**-0.134**	**0.016**	**-0.033**	**0.317**	**0.356**
P-value	0.679	0.961	0.919	0.316	0.256
Perirenal fat, g/kg FBW	**-0.623**	**-0.447**	**-0.545**	**-0.021**	**0.165**
P-value	0.030	0.145	0.067	0.949	0.609
Kidney, g	**0.528**	**0.492**	**0.543**	**-0.055**	**-0.368**
P-value	0.078	0.104	0.068	0.865	0.240

Table A10. (Continued). Pearson correlation coefficients for maternal E2 and fetal organ masses in CON.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Kidney, g/kg FBW	**0.125**	**0.065**	**0.092**	**-0.471**	**-0.674**
P-value	0.699	0.842	0.776	0.122	0.016
Liver, g	**0.350**	**0.381**	**0.393**	**0.324**	**0.107**
P-value	0.265	0.222	0.206	0.304	0.742
Liver, g/kg FBW	**-0.010**	**0.040**	**0.016**	**0.086**	**-0.040**
P-value	0.975	0.902	0.960	0.791	0.901
Stomach complex, g	**0.616**	**0.582**	**0.644**	**0.341**	**0.334**
P-value	0.033	0.047	0.024	0.277	0.288
Small intestine, g	**0.228**	**0.289**	**0.287**	**-0.114**	**-0.162**
P-value	0.475	0.362	0.365	0.723	0.615
Large intestine, g	**0.160**	**0.066**	**0.106**	**0.352**	**0.364**
P-value	0.619	0.839	0.744	0.262	0.245
GIT, g	**0.419**	**0.442**	**0.469**	**0.070**	**0.065**
P-value	0.175	0.150	0.124	0.828	0.840
GIT, g/kg FBW	**-0.106**	**-0.081**	**-0.096**	**-0.423**	**-0.200**
P-value	0.743	0.802	0.767	0.170	0.534
Spleen, g	**0.272**	**-0.060**	**0.068**	**0.357**	**0.340**
P-value	0.393	0.853	0.833	0.255	0.279
Spleen, g/kg FBW	**0.001**	**-0.333**	**-0.224**	**0.108**	**0.200**
P-value	0.999	0.290	0.484	0.738	0.533
Lung, g	**0.446**	**0.576**	**0.566**	**0.150**	**0.052**
P-value	0.146	0.050	0.055	0.641	0.872
Lung, g/kg FBW	**0.051**	**0.209**	**0.158**	**-0.140**	**-0.104**
P-value	0.875	0.514	0.624	0.663	0.747
Heart, g	**-0.184**	**-0.129**	**-0.159**	**-0.199**	**-0.267**
P-value	0.567	0.689	0.621	0.535	0.401
Heart, g/kg FBW	**-0.365**	**-0.314**	**-0.358**	**-0.321**	**-0.329**
P-value	0.244	0.321	0.254	0.309	0.296

Table A11. Pearson correlation coefficients for maternal E2 and fetal organ masses in HI.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
FBW, g	**0.437**	**0.645**	**0.588**	**0.874**	**0.854**
P-value	0.118	0.013	0.027	<.0001	0.000
EFBW, g	**0.451**	**0.644**	**0.594**	**0.873**	**0.851**
P-value	0.106	0.013	0.025	<.0001	0.000
Placentome Wt., g	**0.503**	**0.643**	**0.620**	**0.937**	**0.933**
P-value	0.067	0.013	0.018	<.0001	<.0001
Fetal:placentome	**-0.246**	**-0.322**	**-0.309**	**-0.587**	**-0.603**
P-value	0.396	0.261	0.282	0.027	0.022
BPHD	**0.435**	**0.635**	**0.581**	**0.567**	**0.550**
P-value	0.120	0.015	0.029	0.034	0.042
Girth	**0.358**	**0.647**	**0.549**	**0.777**	**0.743**
P-value	0.209	0.012	0.042	0.001	0.002
Brain, g	**0.504**	**0.602**	**0.596**	**0.595**	**0.613**
P-value	0.066	0.023	0.025	0.025	0.020
Brain, g/kg FBW	**0.105**	**-0.027**	**0.035**	**-0.408**	**-0.363**
P-value	0.722	0.926	0.904	0.148	0.203
Brain:liver	**-0.017**	**-0.122**	**-0.080**	**-0.435**	**-0.423**
P-value	0.955	0.677	0.787	0.120	0.132
Pancreas, g	**0.093**	**0.468**	**0.315**	**0.444**	**0.373**
P-value	0.752	0.092	0.273	0.112	0.189
Pancreas, g/kg FBW	**-0.350**	**-0.156**	**-0.263**	**-0.344**	**-0.374**
P-value	0.220	0.595	0.363	0.229	0.188
Perirenal fat, g	**0.413**	**0.428**	**0.452**	**0.738**	**0.747**
P-value	0.142	0.127	0.105	0.003	0.002
Perirenal fat, g/kg FBW	**-0.164**	**-0.372**	**-0.294**	**-0.383**	**-0.347**
P-value	0.575	0.191	0.307	0.177	0.224
Kidney, g	**0.253**	**0.476**	**0.399**	**0.783**	**0.730**
P-value	0.383	0.086	0.157	0.001	0.003

Table A11. (Continued). Pearson correlation coefficients for maternal E2 and fetal organ masses in HI.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Kidney, g/kg FBW	**-0.375**	**-0.427**	**-0.431**	**-0.302**	**-0.327**
P-value	0.187	0.128	0.124	0.294	0.253
Liver, g	**0.429**	**0.576**	**0.544**	**0.772**	**0.780**
P-value	0.126	0.031	0.044	0.001	0.001
Liver, g/kg FBW	**0.258**	**0.274**	**0.286**	**0.362**	**0.411**
P-value	0.372	0.344	0.321	0.203	0.144
Stomach complex, g	**0.612**	**0.798**	**0.762**	**0.824**	**0.814**
P-value	0.020	0.001	0.002	0.000	0.000
Small intestine, g	**0.485**	**0.624**	**0.599**	**0.811**	**0.797**
P-value	0.079	0.017	0.024	0.000	0.001
Large intestine, g	**0.418**	**0.510**	**0.500**	**0.668**	**0.641**
P-value	0.137	0.063	0.069	0.009	0.013
GIT, g	**0.546**	**0.688**	**0.666**	**0.823**	**0.812**
P-value	0.044	0.007	0.009	0.000	0.000
GIT, g/kg FBW	**0.349**	**0.235**	**0.308**	**-0.044**	**-0.032**
P-value	0.222	0.419	0.284	0.881	0.914
Spleen, g	**0.378**	**0.520**	**0.487**	**0.638**	**0.652**
P-value	0.183	0.057	0.077	0.014	0.012
Spleen, g/kg FBW	**0.127**	**0.146**	**0.149**	**0.167**	**0.210**
P-value	0.664	0.618	0.612	0.568	0.472
Lung, g	**0.545**	**0.651**	**0.645**	**0.765**	**0.757**
P-value	0.044	0.012	0.013	0.001	0.002
Lung, g/kg FBW	**0.350**	**0.299**	**0.345**	**0.061**	**0.082**
P-value	0.220	0.299	0.227	0.835	0.780
Heart, g	**0.482**	**0.790**	**0.693**	**0.705**	**0.654**
P-value	0.081	0.001	0.006	0.005	0.011
Heart, g/kg FBW	**0.216**	**0.377**	**0.324**	**0.107**	**0.065**
P-value	0.458	0.185	0.259	0.715	0.826

Table A12. Pearson correlation coefficients for maternal E2 and fetal organ masses in HI+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
FBW, g	**-0.394**	**0.190**	**-0.179**	**0.547**	**0.544**
P-value	0.206	0.555	0.578	0.065	0.068
EFBW, g	**-0.401**	**0.187**	**-0.185**	**0.552**	**0.547**
P-value	0.197	0.561	0.565	0.063	0.066
Placentome Wt., g	**-0.280**	**0.271**	**-0.055**	**0.618**	**0.625**
P-value	0.378	0.395	0.864	0.032	0.030
Fetal:placentome	**0.106**	**-0.252**	**-0.060**	**-0.500**	**-0.518**
P-value	0.743	0.429	0.854	0.098	0.084
BPHD	**-0.485**	**0.073**	**-0.303**	**0.426**	**0.421**
P-value	0.110	0.821	0.339	0.168	0.173
Girth	**-0.544**	**-0.067**	**-0.420**	**0.560**	**0.530**
P-value	0.067	0.836	0.174	0.058	0.076
Brain, g	**-0.214**	**0.215**	**-0.037**	**0.434**	**0.472**
P-value	0.504	0.503	0.908	0.159	0.122
Brain, g/kg FBW	**0.397**	**-0.089**	**0.232**	**-0.457**	**-0.441**
P-value	0.201	0.783	0.468	0.135	0.151
Brain:liver	**0.553**	**-0.177**	**0.293**	**-0.427**	**-0.392**
P-value	0.062	0.583	0.356	0.166	0.208
Pancreas, g	**-0.503**	**-0.125**	**-0.421**	**0.569**	**0.572**
P-value	0.096	0.698	0.173	0.053	0.052
Pancreas, g/kg FBW	**-0.470**	**-0.325**	**-0.501**	**0.380**	**0.390**
P-value	0.123	0.302	0.097	0.224	0.211
Perirenal fat, g	**-0.294**	**0.244**	**-0.084**	**0.577**	**0.526**
P-value	0.353	0.444	0.795	0.050	0.079
Perirenal fat, g/kg FBW	**0.107**	**0.204**	**0.178**	**0.150**	**0.082**
P-value	0.742	0.524	0.581	0.642	0.800
Kidney, g	**-0.540**	**-0.011**	**-0.387**	**0.593**	**0.601**
P-value	0.070	0.973	0.214	0.042	0.039

Table A12. (Continued). Pearson correlation coefficients for maternal E2 and fetal organ masses in HI+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Kidney, g/kg FBW	**-0.501**	**-0.285**	**-0.501**	**0.340**	**0.359**
P-value	0.097	0.369	0.097	0.279	0.252
Liver, g	**-0.482**	**0.114**	**-0.281**	**0.474**	**0.450**
P-value	0.112	0.723	0.377	0.120	0.142
Liver, g/kg FBW	**-0.531**	**0.050**	**-0.344**	**0.210**	**0.158**
P-value	0.076	0.878	0.273	0.513	0.625
Stomach complex, g	**-0.411**	**0.216**	**-0.174**	**0.735**	**0.735**
P-value	0.185	0.501	0.588	0.006	0.007
Small intestine, g	**-0.358**	**0.007**	**-0.250**	**0.458**	**0.414**
P-value	0.254	0.982	0.434	0.135	0.181
Large intestine, g	**-0.208**	**0.215**	**-0.034**	**0.616**	**0.627**
P-value	0.517	0.503	0.917	0.033	0.029
GIT, g	**-0.354**	**0.145**	**-0.174**	**0.635**	**0.607**
P-value	0.259	0.654	0.588	0.027	0.036
GIT, g/kg FBW	**0.111**	**-0.108**	**0.021**	**0.059**	**0.018**
P-value	0.731	0.738	0.949	0.855	0.957
Spleen, g	**-0.383**	**0.339**	**-0.086**	**0.377**	**0.361**
P-value	0.219	0.281	0.790	0.227	0.249
Spleen, g/kg FBW	**-0.351**	**0.340**	**-0.057**	**0.109**	**0.084**
P-value	0.263	0.279	0.861	0.736	0.796
Lung, g	**-0.357**	**0.174**	**-0.158**	**0.651**	**0.686**
P-value	0.255	0.589	0.624	0.022	0.014
Lung, g/kg FBW	**-0.096**	**0.087**	**-0.016**	**0.318**	**0.383**
P-value	0.766	0.788	0.961	0.314	0.219
Heart, g	**-0.281**	**-0.098**	**-0.253**	**0.494**	**0.501**
P-value	0.377	0.762	0.428	0.103	0.097
Heart, g/kg FBW	**0.163**	**-0.504**	**-0.156**	**-0.060**	**-0.039**
P-value	0.612	0.095	0.629	0.852	0.905

Table A13. Pearson correlation coefficients for maternal E2 and fetal organ masses in HIIUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
FBW, g	**0.383**	**0.466**	**0.435**	**0.771**	**0.785**
P-value	0.349	0.245	0.282	0.025	0.021
EFBW, g	**0.429**	**0.479**	**0.465**	**0.809**	**0.814**
P-value	0.290	0.230	0.246	0.015	0.014
Placentome Wt., g	**0.289**	**0.393**	**0.351**	**0.765**	**0.802**
P-value	0.488	0.335	0.394	0.027	0.017
Fetal:placentome	**0.320**	**0.284**	**0.307**	**0.161**	**0.125**
P-value	0.439	0.495	0.460	0.703	0.769
BPHD	**0.541**	**0.656**	**0.612**	**0.718**	**0.744**
P-value	0.167	0.077	0.107	0.045	0.035
Girth	**0.282**	**0.394**	**0.346**	**0.781**	**0.822**
P-value	0.499	0.334	0.401	0.022	0.012
Brain, g	**0.565**	**0.683**	**0.638**	**0.772**	**0.795**
P-value	0.144	0.062	0.089	0.025	0.019
Brain, g/kg FBW	**0.539**	**0.692**	**0.628**	**0.385**	**0.415**
P-value	0.168	0.057	0.096	0.347	0.306
Brain:liver	**0.551**	**0.640**	**0.607**	**0.396**	**0.416**
P-value	0.157	0.087	0.111	0.331	0.305
Pancreas, g	**-0.212**	**0.141**	**-0.036**	**0.054**	**0.110**
P-value	0.615	0.738	0.933	0.900	0.795
Pancreas, g/kg FBW	**-0.453**	**-0.190**	**-0.329**	**-0.476**	**-0.429**
P-value	0.260	0.653	0.426	0.233	0.289
Perirenal fat, g	**0.455**	**0.635**	**0.558**	**0.731**	**0.749**
P-value	0.257	0.091	0.151	0.039	0.032
Perirenal fat, g/kg FBW	**0.071**	**0.274**	**0.176**	**-0.059**	**-0.036**
P-value	0.868	0.512	0.676	0.890	0.932
Kidney, g	**-0.297**	**-0.374**	**-0.341**	**0.063**	**0.024**
P-value	0.475	0.361	0.409	0.883	0.956

Table A13. (Continued). Pearson correlation coefficients for maternal E2 and fetal organ masses in HIIUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Kidney, g/kg FBW	**-0.495**	**-0.619**	**-0.568**	**-0.519**	**-0.552**
P-value	0.212	0.102	0.142	0.188	0.156
Liver, g	**0.195**	**0.302**	**0.255**	**0.556**	**0.573**
P-value	0.644	0.467	0.543	0.152	0.138
Liver, g/kg FBW	**-0.402**	**-0.327**	**-0.371**	**-0.268**	**-0.255**
P-value	0.324	0.429	0.365	0.521	0.542
Stomach complex, g	**0.650**	**0.787**	**0.734**	**0.678**	**0.678**
P-value	0.081	0.020	0.038	0.065	0.064
Small intestine, g	**0.419**	**0.519**	**0.480**	**0.523**	**0.496**
P-value	0.301	0.187	0.228	0.183	0.211
Large intestine, g	**0.171**	**0.072**	**0.125**	**-0.196**	**-0.270**
P-value	0.686	0.865	0.769	0.642	0.519
GIT, g	**0.547**	**0.646**	**0.610**	**0.598**	**0.571**
P-value	0.161	0.084	0.108	0.117	0.140
GIT, g/kg FBW	**0.405**	**0.487**	**0.455**	**-0.068**	**-0.124**
P-value	0.320	0.221	0.257	0.874	0.771
Spleen, g	**0.141**	**0.236**	**0.195**	**0.670**	**0.702**
P-value	0.739	0.574	0.644	0.069	0.052
Spleen, g/kg FBW	**-0.225**	**-0.188**	**-0.208**	**0.278**	**0.309**
P-value	0.593	0.656	0.621	0.506	0.456
Lung, g	**0.404**	**0.544**	**0.485**	**0.570**	**0.572**
P-value	0.321	0.163	0.224	0.140	0.138
Lung, g/kg FBW	**0.371**	**0.564**	**0.477**	**0.269**	**0.278**
P-value	0.365	0.145	0.232	0.519	0.505
Heart, g	**0.443**	**0.444**	**0.456**	**0.725**	**0.672**
P-value	0.272	0.270	0.256	0.042	0.068
Heart, g/kg FBW	**0.023**	**-0.073**	**-0.023**	**0.012**	**-0.061**
P-value	0.957	0.863	0.956	0.977	0.886

Table A14. Pearson correlation coefficients for maternal E2 and fetal organ masses in HIIUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
FBW, g	**-0.532**	**-0.332**	**-0.588**	**0.512**	**0.415**
P-value	0.356	0.585	0.297	0.378	0.488
EFBW, g	**-0.537**	**-0.421**	**-0.631**	**0.428**	**0.344**
P-value	0.350	0.481	0.254	0.473	0.571
Placentome Wt., g	**-0.609**	**-0.447**	**-0.702**	**0.406**	**0.344**
P-value	0.276	0.451	0.187	0.498	0.571
Fetal:placentome	**0.495**	**0.640**	**0.690**	**-0.137**	**-0.179**
P-value	0.397	0.245	0.197	0.826	0.774
BPHD	**-0.484**	**-0.264**	**-0.519**	**0.608**	**0.514**
P-value	0.409	0.668	0.371	0.277	0.376
Girth	**-0.608**	**-0.342**	**-0.656**	**0.393**	**0.274**
P-value	0.276	0.574	0.230	0.513	0.656
Brain, g	**-0.754**	**-0.308**	**-0.763**	**0.087**	**0.013**
P-value	0.141	0.614	0.134	0.890	0.984
Brain, g/kg FBW	**0.362**	**0.361**	**0.458**	**-0.555**	**-0.490**
P-value	0.549	0.550	0.437	0.332	0.403
Brain:liver	**0.657**	**-0.214**	**0.456**	**-0.577**	**-0.362**
P-value	0.229	0.730	0.441	0.309	0.549
Pancreas, g	**-0.564**	**-0.181**	**-0.549**	**0.511**	**0.366**
P-value	0.322	0.771	0.338	0.379	0.545
Pancrease, g/kg FBW	**-0.554**	**-0.124**	**-0.516**	**0.532**	**0.377**
P-value	0.333	0.843	0.374	0.356	0.532
Perirenal fat, g	**0.005**	**0.276**	**0.123**	**-0.652**	**-0.728**
P-value	0.994	0.654	0.844	0.233	0.163
Perirenal fat, g/kg FBW	**0.348**	**0.427**	**0.476**	**-0.599**	**-0.584**
P-value	0.566	0.473	0.418	0.286	0.301
Kidney, g	**-0.622**	**-0.282**	**-0.641**	**0.269**	**0.218**
P-value	0.263	0.646	0.243	0.662	0.724

