2014-03-10

Different Expression of Placental Pyruvate Kinase M2 in Normal, Preeclamptic, and Intrauterine Growth Restriction Pregnancies

Brigham L. Bahr
Brigham Young University - Provo

Different Expression of Placental Pyruvate Kinase M2 in Normal, Preeclamptic, and Intrauterine Growth Restriction Pregnancies

Brigham L. Bahr

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

Juan A. Arroyo, Chair
Benjamin T. Bikman
Joshua Andersen

Department of Physiology and Developmental Biology

Brigham Young University

March 2014

ABSTRACT

Different Expression of Placental Pyruvate Kinase M2 in Normal, Preeclamptic, and Intrauterine Growth Restriction Pregnancies

Brigham L. Bahr
Department of Physiology and Developmental Biology, BYU
Master of Science

This thesis will be organized into two chapters discussing the placental expression of two proteins, pyruvate kinase M2 (PKM2) and heat shock protein 27 (HSP 27), in human placentas. Understanding the mechanisms of placental metabolism in healthy and diseased placentas helps us understand how placenta disorders occur and how we can treat these disorders. The goal is to investigate these proteins to gain an understanding of their roles in placental disorders and help decrease maternal and fetal mortality rates.

Chapter one covers the background of pyruvate kinase M2 (PKM2) in cancer and embryonic tissues, and the expression of PKM2 in the human placenta. Cancer PKM2 has been studied extensively, but little is know about the role of placental PKM2. Expression of PKM2 is confirmed in normal human placenta samples and described in preeclamptic and intrauterine growth restriction (IUGR) affected human placentas. Proteins associated with elevated PKM2 in cancer are also associated with elevated PKM2 in human placentas. Comparing normal and diseased placenta samples helps understand the similarities between cancer PKM2 and placental PKM2. Understanding the mechanisms of placental metabolism and PKM2 expression in the human placenta will clarify how the placenta is affected by preeclampsia and IUGR and the role placental PKM2 plays in each of these diseases.

Chapter two will cover a paper that I wrote on the expression of phosphorylated heat shock protein 27 (HSP27) in the human placenta. Heat shock proteins are involved in the stress response and help inhibit apoptosis. The object of the study was to look for correlations between p-HSP27 and apoptosis in human and ovine placenta samples. P-HSP27 was quantified in human placenta samples and in placenta sampled collected from ovine models. Pregnant control and hyperthermic sheep models were used to quantify expression of p-HSP27 across gestation. This study showed similarities between human IUGR and our ovine IUGR model, suggesting a link between decreased p-HSP27 and increased apoptosis in IUGR.

Keywords: placenta, pyruvate kinase M2 (PKM2), preeclampsia (PE), intrauterine growth restriction (IUGR), metabolism

ACKNOWLEDGMENTS

Thank you to Dr. Juan Arroyo for being my graduate chair/advisor and helping me complete my research and thesis, for pushing me to stretch myself. I would also like to thank Dr. David Bearss for getting me started on my research. Thank you to Dr. Joshua Andersen and Dr. Benjamin Bikman for being on my graduate committee, and to the Arroyo and Bearss labs and all the undergraduate students who helped with the research.

To Emily, thank you for your love and continued support, your encouragement, and for your patience. To my parents, thank you for your advice and continued support.

This research was supported by grant 7R00HD055054-06

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: Different Expression of Placental Pyruvate Kinase M2 in Normal, Preeclamptic, and Intrauterine Growth Restriction Pregnancies

Brigham L. Bahr, Matthew D. Price, Daniel Merrill, Camilo Mejia, Lee Call, David Bearss, Juan A. Arroyo

Department of Physiology and Developmental Biology,
Brigham Young University, Provo UT 84602, USA

Abstract

Introduction: Preeclampsia (PE) and intrauterine growth restriction (IUGR) are two diseases that affect pregnant women and their unborn children. These diseases cause low birth weight, pre-term delivery, neurological and cardiovascular disorders and combined they account for 20% of preterm delivery. Pyruvate kinase M2 (PKM2) is a metabolism enzyme found in developing embryonic and cancer tissues. Our objective is to determine the expression of PKM2 in human PE and IUGR compared to normal pregnancies.

Methods: Human placental tissues were obtained for PKM2 determination and analyzed by immunohistochemistry, western blot, and a pyruvate assay. Placental samples were homogenized and cytoplasmic and nuclear proteins were extracted for western blot analysis.

Results: Preeclampsia samples had elevated levels of p-PKM2, p-ERK, and ERK in the cytoplasm. Beta-catenin and lactose dehydrogenase (LDH) were also elevated in preeclampsia placenta samples.

Discussion and Conclusion: We conclude that PKM2 is expressed in normal, PE and IUGR pregnancies. Also, that this expression is increased in the PE placenta. These results suggest placental metabolism through PKM2 could be involved in the development of human preeclampsia.