Table A14. (Continued). Pearson correlation coefficients for maternal E2 and fetal organ masses in HIIUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Kidney, g/kg FBW	**-0.267**	**-0.009**	**-0.227**	**-0.165**	**-0.140**
P-value	0.664	0.988	0.714	0.791	0.823
Liver, g	**-0.742**	**0.025**	**-0.609**	**0.476**	**0.294**
P-value	0.151	0.968	0.276	0.418	0.631
Liver, g/kg FBW	**-0.585**	**0.572**	**-0.240**	**0.195**	**-0.016**
P-value	0.301	0.314	0.697	0.753	0.980
Stomach complex, g	**-0.577**	**-0.282**	**-0.604**	**0.491**	**0.371**
P-value	0.309	0.645	0.281	0.402	0.539
Small intestine, g	**-0.781**	**-0.578**	**-0.902**	**-0.571**	**-0.653**
P-value	0.119	0.308	0.036	0.315	0.232
Large intestine, g	**-0.770**	**-0.315**	**-0.779**	**0.202**	**0.045**
P-value	0.128	0.606	0.121	0.745	0.943
GIT, g	**-0.787**	**-0.583**	**-0.909**	**-0.156**	**-0.255**
P-value	0.114	0.303	0.033	0.802	0.680
GIT, g/kg FBW	**-0.205**	**-0.259**	**-0.284**	**-0.992**	**-0.969**
P-value	0.740	0.674	0.644	0.001	0.007
Spleen, g	**-0.811**	**-0.219**	**-0.772**	**0.299**	**0.159**
P-value	0.096	0.723	0.126	0.626	0.799
Spleen, g/kg FBW	**-0.834**	**-0.102**	**-0.740**	**0.183**	**0.038**
P-value	0.079	0.870	0.153	0.768	0.952
Lung, g	**-0.408**	**0.078**	**-0.307**	**0.739**	**0.583**
P-value	0.495	0.900	0.616	0.154	0.302
Lung, g/kg FBW	**-0.202**	**0.595**	**0.089**	**0.799**	**0.597**
P-value	0.745	0.290	0.887	0.105	0.288
Heart, g	**-0.294**	**-0.869**	**-0.621**	**-0.116**	**0.017**
P-value	0.631	0.056	0.263	0.852	0.979
Heart, g/kg FBW	**0.119**	**-0.742**	**-0.222**	**-0.479**	**-0.251**
P-value	0.849	0.151	0.719	0.415	0.683

Table A15. Pearson correlation coefficients for maternal E2 and fetal organ masses in HI non-IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
FBW, g	**0.150**	**0.001**	**0.091**	**0.621**	**0.570**
P-value	0.777	0.998	0.863	0.188	0.238
EFBW, g	**0.113**	**-0.033**	**0.050**	**0.623**	**0.560**
P-value	0.831	0.951	0.925	0.187	0.248
Placentome Wt., g	**0.677**	**0.164**	**0.505**	**0.888**	**0.919**
P-value	0.139	0.756	0.306	0.018	0.010
Fetal:placentome	**-0.978**	**-0.328**	**-0.782**	**-0.739**	**-0.857**
P-value	0.001	0.526	0.066	0.094	0.029
BPHD	**-0.505**	**-0.011**	**-0.313**	**-0.148**	**-0.339**
P-value	0.307	0.984	0.546	0.779	0.511
Girth	**0.007**	**0.371**	**0.217**	**0.331**	**0.180**
P-value	0.990	0.469	0.680	0.522	0.734
Brain, g	**-0.174**	**-0.858**	**-0.598**	**-0.178**	**0.006**
P-value	0.742	0.029	0.210	0.736	0.991
Brain, g/kg FBW	**-0.151**	**-0.275**	**-0.249**	**-0.618**	**-0.502**
P-value	0.776	0.598	0.634	0.191	0.310
Brain:liver	**-0.539**	**-0.277**	**-0.486**	**-0.399**	**-0.468**
P-value	0.270	0.595	0.328	0.433	0.349
Pancreas, g	**0.097**	**0.491**	**0.341**	**0.332**	**0.141**
P-value	0.854	0.323	0.508	0.521	0.790
Pancreas, g/kg FBW	**0.046**	**0.480**	**0.303**	**0.098**	**-0.068**
P-value	0.931	0.335	0.559	0.854	0.898
Perirenal fat, g	**0.159**	**-0.262**	**-0.054**	**0.663**	**0.655**
P-value	0.764	0.616	0.919	0.151	0.158
Perirenal fat, g/kg FBW	**0.053**	**-0.418**	**-0.207**	**0.520**	**0.532**
P-value	0.921	0.410	0.693	0.290	0.277
Kidney, g	**0.382**	**0.509**	**0.524**	**0.760**	**0.607**
P-value	0.454	0.302	0.286	0.080	0.201

Table A15. (Continued). Pearson correlation coefficients for maternal E2 and fetal organ masses in HI non-IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Kidney, g/kg FBW	**0.318**	**0.601**	**0.538**	**0.196**	**0.088**
P-value	0.539	0.207	0.271	0.710	0.869
Liver, g	**0.383**	**0.002**	**0.233**	**0.238**	**0.346**
P-value	0.454	0.998	0.656	0.649	0.502
Liver, g/kg FBW	**0.448**	**0.050**	**0.301**	**-0.091**	**0.085**
P-value	0.373	0.925	0.563	0.865	0.873
Stomach complex, g	**0.512**	**0.363**	**0.519**	**0.512**	**0.531**
P-value	0.299	0.479	0.291	0.300	0.279
Small intestine, g	**0.301**	**0.083**	**0.230**	**0.678**	**0.673**
P-value	0.563	0.876	0.661	0.139	0.143
Large intestine, g	**0.403**	**0.153**	**0.332**	**0.548**	**0.569**
P-value	0.429	0.773	0.520	0.260	0.239
GIT, g	**0.349**	**0.111**	**0.276**	**0.614**	**0.627**
P-value	0.498	0.834	0.597	0.195	0.182
GIT, g/kg FBW	**0.540**	**0.266**	**0.480**	**0.428**	**0.523**
P-value	0.269	0.610	0.335	0.397	0.287
Spleen, g	**0.278**	**0.011**	**0.175**	**-0.169**	**-0.064**
P-value	0.594	0.983	0.740	0.748	0.904
Spleen, g/kg FBW	**0.272**	**0.042**	**0.189**	**-0.407**	**-0.273**
P-value	0.602	0.937	0.719	0.423	0.601
Lung, g	**0.578**	**0.261**	**0.501**	**0.768**	**0.735**
P-value	0.230	0.617	0.312	0.075	0.096
Lung, g/kg FBW	**0.715**	**0.386**	**0.655**	**0.499**	**0.513**
P-value	0.110	0.450	0.158	0.314	0.298
Heart, g	**0.321**	**0.846**	**0.680**	**0.071**	**-0.018**
P-value	0.535	0.034	0.137	0.894	0.973
Heart, g/kg FBW	**0.296**	**0.727**	**0.597**	**-0.142**	**-0.180**
P-value	0.569	0.102	0.211	0.788	0.732

Table A16. Pearson correlation coefficients for maternal E2 and fetal organ masses in HI non-IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
FBW, g	**-0.297**	**0.028**	**-0.151**	**0.302**	**0.322**
P-value	0.517	0.953	0.746	0.510	0.481
EFBW, g	**-0.297**	**0.087**	**-0.117**	**0.333**	**0.354**
P-value	0.518	0.853	0.803	0.466	0.436
Placentome Wt., g	**0.099**	**0.275**	**0.195**	**0.458**	**0.490**
P-value	0.833	0.550	0.675	0.302	0.265
Fetal:placentome	**-0.385**	**-0.470**	**-0.457**	**-0.423**	**-0.451**
P-value	0.393	0.287	0.303	0.344	0.310
BPHD	**-0.486**	**-0.108**	**-0.323**	**0.163**	**0.163**
P-value	0.269	0.818	0.480	0.727	0.728
Girth	**-0.545**	**-0.348**	**-0.484**	**0.436**	**0.400**
P-value	0.206	0.444	0.271	0.328	0.374
Brain, g	**0.185**	**0.183**	**0.190**	**0.260**	**0.330**
P-value	0.691	0.695	0.683	0.574	0.470
Brain, g/kg FBW	**0.540**	**0.148**	**0.370**	**-0.148**	**-0.104**
P-value	0.210	0.752	0.414	0.751	0.824
Brain:liver	**0.596**	**0.563**	**0.631**	**-0.019**	**0.045**
P-value	0.158	0.188	0.129	0.967	0.924
Pancreas, g	**-0.432**	**-0.352**	**-0.426**	**0.538**	**0.553**
P-value	0.333	0.438	0.341	0.213	0.198
Pancreas, g/kg FBW	**-0.419**	**-0.483**	**-0.487**	**0.557**	**0.571**
P-value	0.350	0.273	0.267	0.194	0.180
Perirenal fat, g	**-0.462**	**-0.036**	**-0.269**	**0.385**	**0.329**
P-value	0.297	0.939	0.559	0.394	0.471
Perirenal fat, g/kg FBW	**-0.431**	**-0.083**	**-0.273**	**0.334**	**0.226**
P-value	0.334	0.859	0.553	0.464	0.626
Kidney, g	**-0.719**	**-0.414**	**-0.607**	**0.422**	**0.461**
P-value	0.069	0.356	0.149	0.346	0.298

Table A16. (Continued). Pearson correlation coefficients for maternal E2 and fetal organ masses in HI non-IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Kidney, g/kg FBW	**-0.827**	**-0.686**	**-0.805**	**0.353**	**0.394**
P-value	0.022	0.089	0.029	0.438	0.382
Liver, g	**-0.429**	**-0.346**	**-0.428**	**0.130**	**0.115**
P-value	0.337	0.448	0.339	0.781	0.807
Liver, g/kg FBW	**-0.419**	**-0.700**	**-0.614**	**-0.133**	**-0.187**
P-value	0.350	0.080	0.143	0.776	0.688
Stomach complex, g	**-0.163**	**0.199**	**0.021**	**0.776**	**0.794**
P-value	0.727	0.669	0.964	0.040	0.033
Small intestine, g	**0.058**	**0.026**	**0.033**	**0.399**	**0.369**
P-value	0.902	0.957	0.945	0.375	0.415
Large intestine, g	**0.115**	**0.014**	**0.059**	**0.420**	**0.468**
P-value	0.805	0.976	0.900	0.348	0.290
GIT, g	**0.038**	**0.139**	**0.086**	**0.559**	**0.541**
P-value	0.936	0.767	0.855	0.192	0.210
GIT, g/kg FBW	**0.383**	**0.213**	**0.312**	**0.593**	**0.544**
P-value	0.396	0.646	0.496	0.160	0.207
Spleen, g	**0.614**	**0.582**	**0.631**	**-0.143**	**-0.132**
P-value	0.143	0.171	0.129	0.760	0.779
Spleen, g/kg FBW	**0.792**	**0.537**	**0.709**	**-0.383**	**-0.394**
P-value	0.034	0.214	0.075	0.396	0.382
Lung, g	**-0.205**	**-0.059**	**-0.142**	**0.599**	**0.675**
P-value	0.659	0.901	0.762	0.155	0.096
Lung, g/kg FBW	**-0.059**	**-0.119**	**-0.091**	**0.570**	**0.663**
P-value	0.900	0.800	0.846	0.182	0.105
Heart, g	**-0.093**	**-0.128**	**-0.130**	**0.363**	**0.386**
P-value	0.843	0.785	0.781	0.423	0.393
Heart, g/kg FBW	**0.237**	**-0.284**	**-0.038**	**0.223**	**0.238**
P-value	0.609	0.537	0.935	0.631	0.608

Table A17. Pearson correlation coefficients for maternal E2 and CAR mRNA in 38 animals.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**-0.202**	**-0.260**	**-0.240**	**-0.173**	**-0.134**
P-value	0.225	0.115	0.147	0.298	0.421
Flt	**-0.055**	**-0.189**	**-0.117**	**-0.203**	**-0.237**
P-value	0.741	0.255	0.484	0.221	0.152
KDR	**-0.265**	**-0.265**	**-0.278**	**0.028**	**0.068**
P-value	0.108	0.108	0.091	0.870	0.686
HIF	**-0.266**	**-0.189**	**-0.244**	**-0.230**	**-0.272**
P-value	0.118	0.270	0.152	0.178	0.108
eNOS	**0.264**	**0.164**	**0.235**	**-0.116**	**-0.086**
P-value	0.109	0.326	0.155	0.488	0.606
sGC	**-0.257**	**-0.158**	**-0.227**	**0.077**	**0.139**
P-value	0.142	0.373	0.197	0.664	0.434
ANGPT1	**-0.381**	**-0.250**	**-0.343**	**0.233**	**0.237**
P-value	0.022	0.141	0.041	0.171	0.164
TIE2	**-0.192**	**-0.116**	**-0.160**	**0.146**	**0.208**
P-value	0.283	0.521	0.373	0.419	0.247
PlGF	**-0.281**	**-0.323**	**-0.313**	**0.146**	**0.172**
P-value	0.087	0.048	0.056	0.382	0.302
ANGPT2	**-0.359**	**-0.310**	**-0.358**	**0.009**	**-0.014**
P-value	0.078	0.132	0.079	0.967	0.948
Np1	**-0.203**	**-0.180**	**-0.202**	**0.078**	**0.099**
P-value	0.222	0.279	0.223	0.640	0.555
Np2	**0.036**	**0.026**	**0.033**	**0.120**	**0.121**
P-value	0.835	0.879	0.846	0.478	0.476
FGF	**-0.067**	**-0.029**	**-0.053**	**0.165**	**0.237**
P-value	0.697	0.868	0.759	0.337	0.163
FGFR	**-0.126**	**-0.075**	**-0.102**	**0.259**	**0.342**
P-value	0.459	0.660	0.549	0.121	0.038

Table A17. (Continued). Pearson correlation coefficients for maternal E2 and CAR mRNA in 38 animals.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
ERα	**0.138**	**-0.117**	**0.031**	**-0.286**	**-0.271**
P-value	0.407	0.482	0.855	0.082	0.099

Table A18. Pearson correlation coefficients for maternal E2 and CAR mRNA in CON.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**-0.157**	**0.104**	**0.013**	**0.217**	**0.269**
P-value	0.627	0.749	0.968	0.498	0.398
Flt	**0.744**	**0.747**	**0.797**	**0.237**	**-0.059**
P-value	0.006	0.005	0.002	0.459	0.855
KDR	**-0.236**	**0.048**	**-0.070**	**0.215**	**0.283**
P-value	0.461	0.883	0.829	0.503	0.373
HIF	**-0.065**	**0.261**	**0.141**	**-0.029**	**-0.292**
P-value	0.849	0.438	0.680	0.933	0.384
eNOS	**0.265**	**0.523**	**0.457**	**0.349**	**0.336**
P-value	0.406	0.081	0.136	0.266	0.286
sGC	**-0.523**	**-0.163**	**-0.318**	**-0.165**	**0.144**
P-value	0.099	0.632	0.340	0.629	0.674
ANGPT1	**-0.160**	**0.245**	**0.086**	**0.171**	**0.113**
P-value	0.619	0.443	0.789	0.594	0.726
TIE2	**-0.078**	**0.140**	**0.061**	**0.145**	**0.333**
P-value	0.821	0.681	0.858	0.672	0.316
PlGF	**0.283**	**0.315**	**0.337**	**0.270**	**0.256**
P-value	0.373	0.318	0.285	0.395	0.422
ANGPT2	**0.073**	**0.399**	**0.291**	**0.278**	**0.252**
P-value	0.891	0.434	0.577	0.594	0.631
Np1	**-0.118**	**0.241**	**0.104**	**0.243**	**0.172**
P-value	0.715	0.451	0.747	0.446	0.593
Np2	**-0.069**	**0.333**	**0.188**	**0.096**	**0.104**
P-value	0.831	0.291	0.558	0.767	0.749
FGF	**-0.422**	**-0.227**	**-0.313**	**-0.023**	**0.239**
P-value	0.196	0.502	0.349	0.946	0.480
FGFR	**0.125**	**0.032**	**0.095**	**0.327**	**0.476**
P-value	0.699	0.920	0.768	0.300	0.118

Table A18. (Continued). Pearson correlation coefficients for maternal E2 and CAR mRNA in CON.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
ERα	**-0.235**	**0.086**	**-0.051**	**-0.056**	**-0.010**
P-value	0.462	0.791	0.874	0.864	0.976

Table A19. Pearson correlation coefficients for maternal E2 and CAR mRNA in HI.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**-0.531**	**-0.514**	**-0.558**	**-0.432**	**-0.374**
P-value	0.051	0.060	0.038	0.123	0.188
Flt	**-0.620**	**-0.684**	**-0.700**	**-0.467**	**-0.420**
P-value	0.018	0.007	0.005	0.093	0.135
KDR	**-0.358**	**-0.491**	**-0.459**	**-0.184**	**-0.106**
P-value	0.208	0.075	0.099	0.529	0.719
HIF	**-0.651**	**-0.748**	**-0.752**	**-0.384**	**-0.347**
P-value	0.012	0.002	0.002	0.175	0.224
eNOS	**-0.473**	**-0.388**	**-0.457**	**-0.258**	**-0.237**
P-value	0.088	0.170	0.100	0.374	0.414
sGC	**-0.476**	**-0.390**	**-0.455**	**-0.257**	**-0.220**
P-value	0.139	0.235	0.160	0.446	0.516
ANGPT1	**-0.488**	**-0.464**	**-0.509**	**-0.193**	**-0.137**
P-value	0.091	0.111	0.076	0.528	0.655
TIE2	**-0.124**	**-0.109**	**-0.125**	**-0.204**	**-0.195**
P-value	0.687	0.724	0.684	0.503	0.524
PlGF	**-0.501**	**-0.432**	**-0.496**	**-0.345**	**-0.308**
P-value	0.068	0.123	0.071	0.227	0.284
ANGPT2	**-0.503**	**-0.356**	**-0.447**	**-0.364**	**-0.347**
P-value	0.167	0.347	0.227	0.335	0.361
Np1	**-0.149**	**-0.283**	**-0.235**	**-0.042**	**0.044**
P-value	0.612	0.326	0.418	0.887	0.882
Np2	**-0.205**	**-0.080**	**-0.148**	**-0.074**	**-0.048**
P-value	0.481	0.785	0.614	0.802	0.870
FGF	**-0.617**	**-0.210**	**-0.423**	**-0.190**	**-0.264**
P-value	0.025	0.491	0.150	0.533	0.384
FGFR	**0.209**	**0.060**	**0.138**	**-0.172**	**-0.178**
P-value	0.474	0.839	0.638	0.557	0.543