Introduction

Preterm Birth, Intrauterine Growth Restriction, and Preeclampsia

Preterm birth (PTB) is associated with up to 70% of neonatal deaths and leads to an increased incidence of cerebral palsy, neurological defects, and pulmonary disorders in the neonate (Challis et al., 2001). There is an association between the development of intrauterine growth restriction (IUGR) and PTB (Pilling, Elder, & Gibson, 2008). Studies show that there is a risk of up to 44% of PTB associated with IUGR (Delpisheh, Brabin, Attia, & Brabin, 2008). IUGR is a complication of pregnancy that affects up to 10% of all pregnancies and significantly increases risks of fetal and neonatal morbidity and mortality (Brar & Rutherford, 1988; Gray, O'Callaghan, Harvey, Burke, & Payton, 1999; Pollack & Divon, 1992). IUGR complications include perinatal hypoxia and asphyxia, cerebral palsy, and persistent pulmonary hypertension of the newborn (Brar & Rutherford, 1988; Gray et al., 1999; Pollack & Divon, 1992). Preeclampsia is defined as the excretion of protein (≥300 mg in 24 hr) in the urine and high blood pressure, after the 20th week, in pregnant women who are not typically hypertensive (Leung, Smith, To, Sahota, & Baker, 2001; Lindheimer, Taler, & Cunningham, 2010; X. Xiong et al., 1999). Preeclampsia accounts for about 20% of induced PTB (Goldenberg & Rouse, 1998). Preterm birth (PTB) observed during PE results from early delivery of the placenta and fetus to alleviate symptoms associated with PE (Goldenberg & Rouse, 1998). Preeclampsia (PE) can also cause intrauterine fetal demise (IUFD), or death of the fetus before delivery (Alexander & Wilson, 2013). In addition, several studies reported long-term consequences of PE and IUGR, including adult hypertension, heart disease, stroke, and diabetes (Barker, 1993a, 1993b, 2004; Barker et al., 1993; Holemans, Van, Verhaeghe, Aerts, & Van Assche, 1993; Phipps et al., 1993; Reusens-Billen,

Remacle, & Hoet, 1989). Placental dysfunction is a major cause these obstetric complications. PE and IUGR diseased placentas are characterized by a number of pathologic findings including: reduced syncytiotrophoblast surface area, decreased trophoblast invasion, and increased placental trophoblast apoptosis (Allaire, Ballenger, Wells, McMahon, & Lessey, 2000; DiFederico, Genbacev, & Fisher, 1999; Hung, Skepper, Charnock-Jones, & Burton, 2002; Levy & Nelson, 2000; Mayhew et al., 2003; Roos et al., 2007; Smith, Baker, & Symonds, 1997). Normal placental development is critical for a successful pregnancy. Studies have shown that aberrant trophoblast function has been associated with clinical obstetric pathologies including PE and IUGR (Krebs et al., 1996).

Pyruvate Kinase M2 (PKM2) Protein

Pyruvate Kinase is a metabolic enzyme that exists in four isoforms: Isoform L, found in liver, kidney, and intestinal tissues, supports high levels of gluconeogenesis. R isoform is similar to the L isoform, but is primarily found in red blood cells (Christofk, Vander Heiden, Harris, et al., 2008; Mazurek, 2011). Isoforms M1 and M2 are similar because they both regulate the final step of glycolysis and differ by having exon 9 (PKM1) replaced with exon 10 (PKM2) (Dombrauckas, Santarsiero, & Mesecar, 2005). This change causes functional differences between the more active PKM1 and the less active PKM2. Active PKM1 readily promotes glycolysis and the conversion of phosphoenolpyruvate (PEP) to pyruvate, which in the presence of oxygen is transported to the mitochondria and used for energy production. Less active PKM2 inhibits overall glycolysis, but interestingly promotes aerobic glycolysis, converting pyruvate to lactate for energy production. The inhibition of glycolysis by PKM2 allows redistribution of glycolytic intermediates to support biosynthesis of macromolecules and cancer proliferation (Cairns, Harris, & Mak, 2011; Christofk, Vander Heiden, Wu,

Asara, & Cantley, 2008). PKM1 is primarily found in adult tissues, including muscle and brain tissue, which require high levels of energy production (Christofk, Vander Heiden, Harris, et al., 2008; Christofk, Vander Heiden, Wu, et al., 2008; Mazurek, 2011). PKM2 is primarily found in cells with high levels of nucleotide synthesis such as proliferating cancer cells and developing embryonic tissues (Christofk, Vander Heiden, Harris, et al., 2008; Christofk, Vander Heiden, Wu, et al., 2008; Dombrauckas et al., 2005; Jurica et al., 1998; Mazurek, 2011). The role of PKM2 in cancer has been studied extensively since Otto Warburg's observations in the 1920's. Warburg noted cancer cells display an increased level of glucose uptake and lactate production, yet decreased overall ATP production even in the presence of sufficient oxygen (Warburg, 1956). This phenomenon has since been termed the Warburg Effect (Heiden, Cantley, & Thompson, 2009; Y. Xiong, Lei, Zhao, & Guan, 2011).

Research is uncovering more about PKM2 in cancer metabolism, but we still know little about embryonic PKM2. Understanding the role of PKM2 in embryonic tissues could help us understand more about placental metabolism during intrauterine growth restriction (IUGR) and preeclampsia (PE). These two diseases play a significant role in fetal development and can contribute to pre-term delivery, fetal mortality rates, and cardiovascular and metabolic diseases in infants and adults (Lindheimer et al., 2010). Understanding metabolic mechanisms of PE and IUGR could find treatments to save lives and reduce complications in neonates and pregnant mothers.

Placental Metabolism

Placental metabolism is important for a successful pregnancy. The placenta is the primary site of nutrient and gas exchange between the mother and the fetus. Trophoblasts within the placenta use large amounts of energy to mediate nutrient

transfer, synthesis of hormones, and secretion of molecules (Bax & Bloxam, 1997). Altered nutrient supply to the fetus can affect its growth and development leading to obstetric complications such as IUGR and PE (Cetin & Antonazzo, 2009). IUGR and PE are associated with abnormal carbohydrate metabolism and aberrant trophoblast differentiation (Bax & Bloxam, 1997). Placenta metabolism, which uses almost 40% of the utero placental oxygen uptake, is correlated with oxygen availability (Cetin & Antonazzo, 2009). A common feature in IUGR and PE complicated pregnancies is increased hypoxia inducible factor (HIF), suggesting the presence of hypoxia in the placenta (Tal et al., 2010). Previous reports show that different tissues can alter metabolic processes to meet their ATP demands (Tissot van Patot et al., 2010). This suggests that perhaps these tissues could promote anaerobic glycolysis to maintain ATP synthesis (Tissot van Patot et al., 2010). Thus placental metabolism could play a role in placental development and altered metabolism could offset complications of IUGR and PE.