Table A19. (Continued). Pearson correlation coefficients for maternal E2 and CAR mRNA in HI.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
ERα	**-0.099**	**-0.147**	**-0.135**	**-0.002**	**-0.013**
P-value	0.737	0.615	0.646	0.995	0.964

Table A20. Pearson correlation coefficients for maternal E2 and CAR mRNA in HI+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**-0.260**	**-0.395**	**-0.397**	**-0.272**	**-0.286**
P-value	0.414	0.204	0.201	0.393	0.368
Flt	**0.305**	**-0.295**	**0.058**	**-0.310**	**-0.291**
P-value	0.335	0.352	0.859	0.328	0.358
KDR	**-0.369**	**-0.284**	**-0.403**	**-0.027**	**-0.064**
P-value	0.237	0.372	0.194	0.934	0.843
HIF	**-0.511**	**-0.017**	**-0.341**	**0.021**	**-0.019**
P-value	0.108	0.960	0.305	0.950	0.956
eNOS	**0.127**	**-0.191**	**-0.010**	**-0.254**	**-0.240**
P-value	0.695	0.552	0.976	0.425	0.452
sGC	**-0.433**	**-0.141**	**-0.373**	**0.415**	**0.370**
P-value	0.159	0.662	0.232	0.179	0.237
ANGPT1	**-0.575**	**-0.184**	**-0.488**	**-0.212**	**-0.229**
P-value	0.064	0.587	0.128	0.531	0.499
TIE2	**-0.242**	**0.074**	**-0.104**	**0.618**	**0.626**
P-value	0.530	0.849	0.791	0.076	0.072
PlGF	**0.486**	**-0.240**	**0.212**	**-0.194**	**-0.159**
P-value	0.109	0.452	0.509	0.545	0.621
ANGPT2	**-0.029**	**0.076**	**0.023**	**0.268**	**0.263**
P-value	0.938	0.834	0.950	0.454	0.463
Np1	**0.017**	**0.152**	**0.103**	**-0.069**	**-0.022**
P-value	0.958	0.636	0.751	0.832	0.945
Np2	**0.106**	**-0.122**	**0.007**	**0.518**	**0.463**
P-value	0.756	0.720	0.984	0.102	0.152
FGF	**0.322**	**0.177**	**0.317**	**0.057**	**0.042**
P-value	0.307	0.583	0.315	0.861	0.898
FGFR	**-0.461**	**-0.242**	**-0.394**	**-0.337**	**-0.357**
P-value	0.153	0.474	0.231	0.311	0.281

Table A20. (Continued). Pearson correlation coefficients for maternal E2 and CAR mRNA in HI+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
ERα	**0.631**	**-0.258**	**0.299**	**-0.386**	**-0.361**
P-value	0.028	0.419	0.346	0.215	0.249

Table A21. Pearson correlation coefficients for maternal E2 and CAR mRNA in HI IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**-0.624**	**-0.369**	**-0.509**	**-0.682**	**-0.593**
P-value	0.098	0.368	0.197	0.062	0.121
Flt	**-0.708**	**-0.708**	**-0.724**	**-0.585**	**-0.533**
P-value	0.049	0.050	0.042	0.128	0.174
KDR	**-0.491**	**-0.430**	**-0.470**	**-0.274**	**-0.203**
P-value	0.217	0.287	0.239	0.511	0.631
HIF	**-0.915**	**-0.881**	**-0.918**	**-0.835**	**-0.783**
P-value	0.001	0.004	0.001	0.010	0.022
eNOS	**-0.560**	**-0.505**	**-0.544**	**-0.323**	**-0.244**
P-value	0.149	0.202	0.163	0.435	0.560
sGC	**-0.749**	**-0.432**	**-0.608**	**-0.635**	**-0.563**
P-value	0.086	0.393	0.200	0.176	0.245
ANGPT1	**-0.829**	**-0.729**	**-0.809**	**-0.556**	**-0.472**
P-value	0.021	0.063	0.028	0.195	0.285
TIE2	**0.008**	**0.080**	**0.045**	**-0.318**	**-0.305**
P-value	0.987	0.865	0.923	0.488	0.507
PlGF	**-0.703**	**-0.598**	**-0.665**	**-0.494**	**-0.410**
P-value	0.052	0.118	0.072	0.214	0.313
ANGPT2	**-0.481**	**-0.060**	**-0.282**	**-0.364**	**-0.281**
P-value	0.334	0.910	0.589	0.478	0.590
Np1	**-0.292**	**-0.226**	**-0.264**	**-0.066**	**0.003**
P-value	0.483	0.590	0.527	0.876	0.995
Np2	**-0.284**	**-0.097**	**-0.195**	**-0.489**	**-0.438**
P-value	0.496	0.819	0.643	0.219	0.278
FGF	**-0.788**	**-0.631**	**-0.735**	**-0.704**	**-0.653**
P-value	0.035	0.129	0.060	0.077	0.112
FGFR	**0.582**	**0.579**	**0.594**	**0.218**	**0.196**
P-value	0.130	0.133	0.121	0.604	0.642

Table A21. (Continued). Pearson correlation coefficients for maternal E2 and CAR mRNA in HI IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
ERα	**-0.285**	**-0.293**	**-0.298**	**-0.416**	**-0.368**
P-value	0.495	0.481	0.474	0.305	0.370

Table A22. Pearson correlation coefficients for maternal E2 and CAR mRNA in HI IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**-0.510**	**-0.555**	**-0.666**	**0.040**	**-0.029**
P-value	0.380	0.332	0.220	0.950	0.963
Flt	**0.435**	**-0.138**	**0.303**	**-0.599**	**-0.501**
P-value	0.464	0.824	0.620	0.286	0.390
KDR	**-0.602**	**-0.435**	**-0.690**	**-0.246**	**-0.356**
P-value	0.283	0.464	0.197	0.690	0.556
HIF	**-0.952**	**-0.057**	**-0.844**	**0.585**	**0.239**
P-value	0.048	0.943	0.157	0.416	0.761
eNOS	**-0.114**	**-0.216**	**-0.188**	**0.640**	**0.683**
P-value	0.855	0.727	0.762	0.245	0.204
sGC	**-0.715**	**-0.233**	**-0.698**	**0.105**	**-0.070**
P-value	0.174	0.706	0.190	0.867	0.911
ANGPT1	**-0.977**	**-0.339**	**-0.962**	**-0.196**	**-0.347**
P-value	0.004	0.577	0.009	0.752	0.567
TIE2	**-0.997**	**-0.583**	**-0.967**	**-0.213**	**-0.181**
P-value	0.050	0.604	0.165	0.863	0.884
PlGF	**0.450**	**-0.239**	**0.272**	**0.742**	**0.847**
P-value	0.447	0.699	0.658	0.151	0.070
ANGPT2	**-0.587**	**-0.191**	**-0.573**	**0.531**	**0.440**
P-value	0.298	0.758	0.313	0.357	0.458
Np1	**-0.526**	**0.109**	**-0.392**	**0.285**	**0.072**
P-value	0.363	0.861	0.515	0.642	0.908
Np2	**0.736**	**-0.029**	**0.601**	**0.141**	**0.367**
P-value	0.157	0.963	0.283	0.822	0.544
FGF	**0.259**	**0.861**	**0.589**	**0.690**	**0.544**
P-value	0.674	0.061	0.296	0.197	0.343
FGFR	**-0.967**	**-0.740**	**-0.943**	**-0.676**	**-0.700**
P-value	0.033	0.260	0.057	0.325	0.300

Table A22. (Continued). Pearson correlation coefficients for maternal E2 and CAR mRNA in HI IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
ERα	**0.761**	**-0.331**	**0.492**	**-0.368**	**-0.133**
P-value	0.135	0.586	0.400	0.543	0.832

Table A23. Pearson correlation coefficients for maternal E2 and CAR mRNA in HI non-IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**-0.216**	**-0.968**	**-0.686**	**-0.259**	**-0.071**
P-value	0.682	0.002	0.132	0.620	0.893
Flt	**-0.400**	**-0.875**	**-0.745**	**-0.652**	**-0.468**
P-value	0.432	0.022	0.089	0.160	0.349
KDR	**-0.079**	**-0.782**	**-0.497**	**-0.092**	**0.109**
P-value	0.881	0.066	0.316	0.862	0.837
HIF	**-0.051**	**-0.667**	**-0.414**	**0.095**	**0.194**
P-value	0.924	0.148	0.415	0.858	0.713
eNOS	**-0.162**	**0.211**	**0.023**	**-0.070**	**-0.125**
P-value	0.760	0.689	0.966	0.895	0.814
sGC	**0.087**	**-0.172**	**-0.030**	**0.305**	**0.342**
P-value	0.890	0.783	0.962	0.617	0.574
ANGPT1	**-0.461**	**-0.892**	**-0.792**	**-0.543**	**-0.375**
P-value	0.357	0.017	0.060	0.266	0.464
TIE2	**-0.455**	**-0.711**	**-0.684**	**-0.623**	**-0.540**
P-value	0.364	0.113	0.134	0.186	0.269
PlGF	**0.210**	**0.150**	**0.214**	**-0.298**	**-0.208**
P-value	0.690	0.777	0.684	0.567	0.693
ANGPT2	**0.213**	**-0.391**	**0.057**	**0.994**	**0.980**
P-value	0.864	0.745	0.964	0.067	0.128
Np1	**0.108**	**-0.625**	**-0.293**	**-0.068**	**0.164**
P-value	0.838	0.185	0.574	0.899	0.757
Np2	**-0.298**	**-0.590**	**-0.519**	**-0.235**	**-0.126**
P-value	0.566	0.218	0.291	0.654	0.812
FGF	**-0.614**	**0.013**	**-0.365**	**-0.082**	**-0.250**
P-value	0.195	0.981	0.477	0.877	0.633
FGFR	**-0.337**	**-0.382**	**-0.424**	**0.151**	**0.057**
P-value	0.514	0.455	0.402	0.776	0.915

Table A23. (Continued). Pearson correlation coefficients for maternal E2 and CAR mRNA in HI non-IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
ERα	**0.290**	**0.208**	**0.296**	**0.642**	**0.533**
P-value	0.577	0.692	0.570	0.170	0.277

Table A24. Pearson correlation coefficients for maternal E2 and CAR mRNA in HI non-IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**-0.195**	**-0.106**	**-0.176**	**-0.090**	**-0.122**
P-value	0.676	0.822	0.706	0.848	0.794
Flt	**-0.145**	**-0.125**	**-0.134**	**0.158**	**0.189**
P-value	0.757	0.789	0.775	0.734	0.685
KDR	**0.079**	**0.135**	**0.124**	**0.607**	**0.533**
P-value	0.867	0.773	0.791	0.148	0.218
HIF	**0.533**	**0.196**	**0.379**	**0.202**	**0.134**
P-value	0.218	0.674	0.402	0.664	0.775
eNOS	**0.506**	**0.336**	**0.454**	**-0.019**	**-0.097**
P-value	0.247	0.462	0.307	0.968	0.837
sGC	**-0.049**	**-0.105**	**-0.083**	**0.643**	**0.593**
P-value	0.918	0.822	0.860	0.119	0.161
ANGPT1	**0.810**	**0.925**	**0.922**	**0.390**	**0.397**
P-value	0.051	0.008	0.009	0.445	0.436
TIE2	**0.008**	**0.266**	**0.150**	**0.968**	**0.970**
P-value	0.989	0.610	0.777	0.002	0.001
PlGF	**0.531**	**0.078**	**0.317**	**0.128**	**0.115**
P-value	0.220	0.868	0.488	0.784	0.806
ANGPT2	**0.440**	**0.660**	**0.576**	**0.903**	**0.876**
P-value	0.458	0.225	0.309	0.036	0.051
Np1	**0.388**	**0.452**	**0.463**	**0.219**	**0.294**
P-value	0.390	0.309	0.296	0.637	0.523
Np2	**0.153**	**-0.296**	**-0.089**	**0.491**	**0.381**
P-value	0.772	0.570	0.867	0.323	0.457
FGF	**0.323**	**0.305**	**0.330**	**0.603**	**0.548**
P-value	0.480	0.507	0.470	0.151	0.203
FGFR	**0.866**	**0.416**	**0.682**	**-0.204**	**-0.184**
P-value	0.012	0.353	0.092	0.661	0.692

Table A24. (Continued). Pearson correlation coefficients for maternal E2 and CAR mRNA in HI non-IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
ERα	**0.212**	**0.474**	**0.379**	**-0.360**	**-0.370**
P-value	0.648	0.283	0.402	0.428	0.414

Table A25. Pearson correlation coefficients for maternal E2 and COT mRNA in 38 animals.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**0.162**	**0.121**	**0.152**	**0.240**	**0.232**
P-value	0.330	0.471	0.363	0.146	0.160
Flt	**-0.108**	**-0.053**	**-0.089**	**0.259**	**0.247**
P-value	0.520	0.752	0.597	0.116	0.135
KDR	**0.270**	**0.327**	**0.311**	**-0.131**	**-0.149**
P-value	0.102	0.045	0.058	0.433	0.371
HIF	**0.150**	**0.285**	**0.220**	**0.346**	**0.302**
P-value	0.368	0.082	0.185	0.033	0.066
eNOS	**0.180**	**0.154**	**0.178**	**0.039**	**0.001**
P-value	0.281	0.356	0.284	0.816	0.997
sGC	**0.387**	**0.159**	**0.306**	**-0.176**	**-0.181**
P-value	0.018	0.346	0.066	0.298	0.284
ANGPT1	**-0.094**	**-0.186**	**-0.140**	**-0.261**	**-0.203**
P-value	0.581	0.270	0.410	0.119	0.227
TIE2	**0.201**	**0.330**	**0.269**	**-0.128**	**-0.185**
P-value	0.255	0.057	0.124	0.470	0.294
PlGF	**0.117**	**0.119**	**0.123**	**0.086**	**0.062**
P-value	0.498	0.489	0.474	0.618	0.718
ANGPT2	**-0.125**	**-0.162**	**-0.147**	**-0.025**	**0.008**
P-value	0.455	0.332	0.377	0.884	0.962
Np1	**0.150**	**0.093**	**0.133**	**-0.018**	**-0.039**
P-value	0.367	0.580	0.427	0.915	0.815
Np2	**-0.201**	**-0.196**	**-0.212**	**0.058**	**0.005**
P-value	0.226	0.238	0.202	0.728	0.978
FGF	**0.103**	**0.155**	**0.132**	**-0.103**	**-0.114**
P-value	0.539	0.353	0.430	0.539	0.497
FGFR	**0.385**	**0.234**	**0.337**	**-0.074**	**-0.038**

Table A25. (Continued). Pearson correlation coefficients for maternal E2 and COT mRNA in 38 animals.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
P-value	0.017	0.158	0.038	0.658	0.821
ERα	**0.337**	**0.221**	**0.304**	**-0.199**	**-0.217**
P-value	0.038	0.183	0.064	0.232	0.190

Table A26. Pearson correlation coefficients for maternal E2 and COT mRNA in CON.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**-0.192**	**-0.144**	**-0.179**	**-0.195**	**-0.163**
P-value	0.551	0.656	0.579	0.543	0.612
Flt	**-0.123**	**-0.162**	**-0.159**	**-0.144**	**-0.124**
P-value	0.704	0.615	0.621	0.655	0.701
KDR	**-0.066**	**-0.091**	**-0.095**	**-0.279**	**-0.354**
P-value	0.840	0.779	0.768	0.381	0.258
HIF	**0.055**	**0.141**	**0.104**	**-0.307**	**-0.355**
P-value	0.866	0.662	0.747	0.332	0.258
eNOS	**0.372**	**0.642**	**0.568**	**0.145**	**-0.104**
P-value	0.233	0.024	0.054	0.654	0.747
sGC	**0.523**	**0.525**	**0.556**	**0.283**	**-0.071**
P-value	0.081	0.080	0.061	0.372	0.826
ANGPT1	**0.057**	**-0.072**	**-0.005**	**-0.141**	**0.149**
P-value	0.861	0.824	0.988	0.661	0.644
TIE2	**0.538**	**0.674**	**0.662**	**0.173**	**-0.081**
P-value	0.109	0.033	0.037	0.633	0.825
PlGF	**0.566**	**0.102**	**0.307**	**0.091**	**-0.037**
P-value	0.088	0.779	0.388	0.804	0.920
ANGPT2	**-0.376**	**-0.358**	**-0.393**	**-0.111**	**-0.053**
P-value	0.228	0.254	0.206	0.731	0.870
Np1	**0.025**	**0.239**	**0.162**	**0.412**	**0.317**
P-value	0.940	0.454	0.614	0.184	0.316
Np2	**-0.080**	**-0.027**	**-0.057**	**-0.351**	**-0.406**
P-value	0.805	0.934	0.862	0.263	0.191
FGF	**-0.275**	**-0.567**	**-0.487**	**-0.492**	**-0.323**
P-value	0.386	0.055	0.109	0.104	0.306
FGFR	**-0.062**	**-0.047**	**-0.061**	**-0.272**	**-0.101**

Table A26. (Continued). Pearson correlation coefficients for maternal E2 and COT mRNA in CON.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
P-value	0.847	0.885	0.852	0.393	0.755
ERα	**0.378**	**0.422**	**0.419**	**0.245**	**0.003**
P-value	0.225	0.172	0.175	0.444	0.993