Our first objective was to determine the expression and localization of PKM2 in the normal human placenta at term. Because of the involvement of PKM2 in metabolism we extended these studies to determine this protein expression during complicated pregnancies with PE and IUGR. Recent reports have suggested different expression of PKM2 in the nucleus and cytosol of cells. This inspired us to determine differences in PKM2 expression between nuclear and cytosolic fractions in normal, PE and IUGR placentas.

Materials and Methods

Human Placental Tissues

All frozen human term placental samples and slices (Preeclampsia, IUGR, and Control) were purchased from the Research Center for Women's and Infant's Health BioBank, Ontario, Canada.

Immunohistochemistry

Immunohistochemistry was performed as previously done in our lab (Arroyo et al., 2010a). In summary, slides were de-waxed, washed in a 1x Tris buffer solution (TBS), and blocked with Sniper for one hr. This was followed by incubation for one hr with a primary antibody (PKM2-Cell Signaling Technology, Danvers, MA, rabbit #4053, Cytokeratin 7-Dako, Carpinteria, CA, mouse #M7018 (for trophoblast localization), Beta-Catenin-Cell Signaling Technology, Danvers, MA, rabbit #9562, Lactose Dehydrogenase-B-Santa Cruz Biotechnology, Dallas, TX, #sc-100775, IgG negative control-Biocare Medical, Concord, CA). Slides were then incubated with Mach 2 universal stain polymer, followed by color development with diaminobenzidine (DAB). Hematoxylin was used for nuclear counterstain. Slides were mounted using Permount media.

Cytoplasmic and Nuclear Extraction

Approximately 100 mg of placental samples, obtained from the Research Centre for Women's and Infant's Health BioBank, were ground with mortal and pestle in liquid nitrogen. Ground tissues were separated into cytoplasmic and nuclear extracts following the protocol accompanying the NE-PER Nuclear and Cytoplasmic Extraction Reagents kit (Thermo Scientific, Rockford, IL #78835). Ground placental tissue was

combined in a microcentrifuge tube with 500 μL of (ice cold) CER I, and 5 μL of protease inhibitor (Thermo Scientific, Rockford, IL #78441) and homogenized with a tissue grinder (Fisher Scientific Power Gen 125). Microcentrifuge tube was vortexed at the highest setting for 15 s, and incubated on ice for 10 min. 27.5 μL of (ice cold) CER II was added to the tube and vortexed at its highest setting for 5 s, incubated on ice for 5 min, and vortexed again for 5 s. Tubes were centrifuged at 16,000 x g and 4°C for 5 min. Supernatants were collected into clean, chilled, pre-labeled microcentrifuge tubes and kept on ice. Remaining pellets in the old microcentrifuge tubes were re-suspended with 125 μL of ice cold NER and 1.25 μL of protease inhibitor (Thermo Scientific, Rockford, IL #78441), placed on a vortex at the highest setting for 15 s and incubated in ice for 10 min. This was repeated 3 times for a total incubation time of 40 min. Microcentrifuge tubes were again centrifuged at 16,000 x g and 4°C for 10 min. Supernatants were collected into clean, chilled, pre-labeled microcentrifuge tubes and kept on ice.

Bicinchoninic Acid (BCA) Analysis

To determine protein concentrations, cytoplasmic and nuclear supernatants were analyzed with a Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, #23225). Briefly, 2 μL of each sample was combined with 150 μL of Reagent A and B (50:1) in a labeled 96 well plate. The plate was covered and incubated at 37°C for 20 min, cooled to room temperature, and read on a SpectraMax 340PC absorbance microplate reader (Molecular Devices, Sunnyvale, CA). Samples were run in duplicate, averaged, and compared to a bovine serum albumin standard curve to determine protein concentrations. Supernatants, in microcentrifuge tubes, were stored at -80°C for future use.

Western Blotting

Control, IUGR, and Preeclampsia samples were purchased from the Research Centre for Women's and Infant's Health BioBank. Whole tissue lysates (50 μg) or cytoplasmic and nuclear extracts lysates were loaded (15 μL with 15 μg of protein) and separated on 4-12% Bis-Tris Midi Gel (Novex by Life Technologies, Carlsbad, CA, #WG1402BX10), at 160 V for approximately 120 min. Proteins were transferred to nitrocellulose membranes using invitrogen iBlot (Novex by Life Technologies, Carlsbad, CA, # IB301001). Membranes were blocked in 5% milk in TBST for 1 hr followed by overnight incubation with primary antibodies (p-PKM2, Cell Signaling Technology, Danvers, MA, rabbit #3827; p-p44/42, Cell Signaling Technology, Danvers, MA, rabbit #9101; PKM2, Cell Signaling Technology, Danvers, MA, rabbit #4053; p44/42, Cell Signaling Technology, Danvers, MA, rabbit #9102). A secondary anti-rabbit horseradish peroxidase (HRP) conjugated antibody (Pierce Biotechnology, Rockford, IL, goat anti-rabbit HRP #1858415) was incubated for 1 hr at room temperature followed by development using ECL substrate (Advansta, Menlo Park, CA, #K-12045-D50). Proteins were detected by exposure of membranes to x-ray film and development. Nuclear controls were determined by stripping the membranes with warm (37°C) Restore (Thermo Scientific, Rockford, IL, #21063) for 25 min, re-blocking the membranes in 5% milk, and re-probing with primary antibody Lamin B1 (Santa Cruz Biotechnology, Dallas, TX, goat #6216). Loading controls were determined by using a primary antibody against beta-actin (Abcam, Cambridge, MA, mouse #ab6276-100) with a secondary HRP conjugated antibody against mouse (R&D Systems, Minneapolis, MN, goat anti-mouse #HAF007). The presence of these proteins was confirmed and quantified. Bands were analyzed digitally with AlphaEaseFC software (Alpha Innotech Corporation, San Leandro, CA)

Pyruvate Assay

We quantified pyruvate levels in normal, PE, and IURG placentas by extracting cytosolic proteins as described above and analyzing them with a Pyruvate Assay Kit (BioVision Incorporated, Milpitas, CA, #K609-100). We followed the protocol provided in the kit by preparing the standard for a fluorometric assay and the samples for a 96 well plate. The reaction mix was prepared as directed and added to the samples in the 96 well plate, incubated for 30 min at room temperature, and read for fluorescence (Ex/Em 535/590 nm) on a SpectraMax GeminiEM Microplate Reader (Molecular Devices, Sunnyvale, CA).

Statistical Analysis

Control, preeclampsia, and IUGR placental sample end points compared for PKM2, p-PKM2, ERK, and p-ERK expression. Data was analyzed for normality and differences using the Mann-Whitney test with $p < 0.05$ as significantly different.

Results

PKM2 Expression in Human Placenta

We first wanted to determine PKM2 expression in whole lysates of human placenta at term. Figure 1.1A showed a characteristic western blot for PKM2 in the human placenta. To confirm that this expression is on the trophoblast cell of the villi we performed Immunohistochemistry (IHC) for PKM2. IHC showed that PKM2 expression was present in the syncytiotrophobast layer of the placental villi at term (Figure 1.1B). We next investigated PKM2 expression differences between normal placenta and those affected by PE or IUGR. IHC showed increased PKM2 in the PE placenta while there were no differences observed for PKM2 between control and IUGR

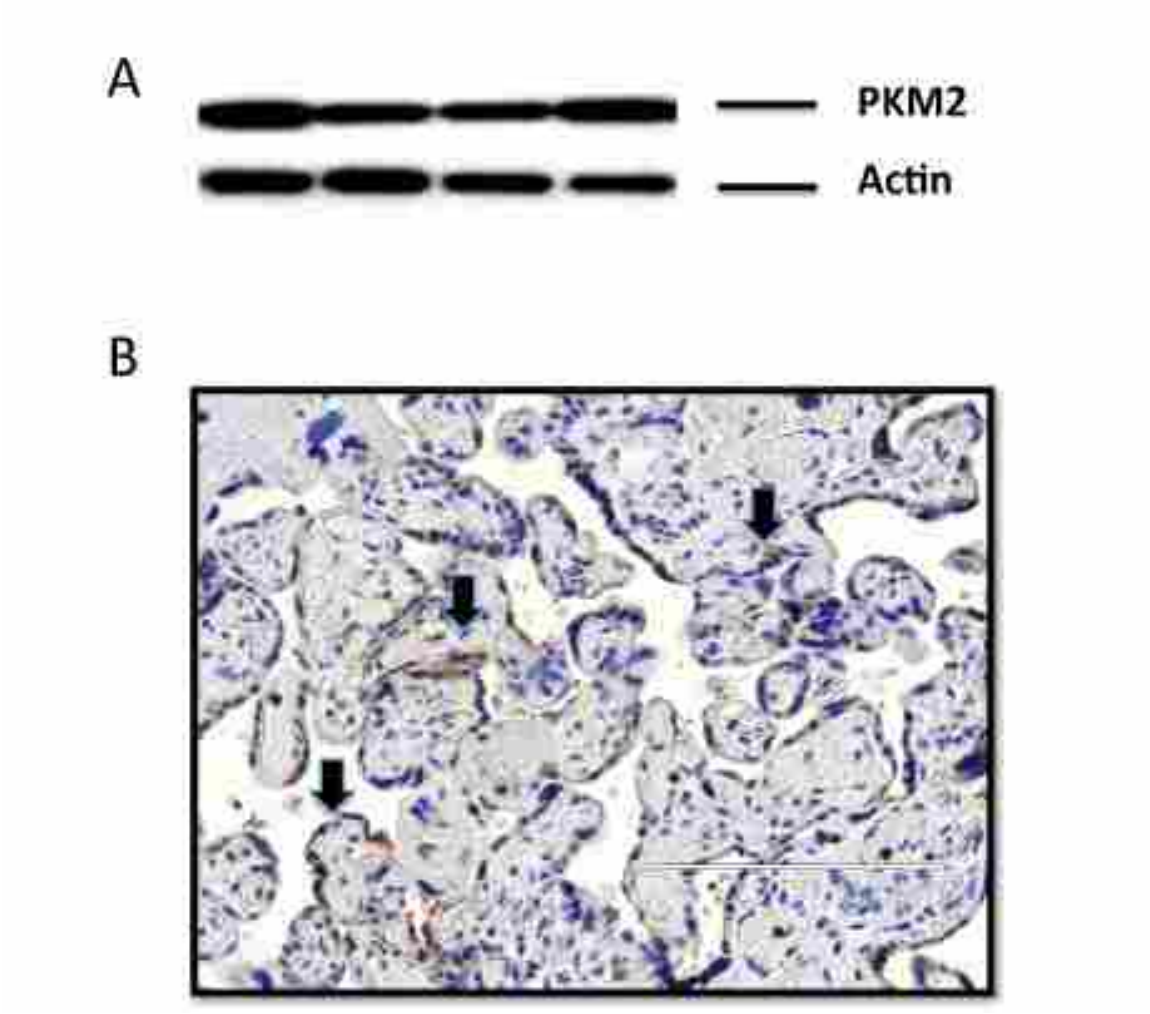

Figure 1.1: PKM2 Expression in Normal Human Placenta Confirmed. Western blot showing expression of PKM2 in normal placenta samples compared to beta actin controls (A). Immunohistochemistry staining for PKM2 in human placenta tissue samples, arrows indicate trophoblast localization (B).