Table A27. Pearson correlation coefficients for maternal E2 and COT mRNA in HI.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**0.453**	**0.539**	**0.532**	**0.429**	**0.362**
P-value	0.104	0.047	0.050	0.126	0.203
Flt	**0.455**	**0.202**	**0.340**	**0.105**	**0.079**
P-value	0.102	0.488	0.234	0.720	0.789
KDR	**0.489**	**0.379**	**0.459**	**0.100**	**0.096**
P-value	0.076	0.182	0.099	0.735	0.743
HIF	**0.704**	**0.361**	**0.557**	**0.728**	**0.775**
P-value	0.005	0.205	0.038	0.003	0.001
eNOS	**-0.144**	**-0.040**	**-0.093**	**-0.023**	**-0.024**
P-value	0.624	0.892	0.751	0.938	0.935
sGC	**0.047**	**-0.385**	**-0.205**	**-0.471**	**-0.449**
P-value	0.878	0.194	0.501	0.104	0.123
ANGPT1	**0.012**	**-0.231**	**-0.129**	**-0.387**	**-0.414**
P-value	0.967	0.428	0.661	0.172	0.141
TIE2	**0.561**	**0.640**	**0.641**	**0.459**	**0.410**
P-value	0.046	0.018	0.018	0.115	0.164
PlGF	**0.171**	**0.083**	**0.130**	**-0.131**	**-0.154**
P-value	0.558	0.778	0.657	0.656	0.598
ANGPT2	**0.084**	**-0.137**	**-0.039**	**-0.244**	**-0.239**
P-value	0.774	0.639	0.895	0.401	0.411
Np1	**-0.086**	**0.105**	**0.017**	**0.029**	**0.003**
P-value	0.769	0.720	0.955	0.921	0.992
Np2	**0.016**	**0.489**	**0.287**	**0.285**	**0.165**
P-value	0.957	0.076	0.320	0.323	0.574
FGF	**0.374**	**0.426**	**0.427**	**0.252**	**0.188**
P-value	0.188	0.129	0.127	0.385	0.520
FGFR	**0.407**	**0.135**	**0.278**	**0.025**	**0.027**

Table A27. (Continued). Pearson correlation coefficients for maternal E2 and COT mRNA in HI.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
P-value	0.149	0.645	0.337	0.932	0.927
ERα	**0.145**	**-0.164**	**-0.024**	**-0.295**	**-0.289**
P-value	0.620	0.576	0.935	0.306	0.316

Table A28. Pearson correlation coefficients for maternal E2 and COT mRNA in HI+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**0.511**	**0.068**	**0.386**	**0.263**	**0.287**
P-value	0.090	0.833	0.215	0.410	0.366
Flt	**-0.003**	**0.182**	**0.099**	**0.463**	**0.414**
P-value	0.993	0.572	0.759	0.130	0.181
KDR	**0.204**	**0.373**	**0.346**	**-0.244**	**-0.252**
P-value	0.524	0.232	0.271	0.444	0.430
HIF	**0.190**	**0.477**	**0.391**	**0.681**	**0.687**
P-value	0.555	0.117	0.209	0.015	0.014
eNOS	**0.374**	**0.035**	**0.276**	**-0.133**	**-0.057**
P-value	0.232	0.914	0.385	0.680	0.860
sGC	**0.356**	**-0.086**	**0.201**	**-0.162**	**-0.129**
P-value	0.257	0.791	0.532	0.616	0.689
ANGPT1	**-0.184**	**-0.338**	**-0.307**	**-0.339**	**-0.317**
P-value	0.589	0.310	0.358	0.308	0.341
TIE2	**-0.264**	**0.188**	**-0.091**	**-0.311**	**-0.267**
P-value	0.433	0.580	0.790	0.352	0.427
PlGF	**0.012**	**-0.024**	**-0.009**	**-0.071**	**-0.107**
P-value	0.969	0.941	0.978	0.827	0.740
ANGPT2	**-0.212**	**-0.282**	**-0.293**	**-0.226**	**-0.193**
P-value	0.509	0.374	0.355	0.480	0.547
Np1	**0.211**	**-0.067**	**0.110**	**-0.155**	**-0.215**
P-value	0.511	0.836	0.735	0.630	0.503
Np2	**-0.157**	**-0.553**	**-0.415**	**-0.247**	**-0.243**
P-value	0.625	0.062	0.179	0.439	0.446
FGF	**0.042**	**0.384**	**0.239**	**0.292**	**0.276**
P-value	0.898	0.218	0.455	0.358	0.385
FGFR	**0.496**	**0.131**	**0.411**	**-0.011**	**0.019**

Table A28. (Continued). Pearson correlation coefficients for maternal E2 and COT mRNA in HI+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
P-value	0.101	0.685	0.184	0.973	0.954
ERα	**0.632**	**0.320**	**0.611**	**-0.196**	**-0.177**
P-value	0.028	0.311	0.035	0.541	0.582

Table A29. Pearson correlation coefficients for maternal E2 and COT mRNA in HI IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**0.627**	**0.455**	**0.551**	**0.319**	**0.270**
P-value	0.096	0.257	0.157	0.441	0.519
Flt	**0.564**	**0.310**	**0.445**	**-0.034**	**-0.116**
P-value	0.146	0.455	0.270	0.937	0.785
KDR	**0.704**	**0.513**	**0.622**	**0.533**	**0.477**
P-value	0.051	0.193	0.100	0.173	0.232
HIF	**0.670**	**0.476**	**0.586**	**0.779**	**0.735**
P-value	0.069	0.233	0.127	0.023	0.038
eNOS	**-0.037**	**0.096**	**0.032**	**0.662**	**0.728**
P-value	0.931	0.821	0.940	0.074	0.041
sGC	**0.133**	**-0.143**	**-0.007**	**-0.469**	**-0.546**
P-value	0.753	0.736	0.988	0.241	0.162
ANGPT1	**0.188**	**-0.073**	**0.057**	**-0.364**	**-0.442**
P-value	0.656	0.864	0.894	0.375	0.273
TIE2	**0.865**	**0.880**	**0.886**	**0.889**	**0.887**
P-value	0.012	0.009	0.008	0.007	0.008
PlGF	**0.263**	**0.186**	**0.226**	**-0.386**	**-0.436**
P-value	0.529	0.660	0.590	0.345	0.280
ANGPT2	**0.189**	**-0.072**	**0.058**	**-0.216**	**-0.263**
P-value	0.654	0.866	0.891	0.607	0.529
Np1	**0.026**	**0.031**	**0.028**	**-0.047**	**-0.005**
P-value	0.952	0.941	0.948	0.913	0.990
Np2	**0.348**	**0.124**	**0.241**	**0.011**	**-0.038**
P-value	0.398	0.769	0.565	0.979	0.928
FGF	**0.373**	**0.193**	**0.287**	**-0.152**	**-0.182**
P-value	0.363	0.647	0.491	0.719	0.666
FGFR	**0.397**	**0.142**	**0.273**	**-0.049**	**-0.118**

Table A29. (Continued). Pearson correlation coefficients for maternal E2 and COT mRNA in HI IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
P-value	0.330	0.737	0.513	0.908	0.782
ERα	**0.313**	**0.098**	**0.208**	**-0.193**	**-0.232**
P-value	0.450	0.818	0.622	0.647	0.580

Table A30. Pearson correlation coefficients for maternal E2 and COT mRNA in HI IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**0.717**	**0.267**	**0.714**	**0.634**	**0.687**
P-value	0.173	0.664	0.176	0.251	0.200
Flt	**0.235**	**0.683**	**0.492**	**-0.404**	**-0.456**
P-value	0.704	0.204	0.400	0.500	0.440
KDR	**-0.383**	**0.305**	**-0.188**	**0.628**	**0.510**
P-value	0.525	0.618	0.762	0.256	0.380
HIF	**0.028**	**0.243**	**0.129**	**0.839**	**0.818**
P-value	0.964	0.694	0.837	0.076	0.090
eNOS	**0.715**	**0.542**	**0.831**	**0.539**	**0.545**
P-value	0.175	0.346	0.081	0.349	0.342
sGC	**0.451**	**0.235**	**0.478**	**0.805**	**0.797**
P-value	0.446	0.704	0.415	0.100	0.107
ANGPT1	**-0.409**	**-0.394**	**-0.512**	**0.315**	**0.230**
P-value	0.494	0.512	0.378	0.606	0.709
TIE2	**-0.313**	**0.596**	**-0.003**	**0.754**	**0.536**
P-value	0.609	0.289	0.996	0.141	0.352
PlGF	**0.319**	**-0.010**	**0.262**	**0.754**	**0.759**
P-value	0.600	0.987	0.670	0.141	0.137
ANGPT2	**-0.375**	**-0.152**	**-0.379**	**0.203**	**0.067**
P-value	0.534	0.807	0.530	0.743	0.915
Np1	**0.207**	**-0.725**	**-0.141**	**-0.743**	**-0.530**
P-value	0.738	0.166	0.822	0.150	0.359
Np2	**-0.040**	**-0.932**	**-0.437**	**-0.427**	**-0.276**
P-value	0.949	0.021	0.462	0.473	0.653
FGF	**-0.100**	**0.570**	**0.163**	**0.634**	**0.433**
P-value	0.873	0.316	0.793	0.251	0.466
FGFR	**0.586**	**0.343**	**0.637**	**0.734**	**0.738**

Table A30. (Continued). Pearson correlation coefficients for maternal E2 and COT mRNA in HI IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
P-value	0.299	0.572	0.247	0.158	0.154
ERα	**0.529**	**0.651**	**0.723**	**0.744**	**0.688**
P-value	0.360	0.234	0.168	0.150	0.199

Table A31. Pearson correlation coefficients for maternal E2 and COT mRNA in HI non-IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**-0.016**	**0.536**	**0.298**	**0.431**	**0.241**
P-value	0.976	0.273	0.566	0.393	0.646
Flt	**0.185**	**-0.225**	**-0.016**	**0.207**	**0.223**
P-value	0.725	0.669	0.975	0.694	0.671
KDR	**0.087**	**0.240**	**0.191**	**-0.442**	**-0.387**
P-value	0.870	0.647	0.718	0.380	0.449
HIF	**0.742**	**0.077**	**0.494**	**0.858**	**0.934**
P-value	0.091	0.885	0.319	0.029	0.006
eNOS	**0.004**	**0.632**	**0.365**	**0.341**	**0.137**
P-value	0.994	0.179	0.477	0.509	0.796
sGC	**0.451**	**-0.448**	**-0.014**	**0.481**	**0.742**
P-value	0.446	0.449	0.982	0.413	0.151
ANGPT1	**-0.180**	**-0.122**	**-0.179**	**-0.461**	**-0.470**
P-value	0.733	0.818	0.734	0.358	0.347
TIE2	**0.426**	**0.852**	**0.747**	**0.717**	**0.537**
P-value	0.399	0.031	0.088	0.109	0.272
PlGF	**-0.213**	**-0.482**	**-0.406**	**-0.300**	**-0.259**
P-value	0.686	0.333	0.425	0.563	0.621
ANGPT2	**-0.002**	**-0.110**	**-0.065**	**-0.424**	**-0.289**
P-value	0.996	0.835	0.903	0.402	0.579
Np1	**-0.380**	**-0.122**	**-0.301**	**-0.356**	**-0.399**
P-value	0.458	0.817	0.563	0.488	0.433
Np2	**-0.374**	**0.544**	**0.085**	**-0.025**	**-0.239**
P-value	0.465	0.265	0.873	0.963	0.648
FGF	**0.310**	**0.898**	**0.703**	**0.660**	**0.456**
P-value	0.550	0.015	0.119	0.154	0.364
FGFR	**0.540**	**0.278**	**0.487**	**0.200**	**0.273**

Table A31. (Continued). Pearson correlation coefficients for maternal E2 and COT mRNA in HI non-IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
P-value	0.269	0.594	0.327	0.704	0.601
ERα	**0.426**	**0.003**	**0.260**	**0.374**	**0.433**
P-value	0.400	0.996	0.618	0.466	0.391

Table A32. Pearson correlation coefficients for maternal E2 and COT mRNA in HI non-IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**-0.171**	**-0.280**	**-0.250**	**0.555**	**0.530**
P-value	0.715	0.543	0.588	0.196	0.221
Flt	**-0.234**	**-0.193**	**-0.218**	**0.622**	**0.573**
P-value	0.613	0.678	0.639	0.136	0.179
KDR	**0.553**	**0.505**	**0.567**	**-0.239**	**-0.253**
P-value	0.198	0.248	0.184	0.606	0.584
HIF	**0.383**	**0.631**	**0.555**	**0.887**	**0.868**
P-value	0.396	0.128	0.196	0.008	0.011
eNOS	**-0.176**	**0.032**	**-0.072**	**0.145**	**0.240**
P-value	0.706	0.945	0.878	0.756	0.604
sGC	**-0.125**	**-0.438**	**-0.307**	**-0.378**	**-0.378**
P-value	0.789	0.326	0.503	0.403	0.404
ANGPT1	**0.138**	**0.518**	**0.347**	**-0.361**	**-0.294**
P-value	0.794	0.292	0.500	0.482	0.571
TIE2	**-0.282**	**0.020**	**-0.150**	**-0.503**	**-0.425**
P-value	0.589	0.970	0.777	0.310	0.402
PlGF	**-0.191**	**-0.218**	**-0.225**	**-0.453**	**-0.526**
P-value	0.681	0.638	0.628	0.308	0.225
ANGPT2	**-0.128**	**-0.312**	**-0.232**	**-0.135**	**-0.015**
P-value	0.784	0.496	0.616	0.773	0.974
Np1	**0.384**	**0.276**	**0.351**	**-0.386**	**-0.484**
P-value	0.396	0.549	0.441	0.392	0.271
Np2	**-0.532**	**-0.243**	**-0.424**	**-0.072**	**-0.082**
P-value	0.219	0.600	0.343	0.877	0.862
FGF	**0.204**	**0.330**	**0.295**	**0.381**	**0.360**
P-value	0.661	0.470	0.520	0.399	0.428
FGFR	**-0.232**	**0.056**	**-0.094**	**0.035**	**-0.008**

Table A32. (Continued). Pearson correlation coefficients for maternal E2 and COT mRNA in HI non-IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
P-value	0.617	0.905	0.841	0.941	0.987
ERα	**0.912**	**0.562**	**0.781**	**-0.073**	**-0.090**
P-value	0.004	0.189	0.038	0.877	0.847

Table A33. Pearson correlation coefficients for maternal E2, total placentome proliferation, CAR and COT vascularity in 38 animals.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Total Placentome					
Proliferating nuclei, %	**0.126**	**0.028**	**0.091**	**0.427**	**0.457**
P-value	0.465	0.870	0.598	0.010	0.005
CAR Vascularity					
CAD[1], %	**-0.125**	**-0.059**	**-0.103**	**0.006**	**-0.013**
P-value	0.467	0.732	0.551	0.973	0.940
CND[2], mm^2	**-0.197**	**-0.166**	**-0.193**	**0.386**	**0.394**
P-value	0.250	0.332	0.260	0.020	0.018
CSD[3], ($\mu m/\mu m^2$) x 10	**-0.115**	**-0.073**	**-0.102**	**0.139**	**0.130**
P-value	0.506	0.672	0.554	0.420	0.449
APC[4], μm^2	**0.126**	**0.174**	**0.152**	**-0.397**	**-0.410**
P-value	0.466	0.310	0.377	0.016	0.013
COT Vascularity					
CAD[1], %	**-0.085**	**-0.003**	**-0.085**	**0.010**	**0.037**
P-value	0.631	0.985	0.632	0.954	0.837
CND[2], mm^2	**-0.064**	**0.022**	**-0.030**	**0.123**	**0.139**
P-value	0.718	0.903	0.868	0.488	0.432
CSD[3], ($\mu m/\mu m^2$) x 10	**-0.103**	**-0.061**	**-0.091**	**0.058**	**0.080**
P-value	0.561	0.732	0.609	0.746	0.654
APC[4], μm^2	**0.176**	**-0.124**	**0.051**	**-0.154**	**-0.120**
P-value	0.318	0.484	0.775	0.383	0.498

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.

[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[4]Area per capillary = capillary area/capillary number per sample area.

Table A34. Pearson correlation coefficients for maternal E2, total placentome proliferation, CAR and COT vascularity in CON.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Total Placentome					
Proliferating nuclei, %	**0.553**	**0.178**	**0.358**	**0.711**	**0.657**
P-value	0.062	0.579	0.254	0.010	0.020
CAR Vascularity					
CAD[1], %	**0.245**	**-0.284**	**-0.079**	**0.460**	**0.485**
P-value	0.469	0.397	0.818	0.155	0.131
CND[2], mm^2	**0.139**	**-0.424**	**-0.207**	**0.492**	**0.502**
P-value	0.684	0.194	0.542	0.125	0.116
CSD[3], ($\mu m/\mu m^2$) x 10	**0.254**	**-0.320**	**-0.096**	**0.505**	**0.495**
P-value	0.451	0.337	0.780	0.114	0.122
APC[4], μm^2	**0.112**	**0.513**	**0.364**	**-0.219**	**-0.284**
P-value	0.742	0.106	0.272	0.518	0.398
COT Vascularity					
CAD[1], %	**-0.236**	**-0.022**	**-0.117**	**-0.482**	**-0.379**
P-value	0.485	0.948	0.732	0.133	0.250
CND[2], mm^2	**-0.180**	**0.083**	**-0.026**	**-0.373**	**-0.308**
P-value	0.596	0.809	0.940	0.258	0.357
CSD[3], ($\mu m/\mu m^2$) x 10	**-0.232**	**0.041**	**-0.075**	**-0.436**	**-0.359**
P-value	0.492	0.905	0.827	0.181	0.278
APC[4], μm^2	**-0.271**	**-0.173**	**-0.229**	**-0.292**	**-0.225**
P-value	0.421	0.611	0.498	0.384	0.506

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.

[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[4]Area per capillary = capillary area/capillary number per sample area.

Table A35. Pearson correlation coefficients for maternal E2, total placentome proliferation, CAR and COT Vascularity in HI.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Total Placentome					
Proliferating nuclei, %	**0.107**	**0.027**	**0.072**	**0.060**	**0.133**
P-value	0.740	0.934	0.825	0.853	0.681
CAR Vascularity					
CAD[1], %	**-0.072**	**0.493**	**0.239**	**0.144**	**0.024**
P-value	0.815	0.087	0.431	0.640	0.938
CND[2], mm^2	**-0.043**	**0.557**	**0.291**	**0.262**	**0.141**
P-value	0.889	0.048	0.335	0.388	0.646
CSD[3], ($\mu m/\mu m^2$) x 10	**-0.056**	**0.529**	**0.268**	**0.173**	**0.053**
P-value	0.856	0.063	0.375	0.571	0.864
APC[4], μm^2	**-0.087**	**-0.060**	**-0.080**	**-0.345**	**-0.367**
P-value	0.778	0.846	0.794	0.249	0.217
COT Vascularity					
CAD[1], %	**-0.389**	**0.117**	**-0.489**	**-0.379**	**-0.388**
P-value	0.212	0.703	0.107	0.224	0.212
CND[2], mm^2	**-0.227**	**-0.389**	**-0.331**	**-0.334**	**-0.329**
P-value	0.478	0.211	0.294	0.289	0.296
CSD[3], ($\mu m/\mu m^2$) x 10	**-0.362**	**-0.504**	**-0.463**	**-0.371**	**-0.374**
P-value	0.247	0.095	0.130	0.235	0.231
APC[4], μm^2	**-0.624**	**-0.680**	**-0.690**	**-0.416**	**-0.416**
P-value	0.030	0.015	0.013	0.179	0.179

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.