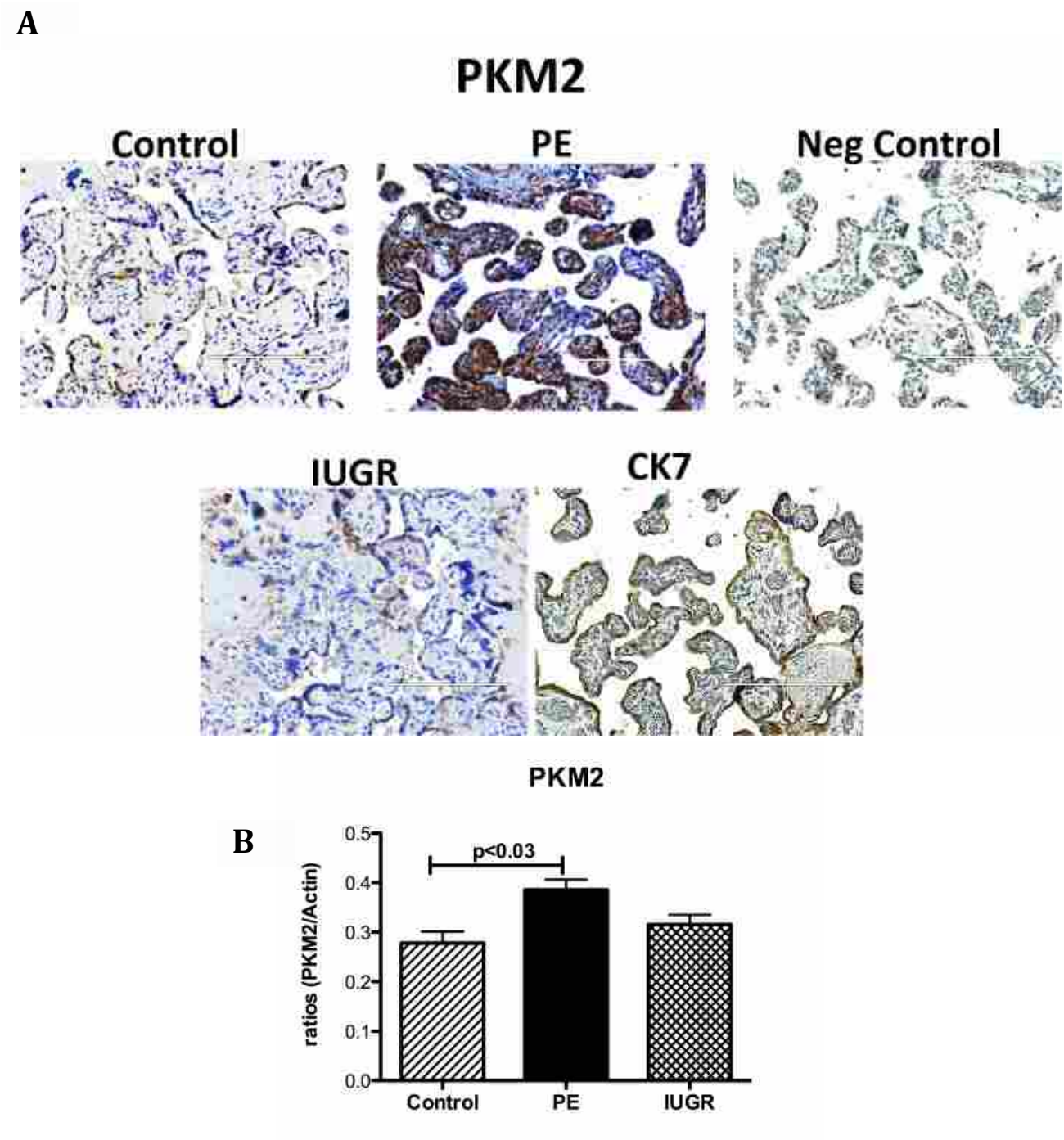

Figure 1.2: PKM2 Levels are Elevated in Preeclampsia Affected Placenta Samples. Immunohistochemistry staining for PKM2 in placental tissue samples; Control, Preeclampsia (PE), Negative control, Intrauterine Growth Restriction (IUGR), and CK7 (for trophoblasts) (A). Graphic quantification of PKM2 expression in placental samples; PKM2 is elevated in preeclampsia compared to control ($p < 0.03$). No significant difference was found between control and IUGR samples. PKM2 was normalized against levels of beta actin (B).