[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[4]Area per capillary = capillary area/capillary number per sample area.

Table A36. Pearson correlation coefficients for maternal E2, total placentome proliferation, CAR and COT Vascularity in HI+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Total Placentome					
Proliferating nuclei, %	**0.470**	**-0.054**	**0.295**	**0.108**	**0.138**
P-value	0.124	0.868	0.352	0.738	0.668
CAR Vascularity					
CAD[1], %	**-0.266**	**-0.020**	**-0.195**	**-0.306**	**-0.237**
P-value	0.404	0.950	0.543	0.334	0.458
CND[2], mm^2	**-0.362**	**-0.311**	**-0.413**	**0.009**	**0.058**
P-value	0.248	0.325	0.182	0.979	0.858
CSD[3], ($\mu m/\mu m^2$) x 10	**-0.339**	**-0.307**	**-0.397**	**-0.297**	**-0.211**
P-value	0.282	0.332	0.201	0.348	0.511
APC[4], μm^2	**0.099**	**0.297**	**0.222**	**-0.243**	**-0.247**
P-value	0.761	0.349	0.489	0.447	0.438
COT Vascularity					
CAD[1], %	**0.223**	**0.190**	**0.094**	**0.063**	**0.103**
P-value	0.511	0.553	0.783	0.854	0.762
CND[2], mm^2	**0.227**	**0.295**	**0.311**	**0.035**	**0.050**
P-value	0.502	0.378	0.352	0.919	0.884
CSD[3], ($\mu m/\mu m^2$) x 10	**0.206**	**-0.031**	**0.116**	**-0.216**	**-0.184**
P-value	0.544	0.929	0.735	0.524	0.588
APC[4], μm^2	**0.423**	**-0.533**	**0.005**	**-0.232**	**-0.186**
P-value	0.195	0.091	0.989	0.492	0.585

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.

[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[4]Area per capillary = capillary area/capillary number per sample area.

Table A37. Pearson correlation coefficients for maternal E2, total placentome proliferation, CAR and COT Vascularity in HI IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Total Placentome					
Proliferating nuclei, %	-0.269	-0.137	-0.212	0.004	0.063
P-value	0.560	0.770	0.648	0.993	0.894
CAR Vascularity					
CAD[1], %	**0.639**	**0.847**	**0.752**	**0.116**	**0.071**
P-value	0.122	0.016	0.051	0.805	0.879
CND[2], mm^2	**-0.102**	**0.167**	**0.022**	**-0.207**	**-0.204**
P-value	0.827	0.721	0.963	0.657	0.662
CSD[3], ($\mu m/\mu m^2$) x 10	**0.475**	**0.735**	**0.609**	**0.057**	**0.018**
P-value	0.281	0.060	0.146	0.904	0.969
APC[4], μm^2	**0.786**	**0.868**	**0.843**	**0.288**	**0.238**
P-value	0.036	0.011	0.017	0.531	0.607
COT Vascularity					
CAD[1], %	**-0.447**	**0.067**	**-0.512**	**-0.468**	**-0.487**
P-value	0.267	0.865	0.195	0.243	0.221
CND[2], mm^2	**-0.271**	**-0.322**	**-0.302**	**-0.389**	**-0.399**
P-value	0.517	0.436	0.467	0.340	0.327
CSD[3], ($\mu m/\mu m^2$) x 10	**-0.428**	**-0.512**	**-0.479**	**-0.453**	**-0.465**
P-value	0.290	0.195	0.230	0.260	0.246
APC[4], μm^2	**-0.719**	**-0.771**	**-0.760**	**-0.417**	**-0.403**
P-value	0.044	0.025	0.029	0.304	0.322

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.

[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[4]Area per capillary = capillary area/capillary number per sample area.

Table A38. Pearson correlation coefficients for maternal E2, placentome proliferation, CAR and COT Vascularity in HI IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Total Placentome					
Proliferating nuclei, %	0.750	-0.532	0.396	-0.047	0.229
P-value	0.144	0.357	0.509	0.940	0.711
CAR Vascularity					
CAD[1], %	**-0.869**	**-0.338**	**-0.871**	**-0.318**	**-0.408**
P-value	0.056	0.579	0.054	0.602	0.496
CND[2], mm^2	**-0.582**	**-0.592**	**-0.742**	**-0.720**	**-0.758**
P-value	0.303	0.293	0.151	0.171	0.138
CSD[3], ($\mu m/\mu m^2$) x 10	**-0.688**	**-0.787**	**-0.915**	**-0.554**	**-0.538**
P-value	0.199	0.115	0.030	0.333	0.350
APC[4], μm^2	**0.295**	**0.419**	**0.428**	**0.707**	**0.732**
P-value	0.630	0.483	0.472	0.182	0.160
COT Vascularity					
CAD[1], %	**0.804**	**0.358**	**0.899**	**0.684**	**0.738**
P-value	0.101	0.486	0.038	0.203	0.155
CND[2], mm^2	**0.365**	**0.937**	**0.710**	**0.106**	**0.015**
P-value	0.546	0.019	0.179	0.865	0.981
CSD[3], ($\mu m/\mu m^2$) x 10	**0.635**	**0.823**	**0.886**	**0.581**	**0.547**
P-value	0.249	0.087	0.045	0.304	0.340
APC[4], μm^2	**0.555**	**-0.716**	**0.154**	**-0.002**	**0.252**
P-value	0.331	0.174	0.805	0.998	0.682

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.
[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.
[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.
[4]Area per capillary = capillary area/capillary number per sample area.

Table A39. Pearson correlation coefficients for maternal E2, placentome proliferation, CAR and COT Vascularity in HI non-IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Total Placentome					
Proliferating nuclei, %	0.608	0.123	0.431	0.008	0.167
P-value	0.277	0.844	0.469	0.990	0.788
CAR Vascularity					
CAD[1], %	**-0.382**	**0.469**	**0.037**	**-0.017**	**-0.212**
P-value	0.455	0.349	0.945	0.975	0.687
CND[2], mm^2	**-0.147**	**0.718**	**0.323**	**0.178**	**-0.032**
P-value	0.781	0.108	0.533	0.736	0.952
CSD[3], ($\mu m/\mu m^2$) x 10	**-0.295**	**0.571**	**0.148**	**0.045**	**-0.157**
P-value	0.570	0.236	0.779	0.933	0.767
APC[4], μm^2	**-0.791**	**-0.619**	**-0.835**	**-0.698**	**-0.695**
P-value	0.061	0.190	0.038	0.123	0.126
COT Vascularity					
CAD[1], %	**0.807**	**0.993**	**0.661**	**0.839**	**0.914**
P-value	0.193	0.001	0.339	0.161	0.086
CND[2], mm^2	**0.814**	**0.338**	**0.661**	**0.825**	**0.905**
P-value	0.186	0.662	0.339	0.175	0.096
CSD[3], ($\mu m/\mu m^2$) x 10	**0.799**	**0.158**	**0.564**	**0.630**	**0.754**
P-value	0.201	0.842	0.436	0.370	0.246
APC[4], μm^2	**-0.743**	**-0.126**	**-0.515**	**-0.673**	**-0.778**
P-value	0.257	0.874	0.485	0.327	0.222

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.

[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[4]Area per capillary = capillary area/capillary number per sample area.

Table A40.Pearson correlation coefficients for maternal E2, placentome proliferation, CAR, COT Vascularity in HI non-IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Total Placentome					
Proliferating nuclei, %	**-0.023**	**0.361**	**0.195**	**0.250**	**0.247**
P-value	0.961	0.426	0.675	0.588	0.593
CAR Vascularity					
CAD[1], %	**-0.113**	**-0.099**	**-0.113**	**-0.561**	**-0.458**
P-value	0.810	0.833	0.810	0.190	0.301
CND[2], mm^2	**-0.096**	**-0.143**	**-0.124**	**0.122**	**0.238**
P-value	0.838	0.759	0.792	0.794	0.607
CSD[3], ($\mu m/\mu m^2$) x 10	**-0.268**	**-0.189**	**-0.240**	**-0.328**	**-0.209**
P-value	0.562	0.686	0.605	0.473	0.653
APC[4], μm^2	**0.052**	**0.175**	**0.118**	**-0.548**	**-0.568**
P-value	0.912	0.707	0.801	0.203	0.184
COT Vascularity					
CAD[1], %	**-0.348**	**0.203**	**-0.406**	**0.046**	**0.072**
P-value	0.499	0.662	0.425	0.931	0.892
CND[2], mm^2	**-0.357**	**-0.317**	**-0.370**	**0.390**	**0.422**
P-value	0.487	0.541	0.471	0.445	0.404
CSD[3], ($\mu m/\mu m^2$) x 10	**-0.348**	**-0.418**	**-0.427**	**-0.252**	**-0.227**
P-value	0.500	0.409	0.399	0.630	0.665
APC[4], μm^2	**0.137**	**-0.351**	**-0.134**	**-0.209**	**-0.188**
P-value	0.796	0.495	0.801	0.692	0.722

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.

[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[4]Area per capillary = capillary area/capillary number per sample area.

Table A41. Pearson correlation coefficients for maternal E2 and JEJ mRNA in 38 animals.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**0.117**	**-0.019**	**0.063**	**-0.043**	**0.042**
P-value	0.486	0.908	0.707	0.797	0.801
Flt	**0.055**	**-0.101**	**-0.011**	**-0.230**	**-0.140**
P-value	0.743	0.548	0.948	0.165	0.404
KDR	**-0.052**	**-0.046**	**-0.053**	**-0.009**	**0.019**
P-value	0.755	0.782	0.754	0.956	0.909
HIF	**-0.353**	**-0.363**	**-0.378**	**0.116**	**0.147**
P-value	0.030	0.025	0.019	0.487	0.379
eNOS	**-0.124**	**-0.098**	**-0.119**	**0.068**	**0.047**
P-value	0.458	0.559	0.477	0.683	0.781
sGC	**0.239**	**0.159**	**0.218**	**0.129**	**0.102**
P-value	0.148	0.341	0.189	0.441	0.544
ANGPT1	**-0.167**	**-0.169**	**-0.176**	**0.315**	**0.372**
P-value	0.316	0.311	0.290	0.054	0.021
TIE2	**-0.019**	**-0.283**	**-0.140**	**-0.082**	**-0.052**
P-value	0.911	0.085	0.403	0.623	0.758
ANGPT2	**-0.052**	**-0.031**	**-0.047**	**-0.164**	**-0.096**
P-value	0.757	0.854	0.780	0.324	0.565
Np1	**-0.051**	**-0.122**	**-0.086**	**0.012**	**0.013**
P-value	0.761	0.466	0.608	0.943	0.940
Np2	**0.222**	**-0.025**	**0.125**	**-0.009**	**0.067**
P-value	0.180	0.882	0.454	0.957	0.689
FGF	**-0.044**	**-0.047**	**-0.046**	**-0.050**	**-0.043**
P-value	0.794	0.780	0.785	0.767	0.798
FGFR	**-0.202**	**-0.282**	**-0.249**	**0.023**	**0.056**
P-value	0.224	0.086	0.132	0.891	0.740
VIP	**-0.021**	**-0.198**	**-0.102**	**0.046**	**0.147**

Table A41. (Continued). Pearson correlation coefficients for maternal E2 and JEJ mRNA in 38 animals.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
P-value	0.902	0.234	0.544	0.785	0.377
VIPR1	**0.248**	**-0.034**	**0.143**	**0.005**	**0.018**
P-value	0.140	0.841	0.397	0.978	0.916
VIPR2	**0.179**	**0.025**	**0.120**	**-0.026**	**-0.033**
P-value	0.283	0.880	0.472	0.876	0.843
ERα	**-0.318**	**-0.279**	**-0.318**	**0.248**	**0.211**
P-value	0.051	0.090	0.052	0.134	0.204

Table A42. Pearson correlation coefficients for maternal E2 and JEJ mRNA in CON.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**0.071**	**-0.036**	**0.019**	**-0.051**	**0.245**
P-value	0.826	0.911	0.952	0.875	0.442
Flt	**-0.145**	**-0.196**	**-0.179**	**0.028**	**0.328**
P-value	0.652	0.543	0.579	0.931	0.298
KDR	**-0.387**	**-0.469**	**-0.450**	**0.192**	**0.305**
P-value	0.213	0.124	0.142	0.550	0.334
HIF	**-0.114**	**-0.508**	**-0.364**	**0.402**	**0.428**
P-value	0.724	0.092	0.244	0.195	0.165
eNOS	**-0.287**	**0.070**	**-0.083**	**-0.108**	**-0.231**
P-value	0.365	0.828	0.797	0.738	0.471
sGC	**0.008**	**-0.046**	**-0.032**	**0.217**	**-0.091**
P-value	0.981	0.888	0.921	0.499	0.779
ANGPT1	**-0.445**	**-0.336**	**-0.388**	**0.143**	**0.347**
P-value	0.148	0.285	0.212	0.657	0.270
TIE2	**-0.406**	**-0.440**	**-0.452**	**0.123**	**0.138**
P-value	0.191	0.152	0.140	0.703	0.668
ANGPT2	**-0.137**	**-0.428**	**-0.325**	**-0.043**	**0.291**
P-value	0.672	0.166	0.303	0.895	0.358
Np1	**-0.302**	**-0.153**	**-0.224**	**0.331**	**0.313**
P-value	0.339	0.635	0.485	0.293	0.321
Np2	**-0.495**	**-0.601**	**-0.584**	**-0.299**	**-0.012**
P-value	0.102	0.039	0.046	0.344	0.970
FGF	**0.081**	**-0.098**	**-0.015**	**0.436**	**0.368**
P-value	0.801	0.762	0.963	0.157	0.239
FGFR	**-0.246**	**-0.441**	**-0.374**	**0.216**	**0.222**
P-value	0.440	0.151	0.231	0.501	0.488
VIP	**-0.193**	**-0.273**	**-0.245**	**0.272**	**0.638**

Table A42. (Continued). Pearson correlation coefficients for maternal E2 and JEJ mRNA in CON.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
P-value	0.548	0.391	0.443	0.392	0.026
VIPR1	**-0.171**	**-0.283**	**-0.261**	**-0.113**	**-0.150**
P-value	0.596	0.372	0.413	0.727	0.642
VIPR2	**0.204**	**0.433**	**0.370**	**0.225**	**0.056**
P-value	0.525	0.160	0.237	0.483	0.862
ERα	**0.032**	**-0.099**	**-0.058**	**-0.139**	**-0.295**
P-value	0.921	0.761	0.858	0.666	0.353

Table A43. Pearson correlation coefficients for maternal E2 and JEJ mRNA in HI.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**-0.055**	**-0.377**	**-0.245**	**-0.395**	**-0.405**
P-value	0.853	0.184	0.399	0.163	0.151
Flt	**0.202**	**0.405**	**0.332**	**-0.056**	**-0.113**
P-value	0.488	0.151	0.246	0.849	0.700
KDR	**-0.010**	**-0.067**	**-0.043**	**-0.132**	**-0.050**
P-value	0.973	0.819	0.884	0.652	0.865
HIF	**-0.045**	**0.064**	**0.013**	**-0.051**	**-0.062**
P-value	0.878	0.829	0.964	0.862	0.834
eNOS	**0.127**	**-0.223**	**-0.065**	**-0.155**	**-0.141**
P-value	0.666	0.445	0.826	0.597	0.631
sGC	**0.651**	**0.354**	**0.528**	**0.591**	**0.687**
P-value	0.012	0.214	0.052	0.026	0.007
ANGPT1	**0.188**	**0.007**	**0.099**	**0.402**	**0.464**
P-value	0.519	0.981	0.735	0.154	0.095
TIE2	**0.143**	**-0.018**	**0.062**	**0.257**	**0.314**
P-value	0.625	0.951	0.833	0.375	0.274
ANGPT2	**-0.348**	**-0.220**	**-0.298**	**0.069**	**0.076**
P-value	0.223	0.451	0.301	0.814	0.795
Np1	**-0.213**	**-0.061**	**-0.141**	**0.183**	**0.206**
P-value	0.464	0.835	0.630	0.532	0.480
Np2	**0.098**	**-0.022**	**0.036**	**0.219**	**0.270**
P-value	0.739	0.940	0.902	0.453	0.351
FGF	**-0.003**	**0.159**	**0.090**	**0.250**	**0.265**
P-value	0.991	0.587	0.758	0.388	0.360
FGFR	**-0.162**	**-0.258**	**-0.228**	**0.021**	**0.069**
P-value	0.581	0.373	0.434	0.942	0.815
VIP	**-0.113**	**0.097**	**-0.001**	**0.230**	**0.235**

Table A43. (Continued). Pearson correlation coefficients for maternal E2 and JEJ mRNA in HI.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
P-value	0.700	0.743	0.997	0.429	0.418
VIPR1	**0.553**	**0.358**	**0.480**	**0.052**	**0.073**
P-value	0.040	0.209	0.083	0.861	0.804
VIPR2	**0.129**	**0.039**	**0.086**	**0.093**	**0.064**
P-value	0.660	0.895	0.771	0.753	0.827
ERα	**0.418**	**0.182**	**0.311**	**0.365**	**0.341**
P-value	0.137	0.535	0.280	0.200	0.233