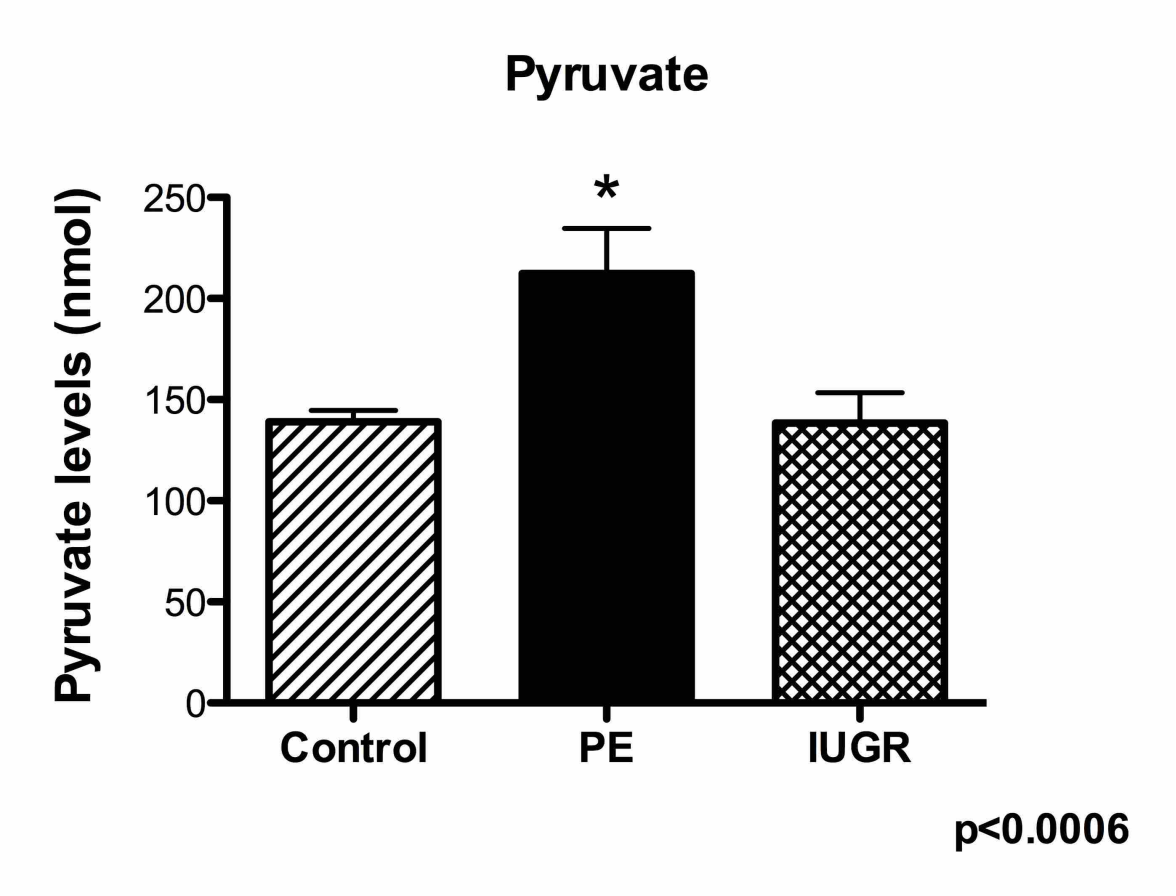

Figure 1.3: Pyruvate is Elevated in PE Diseased Human Placenta Samples. Homogenized human placental samples were analyzed with a BioVision Pyruvate Assay Kit to determine pyruvate levels in control, PE, and IUGR samples. The PE samples showed a significant increase in pyruvate ($p < 0.0006$) when compared to control samples.

(Figure 1.2A). Cytokeratin7 (CK7) staining was utilized to identify the localization of the trophoblast cells in the villi of the placenta (Figure 1.2A). Similarly to the results obtained by IHC, we found increased PKM2 expression in PE (1.4-fold; $p<0.03$) as compared to controls placentas (Figure 1.2B). There were not significant differences in PKM2 expression between control and IUGR placenta (Figure 1.2B).

Pyruvate in the Human Placenta

We next investigated pyruvate levels in the human placenta in control, PE and IUGR pregnancies. Similar to the results obtained for PKM2 protein we detected an increase in pyruvate levels (1.5-fold, $p<0.0006$) for PE affected placentas when compared to controls (Figure 1.3). No differences in pyruvate levels were observed between control and IUGR placentas.

Nuclear and Cytoplasmic Distribution of PKM2 in Placental Samples

Since PKM2 has been reported to have both cytoplasmic and nuclear functions, we examined the levels of PKM2 in both nuclear and cytoplasmic extracts. Human placenta tissue was homogenized and cytosolic and nuclear proteins were extracted. Western blots were done and quantified to determine levels of PKM2. There were no differences in PKM2 cytosolic expression between control and PE or IUGR placentas (Figure 1.4A). In contrast we observed a significant decrease in cytosolic PKM2 (1.3-fold; $p< 0.02$) between PE and IUGR placentas (Figure 1.4A). Interestingly, when nuclear fractions were studied there was a significant increase for PKM2 (1.6-fold; $p< 0.008$) in the PE placenta as compared to controls (Figure 1.4B). There was also a significant increase in IGUR nuclear PKM2 expression (1.5-fold; $p<0.05$) as compared to control placentas (Figure 1.4B).

Phospho-PKM2, Phospho-ERK, and ERK

Next we wanted to investigate the level of PKM2 phosphorylation (activation) in nuclear and cytosolic fractions in the control, PE, and IUGR placentas. There was an increase in phosphorylated PKM2 (p-PKM2) (1.3-fold; $p< 0.03$) in the PE placenta as compared to controls (Figure 1.5A). In contrast, there was a significant decrease in p-PKM2 in the IUGR placenta (1.3-fold; $p<0.05$) with no differences in PE placenta as compared to controls (Figure 1.5B). Previous studies have shown that ERK phosphorylates PKM2 and phosphorylated PKM2 can function as a nuclear transcription factor (Yang, Xia, et al., 2012; Yang et al., 2011; Yang, Zheng, et al., 2012). Thus we ran western blots to determine expression of phospho-ERK (p-ERK), and ERK in the cytoplasm and nucleus. Levels of p-ERK were elevated in the cytoplasm during PE (2.6-fold; $p<0.02$) and IUGR (1.7-fold; $p<0.05$) as compared to controls (Figure 1.5C). Similarly, ERK total protein levels were increased in both PE (1.2-fold; $p<0.02$) and IUGR (1.2-fold; $p<0.008$) samples (Figure 1,5D). Interestingly, nuclear levels of p-ERK and ERK were not significantly different from control samples (Figure 1.5E & F).

β – Catenin and Lactose Dehydrogenase

Previous studies in cancer have shown that PKM2 is a co-activator of beta-catenin in the nucleus (Yang, Xia, et al., 2012; Yang et al., 2011; Yang, Zheng, et al., 2012). This promotes the expression of c-Myc, and demonstrates the use of PKM2 as a transcriptional regulator. As a transcriptional regulator PKM2 increases the production and use of multiple proteins; including lactose dehydrogenase A (LDHA) and GLUT1 (Yang, Zheng, et al., 2012). These prior studies directed us to look at the expression of beta-catenin and LDH in placental samples. Figure 1.6 shows immunohistochemistry for beta-catenin and lactose dehydrogenase in preeclampsia placenta samples. IHC

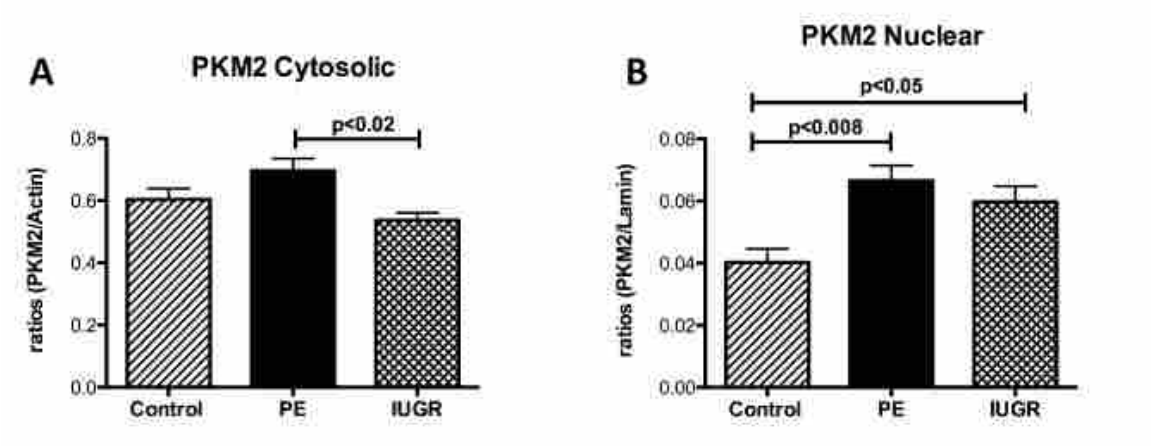

Figure 1.4: Human Placental Samples Show Increased Levels of PKM2 in the Nucleus for Preeclampsia and IUGR Samples. Cytosolic levels of PKM2 as measured by western blot and quantified by Spot Denso analysis. Cytosolic PKM2 levels for preeclampsia and IUGR are not significantly different when compare to control samples, but preeclampsia PKM2 is elevated when compared to IUGR (A). Nuclear PKM2 levels are elevated in both preeclampsia ($p < 0.008$) and IUGR ($p < 0.05$) when compared to control (B).