Table A44. Pearson correlation coefficients for maternal E2 and JEJ mRNA in HI+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**0.210**	**-0.201**	**0.038**	**-0.130**	**-0.131**
P-value	0.512	0.531	0.907	0.686	0.686
Flt	**0.301**	**-0.120**	**0.144**	**-0.463**	**-0.413**
P-value	0.342	0.710	0.655	0.130	0.182
KDR	**-0.287**	**-0.003**	**-0.201**	**0.607**	**0.609**
P-value	0.367	0.992	0.531	0.036	0.036
HIF	**-0.514**	**-0.473**	**-0.613**	**-0.355**	**-0.360**
P-value	0.088	0.121	0.034	0.258	0.251
eNOS	**-0.116**	**-0.207**	**-0.189**	**-0.387**	**-0.362**
P-value	0.719	0.520	0.557	0.214	0.247
sGC	**0.172**	**-0.103**	**0.070**	**-0.325**	**-0.306**
P-value	0.592	0.750	0.829	0.302	0.334
ANGPT1	**-0.405**	**-0.470**	**-0.532**	**-0.009**	**0.004**
P-value	0.191	0.123	0.075	0.977	0.991
TIE2	**-0.002**	**-0.810**	**-0.439**	**-0.531**	**-0.523**
P-value	0.995	0.001	0.154	0.076	0.081
ANGPT2	**-0.411**	**-0.155**	**-0.371**	**-0.063**	**-0.045**
P-value	0.185	0.630	0.235	0.846	0.890
Np1	**-0.117**	**-0.335**	**-0.261**	**-0.195**	**-0.252**
P-value	0.717	0.287	0.413	0.544	0.429
Np2	**0.235**	**-0.391**	**-0.041**	**-0.044**	**0.022**
P-value	0.461	0.209	0.900	0.892	0.946
FGF	**-0.284**	**-0.175**	**-0.285**	**-0.418**	**-0.411**
P-value	0.372	0.587	0.369	0.177	0.185
FGFR	**-0.364**	**-0.495**	**-0.518**	**-0.255**	**-0.216**
P-value	0.245	0.102	0.084	0.424	0.501
VIP	**0.132**	**-0.456**	**-0.157**	**-0.378**	**-0.345**

Table A44. (Continued). Pearson correlation coefficients for maternal E2 and JEJ mRNA in HI+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
P-value	0.682	0.136	0.627	0.226	0.272
VIPR1	**0.611**	**-0.454**	**0.289**	**-0.402**	**-0.364**
P-value	0.046	0.161	0.388	0.221	0.271
VIPR2	**0.181**	**-0.490**	**-0.136**	**-0.637**	**-0.617**
P-value	0.574	0.106	0.673	0.026	0.033
ERα	**-0.298**	**-0.270**	**-0.348**	**-0.495**	**-0.454**
P-value	0.347	0.397	0.267	0.102	0.138

Table A45. Pearson correlation coefficients for maternal E2 and JEJ mRNA in HI IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**0.227**	**0.018**	**0.124**	**-0.101**	**-0.194**
P-value	0.589	0.965	0.770	0.812	0.646
Flt	**0.516**	**0.473**	**0.506**	**0.208**	**0.112**
P-value	0.190	0.237	0.200	0.621	0.791
KDR	**-0.260**	**-0.014**	**-0.142**	**-0.313**	**-0.246**
P-value	0.535	0.974	0.738	0.450	0.558
HIF	**-0.010**	**0.073**	**0.031**	**0.095**	**0.117**
P-value	0.982	0.864	0.942	0.823	0.782
eNOS	**-0.116**	**-0.451**	**-0.289**	**-0.277**	**-0.334**
P-value	0.784	0.262	0.487	0.507	0.419
sGC	**0.393**	**0.341**	**0.376**	**0.809**	**0.838**
P-value	0.336	0.409	0.358	0.015	0.009
ANGPT1	**-0.313**	**-0.050**	**-0.184**	**0.270**	**0.360**
P-value	0.450	0.907	0.662	0.519	0.381
TIE2	**-0.233**	**-0.095**	**-0.167**	**0.399**	**0.501**
P-value	0.579	0.823	0.693	0.327	0.206
ANGPT2	**-0.766**	**-0.636**	**-0.716**	**-0.219**	**-0.134**
P-value	0.027	0.090	0.046	0.603	0.751
Np1	**-0.451**	**-0.384**	**-0.428**	**-0.264**	**-0.176**
P-value	0.262	0.348	0.290	0.528	0.677
Np2	**-0.139**	**-0.002**	**-0.074**	**-0.074**	**0.009**
P-value	0.743	0.996	0.862	0.862	0.983
FGF	**-0.257**	**-0.056**	**-0.160**	**0.165**	**0.277**
P-value	0.539	0.896	0.706	0.695	0.507
FGFR	**-0.430**	**-0.399**	**-0.423**	**-0.194**	**-0.128**
P-value	0.288	0.328	0.296	0.645	0.762
VIP	**0.049**	**0.316**	**0.185**	**0.208**	**0.304**

Table A45. (Continued). Pearson correlation coefficients for maternal E2 and JEJ mRNA in HI IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
P-value	0.908	0.445	0.661	0.621	0.465
VIPR1	**0.756**	**0.533**	**0.659**	**0.631**	**0.564**
P-value	0.030	0.174	0.076	0.093	0.146
VIPR2	**0.569**	**0.446**	**0.519**	**0.629**	**0.583**
P-value	0.141	0.269	0.188	0.095	0.130
ERα	**0.418**	**0.111**	**0.270**	**0.283**	**0.235**
P-value	0.303	0.794	0.518	0.498	0.576

Table A46. Pearson correlation coefficients for maternal E2 and JEJ mRNA in HI IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**0.351**	**-0.003**	**0.292**	**-0.780**	**-0.657**
P-value	0.562	0.996	0.634	0.120	0.228
Flt	**0.573**	**0.708**	**0.785**	**-0.116**	**-0.092**
P-value	0.312	0.181	0.116	0.853	0.883
KDR	**-0.314**	**0.727**	**0.052**	**0.653**	**0.433**
P-value	0.607	0.164	0.934	0.232	0.466
HIF	**-0.599**	**0.327**	**-0.358**	**-0.141**	**-0.280**
P-value	0.286	0.591	0.554	0.821	0.648
eNOS	**0.162**	**0.613**	**0.401**	**-0.502**	**-0.556**
P-value	0.795	0.272	0.504	0.388	0.331
sGC	**-0.036**	**0.170**	**0.043**	**0.755**	**0.744**
P-value	0.954	0.785	0.945	0.140	0.149
ANGPT1	**-0.319**	**-0.169**	**-0.339**	**0.140**	**0.170**
P-value	0.601	0.786	0.576	0.823	0.785
TIE2	**0.183**	**-0.786**	**-0.188**	**-0.436**	**-0.194**
P-value	0.769	0.115	0.762	0.463	0.755
ANGPT2	**-0.343**	**0.170**	**-0.213**	**0.016**	**-0.018**
P-value	0.572	0.785	0.732	0.979	0.977
Np1	**-0.385**	**-0.093**	**-0.361**	**-0.872**	**-0.885**
P-value	0.523	0.882	0.550	0.054	0.046
Np2	**0.430**	**-0.551**	**0.121**	**-0.398**	**-0.137**
P-value	0.470	0.336	0.847	0.507	0.827
FGF	**-0.659**	**0.343**	**-0.402**	**-0.333**	**-0.498**
P-value	0.226	0.572	0.502	0.584	0.393
FGFR	**-0.471**	**-0.416**	**-0.573**	**-0.190**	**-0.146**
P-value	0.424	0.486	0.312	0.760	0.815
VIP	**0.073**	**-0.832**	**-0.299**	**0.148**	**0.340**

Table A46. (Continued). Pearson correlation coefficients for maternal E2 and JEJ mRNA in HI IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
P-value	0.907	0.081	0.625	0.813	0.575
VIPR1	**0.856**	**-0.224**	**0.618**	**0.232**	**0.436**
P-value	0.064	0.718	0.267	0.708	0.463
VIPR2	**0.200**	**-0.378**	**0.003**	**-0.867**	**-0.702**
P-value	0.748	0.530	0.996	0.057	0.186
ERα	**-0.501**	**0.424**	**-0.235**	**-0.504**	**-0.652**
P-value	0.390	0.477	0.704	0.387	0.233

Table A47. Pearson correlation coefficients for maternal E2 and JEJ mRNA in HI non-IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**0.038**	**-0.238**	**-0.113**	**0.204**	**0.180**
P-value	0.943	0.650	0.831	0.699	0.733
Flt	**-0.054**	**0.597**	**0.310**	**-0.226**	**-0.302**
P-value	0.919	0.211	0.550	0.667	0.561
KDR	**0.266**	**-0.383**	**-0.058**	**-0.386**	**-0.159**
P-value	0.611	0.454	0.913	0.450	0.764
HIF	**0.024**	**0.578**	**0.346**	**0.113**	**-0.016**
P-value	0.964	0.229	0.501	0.832	0.976
eNOS	**0.714**	**0.366**	**0.644**	**0.212**	**0.286**
P-value	0.111	0.475	0.168	0.687	0.583
sGC	**0.864**	**0.206**	**0.643**	**0.585**	**0.756**
P-value	0.027	0.696	0.169	0.223	0.082
ANGPT1	**0.583**	**-0.063**	**0.318**	**0.676**	**0.730**
P-value	0.225	0.906	0.540	0.140	0.100
TIE2	**0.518**	**0.025**	**0.329**	**0.400**	**0.445**
P-value	0.292	0.963	0.524	0.433	0.377
ANGPT2	**-0.186**	**-0.375**	**-0.328**	**-0.254**	**-0.242**
P-value	0.725	0.465	0.526	0.628	0.645
Np1	**-0.120**	**-0.251**	**-0.217**	**0.141**	**0.185**
P-value	0.821	0.631	0.680	0.790	0.725
Np2	**0.351**	**-0.311**	**0.034**	**0.429**	**0.499**
P-value	0.496	0.548	0.948	0.396	0.313
FGF	**0.501**	**0.700**	**0.705**	**0.702**	**0.577**
P-value	0.312	0.122	0.118	0.120	0.230
FGFR	**0.762**	**-0.039**	**0.440**	**0.670**	**0.788**
P-value	0.078	0.942	0.382	0.145	0.063
VIP	**-0.405**	**-0.314**	**-0.426**	**0.142**	**0.103**

Table A47. (Continued). Pearson correlation coefficients for maternal E2 and JEJ mRNA in HI non-IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
P-value	0.425	0.545	0.400	0.789	0.845
VIPR1	**0.426**	**0.437**	**0.509**	**-0.186**	**-0.101**
P-value	0.399	0.386	0.302	0.724	0.848
VIPR2	**-0.241**	**0.001**	**-0.146**	**0.457**	**0.329**
P-value	0.646	0.998	0.783	0.362	0.524
ERα	**0.466**	**0.371**	**0.496**	**0.829**	**0.738**
P-value	0.352	0.469	0.318	0.041	0.094

Table A48. Pearson correlation coefficients for maternal E2 and JEJ mRNA in HI non-IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
VEGF	**-0.313**	**-0.360**	**-0.359**	**0.132**	**0.148**
P-value	0.494	0.428	0.430	0.779	0.751
Flt	**-0.192**	**-0.498**	**-0.378**	**-0.480**	**-0.402**
P-value	0.681	0.255	0.403	0.276	0.371
KDR	**-0.288**	**-0.375**	**-0.358**	**0.670**	**0.678**
P-value	0.531	0.408	0.430	0.099	0.094
HIF	**-0.606**	**-0.824**	**-0.779**	**-0.418**	**-0.406**
P-value	0.149	0.023	0.039	0.350	0.366
eNOS	**-0.507**	**-0.614**	**-0.597**	**-0.451**	**-0.391**
P-value	0.245	0.142	0.157	0.309	0.386
sGC	**0.537**	**-0.025**	**0.269**	**-0.407**	**-0.450**
P-value	0.214	0.958	0.560	0.365	0.311
ANGPT1	**-0.535**	**-0.738**	**-0.686**	**-0.145**	**-0.129**
P-value	0.216	0.058	0.089	0.757	0.783
TIE2	**-0.478**	**-0.793**	**-0.697**	**-0.478**	**-0.495**
P-value	0.278	0.034	0.082	0.278	0.258
ANGPT2	**-0.605**	**-0.400**	**-0.539**	**-0.055**	**-0.019**
P-value	0.150	0.374	0.212	0.907	0.968
Np1	**0.060**	**-0.471**	**-0.238**	**-0.231**	**-0.282**
P-value	0.898	0.286	0.607	0.618	0.540
Np2	**0.162**	**-0.346**	**-0.107**	**0.019**	**0.089**
P-value	0.728	0.447	0.820	0.968	0.850
FGF	**0.060**	**-0.385**	**-0.185**	**-0.364**	**-0.310**
P-value	0.899	0.394	0.691	0.423	0.499
FGFR	**-0.458**	**-0.475**	**-0.506**	**-0.131**	**-0.066**
P-value	0.302	0.281	0.247	0.780	0.889
VIP	**0.029**	**-0.214**	**-0.117**	**-0.173**	**-0.156**

Table A48. (Continued). Pearson correlation coefficients for maternal E2 and JEJ mRNA in HI non-IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
P-value	0.950	0.644	0.803	0.711	0.738
VIPR1	**-0.381**	**-0.819**	**-0.725**	**-0.584**	**-0.580**
P-value	0.456	0.046	0.103	0.223	0.227
VIPR2	**-0.110**	**-0.535**	**-0.358**	**-0.858**	**-0.802**
P-value	0.814	0.216	0.431	0.014	0.030
ERα	**-0.262**	**-0.577**	**-0.455**	**-0.746**	**-0.669**
P-value	0.570	0.175	0.306	0.054	0.100

Table A49. Pearson correlation coefficients for maternal E2, fetal small intestinal crypt proliferation and vascularity in 38 animals.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Small intestinal crypt proliferation					
Proliferating nuclei, %	**-0.079**	**-0.100**	**-0.092**	**0.069**	**0.108**
P-value	0.638	0.552	0.585	0.680	0.520
Small Intestinal Vascularity					
CAD[1], %	**0.001**	**0.001**	**-0.001**	**-0.028**	**-0.094**
P-value	0.998	0.996	0.994	0.868	0.575
CND[2], mm^2	**-0.031**	**-0.005**	**-0.021**	**0.066**	**0.077**
P-value	0.854	0.976	0.902	0.695	0.645
CSD[3], ($\mu m/\mu m^2$) x 10	**-0.073**	**-0.102**	**-0.090**	**0.012**	**-0.011**
P-value	0.665	0.543	0.591	0.944	0.947
APC[4], μm^2	**0.015**	**0.037**	**0.024**	**0.005**	**-0.035**
P-value	0.927	0.826	0.888	0.978	0.833
Total jejunal vascularity, mL	**-0.091**	**0.057**	**-0.031**	**0.341**	**0.265**
P-value	0.589	0.732	0.855	0.036	0.108

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.

[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[4]Area per capillary = capillary area/capillary number per sample area.

Table A50. Pearson correlation coefficients for maternal E2, fetal small intestinal crypt proliferation and vascularity in CON.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Small intestinal crypt proliferation					
Proliferating nuclei, %	**-0.350**	**-0.549**	**-0.490**	**0.198**	**0.304**
P-value	0.265	0.065	0.106	0.538	0.337
Small Intestinal Vascularity					
CAD[1], %	**0.113**	**0.440**	**0.320**	**-0.137**	**-0.331**
P-value	0.727	0.153	0.310	0.672	0.293
CND[2], mm^2	**0.051**	**-0.157**	**-0.089**	**0.527**	**0.448**
P-value	0.874	0.627	0.784	0.079	0.145
CSD[3], (μm/$μm^2$) x 10	**-0.272**	**-0.148**	**-0.219**	**0.207**	**0.118**
P-value	0.392	0.646	0.493	0.519	0.714
APC[4], $μm^2$	**0.090**	**0.340**	**0.263**	**-0.448**	**-0.434**
P-value	0.782	0.279	0.410	0.144	0.159
Total jejunal vascularity, mL	**0.175**	**0.409**	**0.333**	**-0.185**	**-0.315**
P-value	0.586	0.187	0.290	0.565	0.319

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.

[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[4]Area per capillary = capillary area/capillary number per sample area.

Table A51. Pearson correlation coefficients for maternal E2, fetal small intestinal crypt proliferation and vascularity in HI.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Small intestinal crypt proliferation					
Proliferating nuclei, %	**-0.131**	**0.004**	**-0.063**	**-0.081**	**-0.072**
P-value	0.657	0.988	0.830	0.784	0.806
Small Intestinal Vascularity					
CAD[1], %	**0.517**	**0.558**	**0.575**	**0.024**	**-0.022**
P-value	0.058	0.038	0.031	0.936	0.940
CND[2], mm^2	**-0.031**	**-0.162**	**-0.110**	**-0.411**	**-0.425**
P-value	0.916	0.580	0.709	0.144	0.130
CSD[3], ($\mu m/\mu m^2$) x 10	**0.303**	**0.388**	**0.372**	**-0.130**	**-0.191**
P-value	0.292	0.170	0.190	0.658	0.512
APC[4], μm^2	**0.421**	**0.635**	**0.574**	**0.449**	**0.413**
P-value	0.133	0.015	0.032	0.107	0.142
Total jejunal vascularity, mL	**0.622**	**0.815**	**0.776**	**0.756**	**0.705**
P-value	0.018	0.000	0.001	0.002	0.005

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.

[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[4]Area per capillary = capillary area/capillary number per sample area.

Table A52. Pearson correlation coefficients for maternal E2, fetal small intestinal crypt proliferation and vascularity in HI+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Small intestinal crypt proliferation					
Proliferating nuclei, %	**-0.502**	**-0.472**	**-0.596**	**-0.041**	**-0.063**
P-value	0.096	0.122	0.041	0.899	0.846
Small Intestinal Vascularity					
CAD[1], %	**0.428**	**0.251**	**0.423**	**0.062**	**0.047**
P-value	0.165	0.432	0.171	0.849	0.884
CND[2], mm^2	**0.396**	**0.602**	**0.601**	**-0.272**	**-0.253**
P-value	0.202	0.038	0.039	0.392	0.427
CSD[3], (μm/$μm^2$) x 10	**0.545**	**0.342**	**0.560**	**-0.156**	**-0.158**
P-value	0.067	0.276	0.059	0.628	0.623
APC[4], $μm^2$	**0.114**	**0.025**	**0.081**	**0.230**	**0.210**
P-value	0.724	0.939	0.803	0.472	0.513
Total jejunal vascularity, mL	**-0.015**	**0.139**	**0.055**	**0.324**	**0.286**
P-value	0.962	0.666	0.865	0.305	0.367

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.

[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[4]Area per capillary = capillary area/capillary number per sample area.

Table A53. Pearson correlation coefficients for maternal E2, fetal small intestinal crypt proliferation and vascularity in HI IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Small intestinal crypt proliferation					
Proliferating nuclei, %	**-0.097**	**0.025**	**-0.038**	**-0.039**	**-0.012**
P-value	0.820	0.953	0.928	0.927	0.978
Small Intestinal Vascularity					
CAD[1], %	**0.757**	**0.779**	**0.783**	**0.247**	**0.201**
P-value	0.030	0.023	0.022	0.556	0.633
CND[2], mm^2	**0.105**	**-0.241**	**-0.070**	**-0.105**	**-0.197**
P-value	0.805	0.566	0.869	0.805	0.639
CSD[3], (μm/$μm^2$) x 10	**0.799**	**0.684**	**0.758**	**0.493**	**0.399**
P-value	0.017	0.061	0.029	0.214	0.328
APC[4], $μm^2$	**0.427**	**0.691**	**0.571**	**0.191**	**0.227**
P-value	0.291	0.058	0.140	0.650	0.588
Total jejunal vascularity, mL	**0.627**	**0.691**	**0.673**	**0.503**	**0.453**
P-value	0.096	0.058	0.067	0.204	0.260

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.

[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[4]Area per capillary = capillary area/capillary number per sample area.

Table A54. Pearson correlation coefficients for maternal E2, fetal small intestinal crypt proliferation and vascularity in HI IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Small intestinal crypt proliferation					
Proliferating nuclei, %	**-0.733**	**-0.088**	**-0.650**	**0.235**	**0.031**
P-value	0.159	0.888	0.235	0.704	0.961
Small Intestinal Vascularity					
CAD[1], %	**0.782**	**0.083**	**0.689**	**0.644**	**0.760**
P-value	0.118	0.894	0.198	0.241	0.136
CND[2], mm^2	**0.162**	**0.705**	**0.441**	**-0.393**	**-0.488**
P-value	0.795	0.183	0.458	0.513	0.405
CSD[3], ($\mu m/\mu m^2$) x 10	**0.922**	**-0.008**	**0.766**	**0.106**	**0.287**
P-value	0.026	0.990	0.131	0.866	0.640
APC[4], μm^2	**0.501**	**-0.085**	**0.381**	**0.788**	**0.869**
P-value	0.390	0.892	0.527	0.113	0.056
Total jejunal vascularity, mL	**-0.052**	**-0.722**	**-0.356**	**-0.117**	**-0.047**
P-value	0.934	0.169	0.557	0.852	0.940

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.

[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[4]Area per capillary = capillary area/capillary number per sample area.

Table A55. Pearson correlation coefficients for maternal E2, fetal small intestinal crypt proliferation and vascularity in HI non-IUGR.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Small intestinal crypt proliferation					
Proliferating nuclei, %	**-0.141**	**0.120**	**-0.017**	**-0.047**	**-0.047**
P-value	0.790	0.821	0.974	0.930	0.929
Small Intestinal Vascularity					
CAD[1], %	**0.292**	**0.819**	**0.647**	**0.118**	**-0.019**
P-value	0.575	0.046	0.165	0.825	0.971
CND[2], mm^2	**0.031**	**0.360**	**0.225**	**-0.478**	**-0.444**
P-value	0.954	0.484	0.668	0.337	0.378
CSD[3], (μm/$μm^2$) x 10	**-0.248**	**0.357**	**0.054**	**-0.585**	**-0.671**
P-value	0.636	0.487	0.919	0.223	0.145
APC[4], $μm^2$	**0.228**	**0.379**	**0.356**	**0.324**	**0.205**
P-value	0.664	0.459	0.489	0.531	0.697
Total jejunal vascularity, mL	**0.434**	**0.757**	**0.698**	**0.770**	**0.613**
P-value	0.389	0.082	0.123	0.073	0.195

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.
[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.
[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.
[4]Area per capillary = capillary area/capillary number per sample area.

Table A56. Pearson correlation coefficients for maternal E2, fetal small intestinal crypt proliferation and vascularity in HI non-IUGR+E2.

	Maternal circulating plasma estradiol-17β, pg/mL				
Item	d 55-70	d 75-90	d 55-90	d 99-126	d 126
Small intestinal crypt proliferation					
Proliferating nuclei, %	**-0.263**	**-0.823**	**-0.594**	**-0.222**	**-0.229**
P-value	0.568	0.023	0.160	0.633	0.621
Small Intestinal Vascularity					
CAD[1], %	**0.358**	**0.200**	**0.284**	**-0.225**	**-0.271**
P-value	0.430	0.667	0.538	0.628	0.557
CND[2], mm^2	**0.756**	**0.608**	**0.731**	**-0.352**	**-0.292**
P-value	0.049	0.147	0.062	0.439	0.524
CSD[3], (μm/$μm^2$) x 10	**0.767**	**0.359**	**0.587**	**-0.405**	**-0.412**
P-value	0.044	0.430	0.166	0.368	0.359
APC[4], $μm^2$	**-0.143**	**-0.077**	**-0.132**	**0.036**	**-0.024**
P-value	0.760	0.869	0.779	0.939	0.959
Total jejunal vascularity, mL	**0.199**	**0.075**	**0.130**	**0.091**	**0.051**
P-value	0.670	0.873	0.782	0.846	0.913

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.
[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.
[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.
[4]Area per capillary = capillary area/capillary number per sample area.

Table A57. Correlation between fetal small intestinal proliferation and vascularity with mRNA factors in all 38 animals

Factors	CAD[1]	CND[2]	CSD[3]	APC[4]	Total small intestine, g	Total jejunal vascularity	Proliferating nuclei, %
VEGF	**-0.285**	**-0.030**	**-0.227**	**-0.255**	**-0.173**	**-0.284**	**-0.010**
P-value	0.083	0.858	0.171	0.123	0.299	0.084	0.952
Flt	**-0.104**	**0.203**	**0.094**	**-0.209**	**-0.251**	**-0.228**	**-0.002**
P-value	0.534	0.222	0.573	0.208	0.129	0.168	0.992
KDR	**-0.079**	**-0.231**	**-0.094**	**0.161**	**0.071**	**-0.035**	**0.415**
P-value	0.636	0.164	0.576	0.333	0.671	0.837	0.010
HIF	**0.008**	**0.179**	**0.153**	**-0.106**	**0.095**	**0.071**	**0.457**
P-value	0.964	0.282	0.360	0.528	0.571	0.670	0.004
eNOS	**-0.085**	**0.217**	**-0.012**	**-0.279**	**-0.028**	**-0.010**	**-0.262**
P-value	0.612	0.191	0.943	0.090	0.866	0.953	0.112
sGC	**-0.052**	**0.072**	**-0.013**	**-0.157**	**-0.072**	**-0.085**	**0.114**
P-value	0.757	0.666	0.937	0.346	0.667	0.614	0.496
ANGPT1	**-0.262**	**-0.407**	**-0.261**	**0.095**	**0.296**	**0.120**	**0.189**
P-value	0.112	0.011	0.113	0.571	0.072	0.473	0.257
TIE2	**0.016**	**-0.094**	**0.062**	**0.097**	**-0.140**	**-0.083**	**0.083**
P-value	0.926	0.573	0.711	0.563	0.403	0.619	0.620
ANGPT2	**-0.454**	**-0.217**	**-0.407**	**-0.148**	**0.001**	**-0.192**	**-0.124**
P-value	0.004	0.191	0.011	0.376	0.996	0.247	0.457
Np1	**-0.227**	**-0.228**	**-0.193**	**-0.008**	**0.177**	**0.005**	**0.433**
P-value	0.171	0.169	0.246	0.962	0.287	0.976	0.007
Np2	**-0.245**	**-0.299**	**-0.215**	**-0.016**	**0.162**	**-0.012**	**0.270**
P-value	0.138	0.069	0.194	0.923	0.331	0.941	0.101
FGF	**-0.224**	**-0.041**	**-0.114**	**-0.116**	**-0.074**	**-0.184**	**0.262**
P-value	0.176	0.806	0.495	0.487	0.660	0.269	0.112
FGFR	**-0.376**	**-0.187**	**-0.296**	**-0.127**	**0.014**	**-0.171**	**0.220**
P-value	0.020	0.260	0.072	0.449	0.932	0.305	0.185
VIP	**-0.127**	**-0.208**	**-0.083**	**0.046**	**0.262**	**0.108**	**0.308**
P-value	0.448	0.210	0.620	0.785	0.112	0.518	0.060

Table A57. (Continued). Correlation between fetal small intestinal proliferation and vascularity with mRNA factors in all 38 animals

Factors	CAD[1]	CND[2]	CSD[3]	APC[4]	Total small intestine, g	Total jejunal vascularity	Proliferating nuclei, %
VIPR1	**0.001**	**0.151**	**0.082**	**-0.127**	**-0.241**	**-0.229**	**0.128**
P-value	0.993	0.373	0.631	0.453	0.152	0.172	0.449
VIPR2	**-0.089**	**-0.024**	**-0.026**	**-0.193**	**-0.009**	**-0.059**	**0.172**
P-value	0.596	0.885	0.878	0.247	0.958	0.725	0.302
ERα	**0.093**	**0.245**	**0.174**	**-0.167**	**0.085**	**0.140**	**-0.024**
P-value	0.580	0.138	0.295	0.317	0.611	0.401	0.889

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.

[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[4]Area per capillary = capillary area/capillary number per sample area.

Table A58. Correlation between fetal small intestinal proliferation and vascularity with mRNA factors in CON animals

Factors	CAD[1]	CND[2]	CSD[3]	APC[4]	Total small intestine, g	Total jejunal vascularity	Proliferating nuclei, %
VEGF	**-0.681**	**-0.434**	**-0.698**	**-0.058**	**-0.398**	**-0.618**	**0.118**
P-value	0.015	0.159	0.012	0.857	0.200	0.032	0.716
Flt	**-0.507**	**-0.120**	**-0.293**	**-0.209**	**-0.637**	**-0.639**	**0.102**
P-value	0.093	0.709	0.355	0.514	0.026	0.025	0.752
KDR	**-0.292**	**-0.120**	**0.103**	**0.019**	**-0.194**	**-0.317**	**0.770**
P-value	0.358	0.711	0.750	0.954	0.545	0.315	0.003
HIF	**-0.155**	**0.474**	**0.348**	**-0.430**	**-0.205**	**-0.232**	**0.387**
P-value	0.630	0.120	0.267	0.164	0.524	0.468	0.214
eNOS	**0.241**	**0.047**	**0.128**	**-0.101**	**0.385**	**0.357**	**-0.327**
P-value	0.450	0.884	0.691	0.756	0.216	0.255	0.299
sGC	**0.216**	**0.429**	**0.459**	**-0.339**	**-0.186**	**-0.001**	**0.110**
P-value	0.500	0.164	0.134	0.282	0.562	0.998	0.733
ANGPT1	**-0.159**	**-0.227**	**0.091**	**0.119**	**0.179**	**-0.008**	**0.475**
P-value	0.622	0.479	0.778	0.713	0.578	0.980	0.118
TIE2	**0.086**	**0.306**	**0.425**	**-0.283**	**-0.369**	**-0.185**	**0.293**
P-value	0.791	0.334	0.168	0.373	0.238	0.566	0.356
ANGPT2	**-0.504**	**0.007**	**-0.419**	**-0.334**	**-0.179**	**-0.405**	**0.019**
P-value	0.095	0.983	0.175	0.289	0.578	0.191	0.952
Np1	**-0.522**	**-0.221**	**-0.253**	**-0.184**	**-0.169**	**-0.432**	**0.576**
P-value	0.082	0.490	0.427	0.567	0.600	0.160	0.050
Np2	**-0.466**	**-0.365**	**-0.303**	**0.017**	**0.123**	**-0.213**	**0.476**
P-value	0.127	0.243	0.339	0.957	0.704	0.507	0.118
FGF	**-0.466**	**0.074**	**-0.029**	**-0.410**	**-0.003**	**-0.278**	**0.329**
P-value	0.127	0.818	0.928	0.186	0.992	0.382	0.296
FGFR	**-0.450**	**-0.008**	**-0.001**	**-0.293**	**-0.075**	**-0.333**	**0.610**
P-value	0.142	0.980	0.998	0.356	0.816	0.290	0.035
VIP	**-0.445**	**0.020**	**0.001**	**-0.176**	**-0.002**	**-0.231**	**0.272**
P-value	0.147	0.951	0.997	0.584	0.996	0.471	0.392

Table A58. (Continued). Correlation between fetal small intestinal proliferation and vascularity with mRNA factors in CON animals

Factors	CAD[1]	CND[2]	CSD[3]	APC[4]	Total small intestine, g	Total jejunal vascularity	Proliferating nuclei, %
VIPR1	**-0.612**	**-0.040**	**-0.306**	**-0.492**	**-0.294**	**-0.503**	**0.219**
P-value	0.035	0.903	0.333	0.104	0.354	0.095	0.494
VIPR2	**-0.171**	**-0.309**	**-0.208**	**0.010**	**-0.265**	**-0.259**	**0.091**
P-value	0.596	0.329	0.517	0.974	0.405	0.416	0.778
ERα	**0.254**	**0.276**	**0.373**	**-0.144**	**-0.113**	**0.100**	**-0.030**
P-value	0.425	0.385	0.232	0.656	0.726	0.758	0.926

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.

[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[4]Area per capillary = capillary area/capillary number per sample area.

Table A59. Correlation between fetal small intestinal proliferation and vascularity with mRNA factors in HI animals

Factors	CAD[1]	CND[2]	CSD[3]	APC[4]	Total small intestine, g	Total jejunal vascularity	Proliferating nuclei, %
VEGF	**0.328**	**0.280**	**0.275**	**-0.165**	**-0.245**	**-0.082**	**0.062**
P-value	0.252	0.333	0.340	0.572	0.399	0.781	0.832
Flt	**0.403**	**0.449**	**0.578**	**-0.100**	**0.155**	**0.359**	**0.127**
P-value	0.153	0.107	0.030	0.733	0.597	0.207	0.666
KDR	**-0.049**	**-0.240**	**-0.209**	**0.119**	**0.177**	**0.066**	**0.042**
P-value	0.867	0.408	0.473	0.686	0.545	0.822	0.886
HIF	**0.373**	**0.060**	**0.194**	**0.116**	**0.123**	**0.247**	**0.703**
P-value	0.189	0.838	0.507	0.693	0.676	0.394	0.005
eNOS	**-0.053**	**0.417**	**0.034**	**-0.190**	**-0.471**	**-0.378**	**-0.574**
P-value	0.857	0.139	0.909	0.515	0.089	0.183	0.032
sGC	**-0.077**	**-0.021**	**-0.216**	**-0.008**	**0.358**	**0.237**	**0.103**
P-value	0.793	0.942	0.459	0.979	0.209	0.415	0.725
ANGPT1	**-0.211**	**-0.669**	**-0.489**	**0.377**	**0.304**	**0.204**	**-0.258**
P-value	0.470	0.009	0.076	0.184	0.290	0.485	0.373
TIE2	**-0.064**	**-0.333**	**-0.224**	**0.389**	**-0.091**	**-0.094**	**-0.171**
P-value	0.829	0.244	0.441	0.169	0.757	0.750	0.560
ANGPT2	**-0.450**	**-0.421**	**-0.378**	**0.043**	**0.188**	**0.012**	**-0.299**
P-value	0.106	0.134	0.183	0.885	0.520	0.968	0.299
Np1	**-0.419**	**-0.332**	**-0.633**	**-0.037**	**0.218**	**-0.025**	**0.235**
P-value	0.136	0.246	0.015	0.901	0.453	0.931	0.419
Np2	**-0.038**	**-0.558**	**-0.445**	**0.355**	**0.226**	**0.130**	**0.048**
P-value	0.897	0.038	0.111	0.213	0.437	0.658	0.872
FGF	**0.017**	**-0.392**	**-0.313**	**0.429**	**0.030**	**0.054**	**0.277**
P-value	0.955	0.166	0.276	0.125	0.920	0.856	0.338
FGFR	**-0.307**	**-0.332**	**-0.543**	**0.093**	**-0.015**	**-0.140**	**0.086**
P-value	0.286	0.246	0.045	0.752	0.960	0.633	0.770
VIP	**-0.163**	**-0.446**	**-0.396**	**0.020**	**0.389**	**0.184**	**0.583**
P-value	0.578	0.110	0.161	0.946	0.169	0.530	0.029

Table A59. (Continued). Correlation between fetal small intestinal proliferation and vascularity with mRNA factors in HI animals

Factors	CAD[1]	CND[2]	CSD[3]	APC[4]	Total small intestine, g	Total jejunal vascularity	Proliferating nuclei, %
VIPR1	**0.546**	**0.713**	**0.688**	**-0.019**	**-0.158**	**0.066**	**0.190**
P-value	0.043	0.004	0.007	0.947	0.591	0.823	0.515
VIPR2	**0.243**	**0.063**	**0.159**	**-0.062**	**0.162**	**0.225**	**0.506**
P-value	0.403	0.829	0.588	0.834	0.581	0.439	0.065
ERα	**0.125**	**0.092**	**-0.070**	**-0.072**	**0.123**	**0.179**	**-0.252**
P-value	0.670	0.754	0.811	0.806	0.676	0.539	0.385

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.

[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.

[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.

[4]Area per capillary = capillary area/capillary number per sample area.

Table A60. Correlation between fetal small intestinal proliferation and vascularity with mRNA factors in HI+E2 animals

Factors	CAD[1]	CND[2]	CSD[3]	APC[4]	Total small intestine, g	Total jejunal vascularity	Proliferating nuclei, %
VEGF	**-0.442**	**0.230**	**-0.178**	**-0.479**	**-0.039**	**-0.195**	**-0.328**
P-value	0.150	0.472	0.580	0.115	0.903	0.544	0.298
Flt	**-0.243**	**0.421**	**-0.001**	**-0.345**	**-0.331**	**-0.273**	**-0.119**
P-value	0.447	0.174	0.999	0.272	0.293	0.391	0.713
KDR	**0.019**	**-0.385**	**-0.232**	**0.278**	**0.426**	**0.313**	**0.484**
P-value	0.953	0.216	0.469	0.381	0.167	0.322	0.111
HIF	**-0.234**	**-0.239**	**-0.238**	**-0.021**	**0.199**	**0.104**	**0.435**
P-value	0.464	0.455	0.457	0.948	0.535	0.747	0.158
eNOS	**-0.574**	**0.347**	**-0.341**	**-0.612**	**-0.388**	**-0.509**	**0.001**
P-value	0.051	0.269	0.278	0.035	0.213	0.091	0.998
sGC	**-0.055**	**-0.149**	**-0.071**	**-0.065**	**-0.480**	**-0.359**	**0.031**
P-value	0.865	0.643	0.826	0.841	0.114	0.252	0.925
ANGPT1	**-0.390**	**-0.446**	**-0.355**	**-0.133**	**0.165**	**-0.009**	**0.374**
P-value	0.210	0.146	0.257	0.680	0.609	0.977	0.231
TIE2	**0.004**	**-0.408**	**-0.022**	**0.117**	**0.038**	**0.072**	**0.150**
P-value	0.990	0.188	0.945	0.716	0.907	0.823	0.642
ANGPT2	**-0.482**	**-0.195**	**-0.463**	**-0.233**	**0.057**	**-0.122**	**-0.089**
P-value	0.112	0.545	0.130	0.466	0.859	0.707	0.784
Np1	**0.175**	**-0.107**	**0.332**	**0.147**	**0.495**	**0.502**	**0.507**
P-value	0.586	0.740	0.291	0.649	0.102	0.096	0.093
Np2	**-0.178**	**0.037**	**0.168**	**-0.250**	**0.122**	**0.044**	**0.236**
P-value	0.580	0.908	0.602	0.432	0.706	0.892	0.460
FGF	**-0.265**	**0.262**	**-0.056**	**-0.370**	**-0.148**	**-0.219**	**0.226**
P-value	0.406	0.411	0.863	0.237	0.646	0.495	0.480
FGFR	**-0.507**	**-0.286**	**-0.383**	**-0.282**	**0.096**	**-0.135**	**0.022**
P-value	0.092	0.367	0.219	0.375	0.766	0.676	0.946
VIP	**0.104**	**-0.237**	**0.150**	**0.224**	**0.248**	**0.267**	**0.104**
P-value	0.749	0.459	0.641	0.483	0.437	0.402	0.747

Table A60. (Continued). Correlation between fetal small intestinal proliferation and vascularity with mRNA factors in HI+E2 animals

Factors	CAD[1]	CND[2]	CSD[3]	APC[4]	Total small intestine, g	Total jejunal vascularity	Proliferating nuclei, %
VIPR1	**0.417**	**-0.032**	**0.276**	**0.224**	**-0.561**	**-0.239**	**-0.153**
P-value	0.202	0.925	0.411	0.508	0.073	0.479	0.653
VIPR2	**-0.261**	**0.259**	**0.065**	**-0.418**	**-0.206**	**-0.232**	**-0.195**
P-value	0.413	0.417	0.842	0.176	0.521	0.468	0.543
ERα	**-0.269**	**0.302**	**0.019**	**-0.380**	**-0.201**	**-0.217**	**0.321**
P-value	0.398	0.341	0.954	0.223	0.531	0.498	0.309

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.
[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.
[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.
[4]Area per capillary = capillary area/capillary number per sample area.

Table A61. Correlation between fetal small intestinal proliferation and vascularity with mRNA factors in HI IUGR animals

Factors	CAD[1]	CND[2]	CSD[3]	APC[4]	Total small intestine, g	Total jejunal vascularity	Proliferating nuclei, %
VEGF	**0.526**	**0.745**	**0.651**	**-0.160**	**0.208**	**0.390**	**0.517**
P-value	0.181	0.034	0.080	0.705	0.622	0.339	0.190
Flt	**0.437**	**0.061**	**0.675**	**0.222**	**0.578**	**0.695**	**-0.254**
P-value	0.279	0.886	0.066	0.597	0.133	0.056	0.544
KDR	**0.044**	**-0.598**	**-0.415**	**0.448**	**0.371**	**0.243**	**0.323**
P-value	0.918	0.118	0.307	0.266	0.365	0.562	0.435
HIF	**0.401**	**-0.037**	**0.229**	**0.374**	**0.288**	**0.345**	**0.954**
P-value	0.325	0.931	0.586	0.362	0.490	0.403	0.000
eNOS	**-0.432**	**0.529**	**-0.198**	**-0.656**	**-0.613**	**-0.590**	**-0.617**
P-value	0.285	0.177	0.638	0.078	0.106	0.124	0.103
sGC	**-0.053**	**-0.029**	**0.076**	**-0.050**	**0.129**	**0.046**	**-0.022**
P-value	0.902	0.947	0.859	0.906	0.761	0.914	0.959
ANGPT1	**-0.417**	**-0.669**	**-0.492**	**0.122**	**0.453**	**0.144**	**0.085**
P-value	0.304	0.070	0.216	0.774	0.260	0.734	0.841
TIE2	**-0.392**	**-0.313**	**-0.452**	**-0.068**	**-0.064**	**-0.273**	**0.220**
P-value	0.337	0.450	0.260	0.872	0.881	0.514	0.601
ANGPT2	**-0.703**	**-0.320**	**-0.780**	**-0.282**	**0.027**	**-0.293**	**0.208**
P-value	0.052	0.439	0.023	0.498	0.950	0.482	0.621
Np1	**-0.354**	**-0.313**	**-0.713**	**0.018**	**-0.225**	**-0.383**	**0.267**
P-value	0.389	0.451	0.047	0.965	0.592	0.350	0.523
Np2	**0.105**	**-0.356**	**-0.357**	**0.368**	**0.067**	**0.003**	**0.587**
P-value	0.805	0.387	0.385	0.370	0.876	0.994	0.126
FGF	**-0.223**	**-0.589**	**-0.528**	**0.300**	**0.022**	**-0.145**	**0.385**
P-value	0.595	0.125	0.178	0.471	0.958	0.732	0.346
FGFR	**-0.386**	**-0.344**	**-0.625**	**0.036**	**-0.080**	**-0.247**	**0.244**
P-value	0.345	0.403	0.098	0.932	0.851	0.556	0.561
VIP	**0.331**	**-0.485**	**-0.093**	**0.606**	**0.182**	**0.174**	**0.697**
P-value	0.423	0.223	0.827	0.112	0.666	0.679	0.055

Table A61. (Continued). Correlation between fetal small intestinal proliferation and vascularity with mRNA factors in HI IUGR animals

Factors	CAD[1]	CND[2]	CSD[3]	APC[4]	Total small intestine, g	Total jejunal vascularity	Proliferating nuclei, %
VIPR1	**0.539**	**0.585**	**0.754**	**-0.060**	**0.114**	**0.303**	**0.111**
P-value	0.168	0.128	0.031	0.888	0.788	0.465	0.794
VIPR2	**0.532**	**0.366**	**0.671**	**0.089**	**0.534**	**0.613**	**0.533**
P-value	0.175	0.372	0.068	0.834	0.173	0.106	0.174
ERα	**0.145**	**0.726**	**0.281**	**-0.450**	**-0.246**	**-0.151**	**-0.103**
P-value	0.733	0.042	0.501	0.264	0.557	0.721	0.809

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.
[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.
[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.
[4]Area per capillary = capillary area/capillary number per sample area.

Table A62. Correlation between fetal small intestinal proliferation and vascularity with mRNA factors in HI IUGR+E2 animals

Factors	CAD[1]	CND[2]	CSD[3]	APC[4]	Total small intestine, g	Total jejunal vascularity	Proliferating nuclei, %
VEGF	**-0.269**	**0.616**	**0.312**	**-0.598**	**-0.066**	**-0.235**	**-0.758**
P-value	0.662	0.269	0.609	0.287	0.916	0.703	0.137
Flt	**0.093**	**0.816**	**0.373**	**-0.267**	**-0.683**	**-0.705**	**-0.731**
P-value	0.881	0.092	0.536	0.664	0.204	0.184	0.161
KDR	**-0.041**	**0.116**	**-0.478**	**0.090**	**-0.289**	**-0.551**	**0.424**
P-value	0.948	0.852	0.415	0.885	0.637	0.336	0.476
HIF	**-0.789**	**0.209**	**-0.841**	**-0.701**	**0.099**	**-0.744**	**0.038**
P-value	0.113	0.736	0.074	0.187	0.874	0.149	0.951
eNOS	**-0.340**	**0.962**	**0.065**	**-0.666**	**-0.219**	**-0.608**	**-0.487**
P-value	0.576	0.009	0.918	0.220	0.724	0.277	0.406
sGC	**0.152**	**-0.501**	**-0.320**	**0.356**	**-0.463**	**-0.448**	**0.028**
P-value	0.807	0.390	0.599	0.557	0.433	0.450	0.964
ANGPT1	**-0.412**	**-0.446**	**-0.604**	**-0.219**	**-0.060**	**-0.474**	**-0.135**
P-value	0.491	0.452	0.281	0.724	0.923	0.420	0.828
TIE2	**-0.109**	**-0.438**	**0.148**	**-0.113**	**0.149**	**0.235**	**-0.514**
P-value	0.861	0.461	0.812	0.856	0.811	0.704	0.376
ANGPT2	**-0.575**	**-0.044**	**-0.670**	**-0.483**	**-0.138**	**-0.761**	**-0.216**
P-value	0.310	0.944	0.216	0.410	0.826	0.135	0.728
Np1	**-0.866**	**0.443**	**-0.434**	**-0.970**	**0.421**	**-0.323**	**-0.279**
P-value	0.058	0.455	0.465	0.006	0.480	0.596	0.650
Np2	**0.003**	**-0.214**	**0.321**	**-0.117**	**-0.139**	**0.011**	**-0.770**
P-value	0.996	0.729	0.598	0.852	0.824	0.986	0.127
FGF	**-0.883**	**0.381**	**-0.828**	**-0.838**	**0.246**	**-0.668**	**0.090**
P-value	0.047	0.528	0.083	0.077	0.690	0.218	0.885
FGFR	**-0.617**	**-0.445**	**-0.663**	**-0.420**	**0.258**	**-0.273**	**-0.086**
P-value	0.267	0.453	0.223	0.482	0.676	0.657	0.890
VIP	**0.216**	**-0.912**	**0.077**	**0.421**	**0.125**	**0.471**	**-0.053**
P-value	0.727	0.031	0.902	0.480	0.842	0.423	0.933

Table A62. (Continued). Correlation between fetal small intestinal proliferation and vascularity with mRNA factors in HI IUGR+E2 animals

Factors	CAD[1]	CND[2]	CSD[3]	APC[4]	Total small intestine, g	Total jejunal vascularity	Proliferating nuclei, %
VIPR1	**0.881**	**-0.180**	**0.952**	**0.723**	**-0.441**	**0.462**	**-0.396**
P-value	0.048	0.772	0.013	0.168	0.458	0.434	0.510
VIPR2	**-0.356**	**0.298**	**0.209**	**-0.581**	**0.184**	**0.007**	**-0.656**
P-value	0.557	0.626	0.736	0.304	0.767	0.992	0.229
ERα	**-0.848**	**0.629**	**-0.637**	**-0.921**	**0.200**	**-0.669**	**-0.065**
P-value	0.070	0.256	0.248	0.026	0.747	0.217	0.917

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.
[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.
[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.
[4]Area per capillary = capillary area/capillary number per sample area.

Table A63. Correlation between fetal small intestinal proliferation and vascularity with mRNA factors in HI non-IUGR animals

Factors	CAD[1]	CND[2]	CSD[3]	APC[4]	Total small intestine, g	Total jejunal vascularity	Proliferating nuclei, %
VEGF	**-0.112**	**-0.752**	**-0.283**	**0.360**	**0.241**	**0.234**	**-0.922**
P-value	0.833	0.085	0.586	0.483	0.646	0.655	0.009
Flt	**0.411**	**0.813**	**0.511**	**-0.367**	**-0.084**	**0.219**	**0.534**
P-value	0.418	0.049	0.300	0.474	0.875	0.677	0.275
KDR	**-0.191**	**0.254**	**0.068**	**-0.350**	**-0.154**	**-0.407**	**-0.388**
P-value	0.718	0.627	0.898	0.497	0.771	0.423	0.447
HIF	**0.268**	**0.201**	**0.125**	**-0.275**	**0.425**	**0.683**	**-0.050**
P-value	0.608	0.703	0.814	0.599	0.400	0.135	0.925
eNOS	**0.627**	**0.192**	**0.334**	**0.564**	**-0.297**	**0.154**	**-0.568**
P-value	0.183	0.716	0.518	0.244	0.568	0.771	0.240
sGC	**-0.062**	**0.121**	**-0.440**	**-0.167**	**0.395**	**0.205**	**0.283**
P-value	0.907	0.819	0.382	0.752	0.439	0.697	0.587
ANGPT1	**-0.022**	**-0.710**	**-0.506**	**0.564**	**0.290**	**0.276**	**-0.588**
P-value	0.968	0.114	0.306	0.244	0.577	0.597	0.220
TIE2	**0.320**	**-0.376**	**-0.033**	**0.810**	**-0.220**	**0.034**	**-0.593**
P-value	0.536	0.463	0.950	0.051	0.675	0.950	0.215
ANGPT2	**-0.120**	**-0.426**	**0.017**	**0.132**	**-0.032**	**-0.060**	**-0.906**
P-value	0.821	0.400	0.975	0.803	0.952	0.910	0.013
Np1	**-0.737**	**-0.237**	**-0.692**	**-0.835**	**0.795**	**0.185**	**0.355**
P-value	0.095	0.652	0.128	0.039	0.059	0.726	0.490
Np2	**-0.262**	**-0.799**	**-0.553**	**0.298**	**0.380**	**0.193**	**-0.792**
P-value	0.616	0.057	0.255	0.567	0.457	0.715	0.060
FGF	**0.653**	**-0.085**	**0.072**	**0.767**	**0.002**	**0.548**	**0.042**
P-value	0.160	0.873	0.892	0.075	0.998	0.260	0.938
FGFR	**-0.040**	**-0.543**	**-0.519**	**0.400**	**0.331**	**0.255**	**-0.529**
P-value	0.941	0.266	0.292	0.431	0.522	0.626	0.280
VIP	**-0.716**	**-0.381**	**-0.649**	**-0.555**	**0.591**	**0.045**	**0.559**
P-value	0.110	0.456	0.163	0.253	0.217	0.933	0.249

Table A63. (Continued). Correlation between fetal small intestinal proliferation and vascularity with mRNA factors in HI non-IUGR animals

Factors	CAD[1]	CND[2]	CSD[3]	APC[4]	Total small intestine, g	Total jejunal vascularity	Proliferating nuclei, %
VIPR1	**0.584**	**0.887**	**0.617**	**0.052**	**-0.524**	**-0.166**	**0.294**
P-value	0.224	0.018	0.192	0.923	0.286	0.753	0.572
VIPR2	**-0.333**	**-0.509**	**-0.545**	**-0.008**	**0.483**	**0.280**	**0.466**
P-value	0.518	0.303	0.263	0.988	0.332	0.590	0.352
ERα	**0.100**	**-0.672**	**-0.512**	**0.347**	**0.705**	**0.845**	**-0.484**
P-value	0.850	0.144	0.300	0.500	0.118	0.034	0.330

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.
[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.
[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.
[4]Area per capillary = capillary area/capillary number per sample area.

Table A64. Correlation between fetal small intestinal proliferation and vascularity with mRNA factors in HI non-IUGR+E2 animals

Factors	CAD[1]	CND[2]	CSD[3]	APC[4]	Total small intestine, g	Total jejunal vascularity	Proliferating nuclei, %
VEGF	**-0.670**	**-0.298**	**-0.457**	**-0.356**	**0.220**	**-0.125**	**0.247**
P-value	0.100	0.516	0.302	0.433	0.635	0.789	0.593
Flt	**-0.300**	**0.148**	**0.043**	**-0.273**	**0.040**	**-0.033**	**0.409**
P-value	0.514	0.752	0.928	0.554	0.933	0.944	0.363
KDR	**-0.036**	**-0.630**	**-0.266**	**0.309**	**0.637**	**0.386**	**0.494**
P-value	0.940	0.129	0.564	0.501	0.124	0.392	0.260
HIF	**0.020**	**-0.475**	**-0.099**	**0.316**	**0.326**	**0.303**	**0.657**
P-value	0.965	0.282	0.832	0.489	0.476	0.509	0.109
eNOS	**-0.703**	**-0.030**	**-0.452**	**-0.591**	**-0.468**	**-0.595**	**0.299**
P-value	0.078	0.949	0.308	0.162	0.289	0.159	0.515
sGC	**0.241**	**0.321**	**0.542**	**-0.197**	**-0.303**	**-0.086**	**0.430**
P-value	0.603	0.483	0.209	0.671	0.509	0.855	0.336
ANGPT1	**-0.512**	**-0.455**	**-0.407**	**-0.187**	**0.166**	**-0.071**	**0.592**
P-value	0.241	0.306	0.365	0.689	0.722	0.879	0.161
TIE2	**0.347**	**-0.486**	**0.131**	**0.543**	**0.360**	**0.469**	**0.752**
P-value	0.446	0.269	0.780	0.207	0.428	0.288	0.051
ANGPT2	**-0.451**	**-0.343**	**-0.482**	**-0.027**	**0.261**	**0.020**	**0.035**
P-value	0.309	0.451	0.274	0.954	0.572	0.965	0.941
Np1	**0.506**	**-0.326**	**0.461**	**0.517**	**0.562**	**0.660**	**0.801**
P-value	0.246	0.475	0.298	0.235	0.189	0.107	0.031
Np2	**-0.213**	**0.128**	**0.185**	**-0.283**	**0.259**	**0.109**	**0.602**
P-value	0.646	0.785	0.692	0.539	0.574	0.815	0.153
FGF	**0.419**	**0.182**	**0.446**	**0.161**	**-0.104**	**0.136**	**0.556**
P-value	0.350	0.696	0.316	0.731	0.824	0.772	0.195
FGFR	**-0.335**	**-0.196**	**-0.246**	**-0.027**	**0.337**	**0.137**	**0.246**
P-value	0.463	0.674	0.595	0.955	0.460	0.770	0.595
VIP	**0.403**	**-0.077**	**0.429**	**0.512**	**0.774**	**0.781**	**0.347**
P-value	0.370	0.870	0.336	0.241	0.041	0.038	0.446

Table A64. (Continued). Correlation between fetal small intestinal proliferation and vascularity with mRNA factors in HI non-IUGR+E2 animals

Factors	CAD[1]	CND[2]	CSD[3]	APC[4]	Total small intestine, g	Total jejunal vascularity	Proliferating nuclei, %
VIPR1	**0.207**	**0.050**	**0.080**	**-0.012**	**-0.646**	**-0.346**	**0.426**
P-value	0.695	0.925	0.880	0.982	0.166	0.502	0.400
VIPR2	**0.080**	**0.280**	**0.335**	**-0.104**	**-0.228**	**-0.037**	**0.491**
P-value	0.865	0.543	0.463	0.824	0.622	0.937	0.263
ERα	**-0.281**	**0.255**	**0.018**	**-0.403**	**-0.458**	**-0.376**	**0.404**
P-value	0.541	0.581	0.969	0.370	0.301	0.406	0.368

[1]Capillary area density = (capillary area/tissue area evaluated) x 100.
[2]Capillary number density = (capillary number/tissue area evaluated) x 1,000,000. This calculation provides the number of capillaries per mm^2 of tissue area analyzed.
[3]Capillary surface density = (total capillary circumference/tissue area evaluated) x 10.
[4]Area per capillary = capillary area/capillary number per sample area.