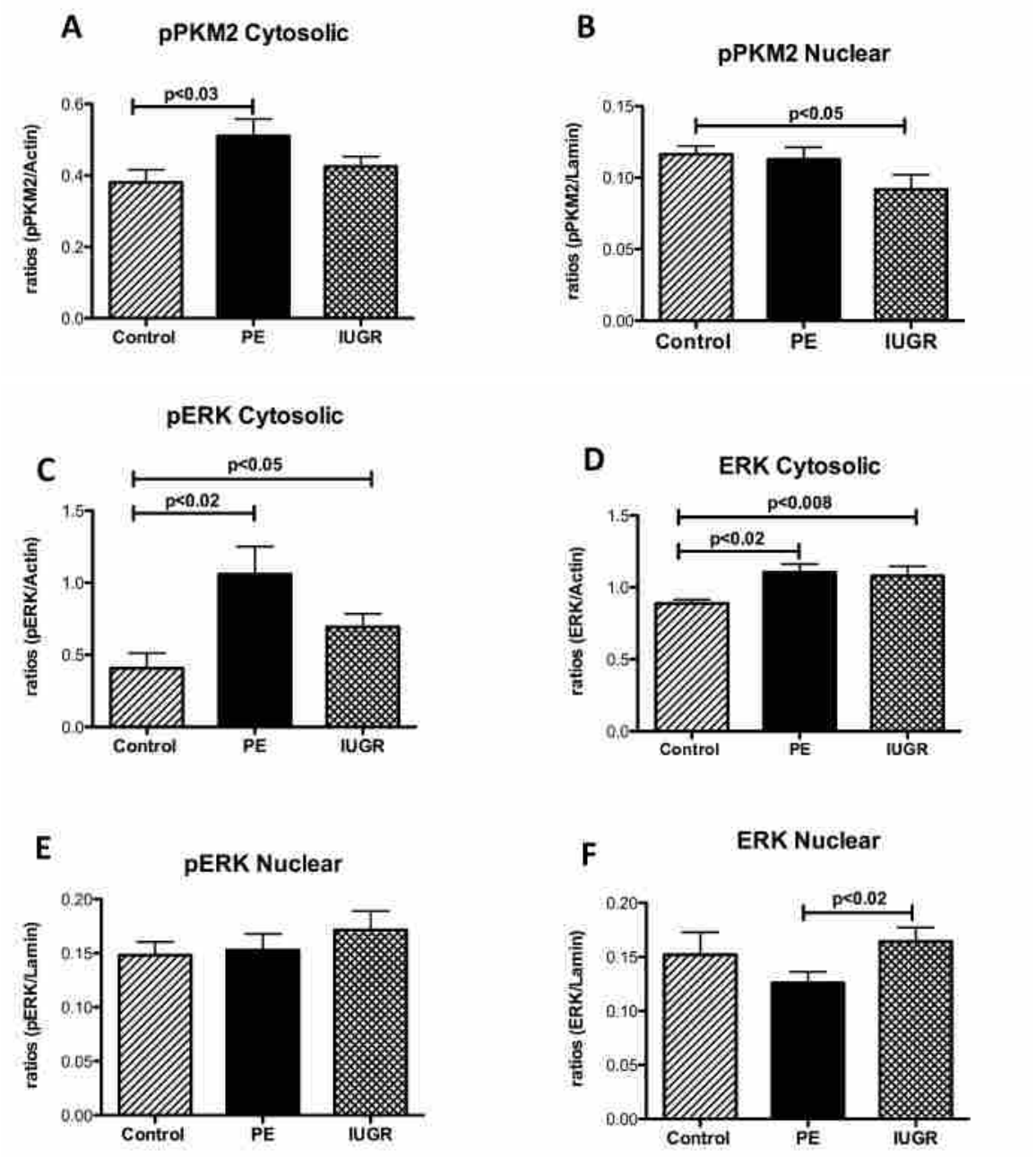

Figure 1.5: Cytosolic Levels of p-PKM2, p-ERK, and ERK are Elevated in Human Diseased Placental Samples. Phospho-PKM2 is elevated ($p<0.03$) in preeclampsia placental samples when compared to control samples (A). In IUGR samples, p-PKM2 is slightly decreased ($p<0.05$) compared to control (B). ERK and p-ERK are both elevated in cytosolic samples of preeclampsia and IUGR placentae (C, D). In nuclear placental samples, neither ERK nor p-ERK appear to be elevated when compared to control placenta samples (E, F).

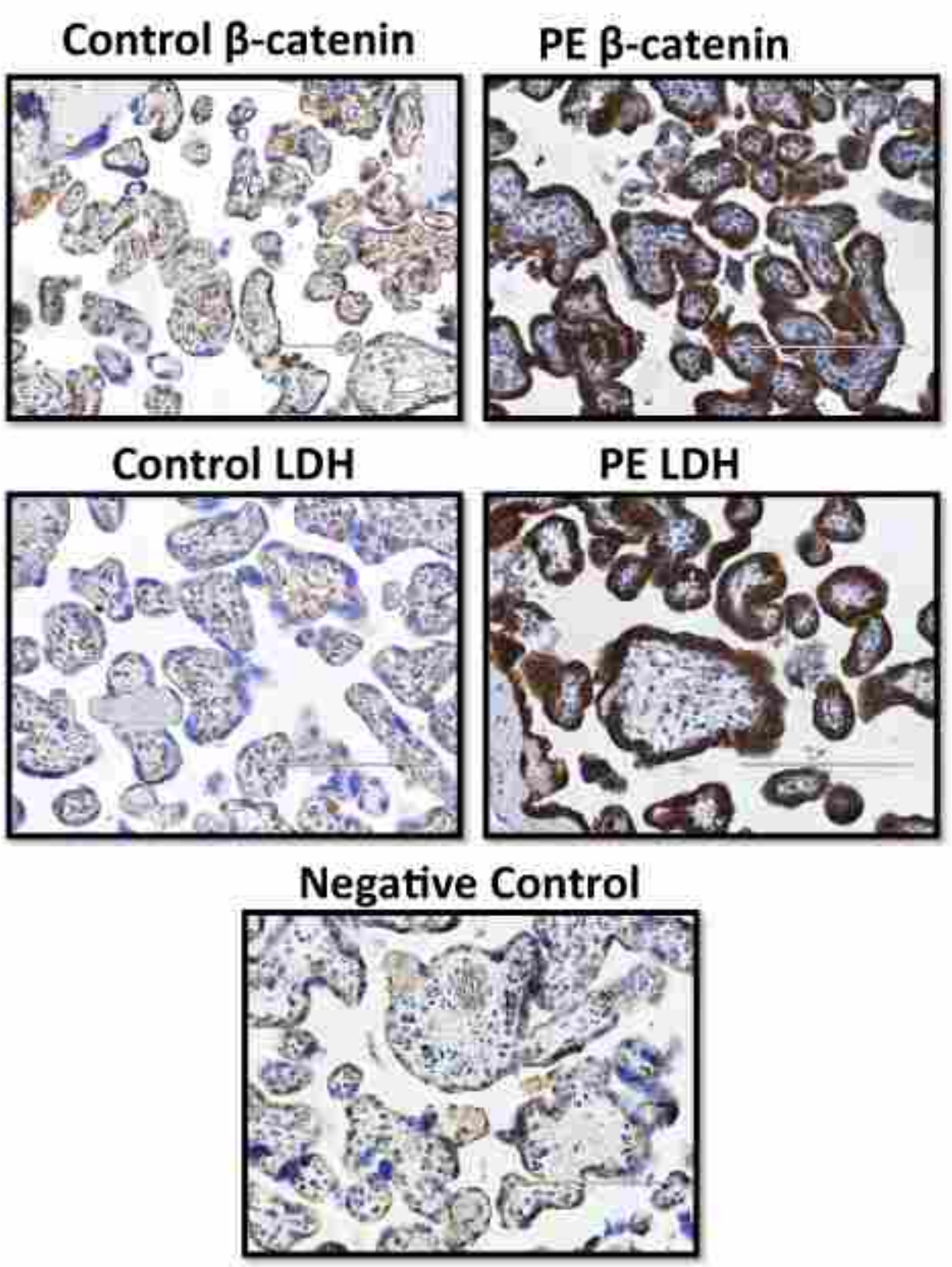

Figure 1.6: Beta-Catenin and Lactose Dehydrogenase (LDH) are Elevated in Preeclampsia Effected Human Placenta Samples. Immunohistochemistry shows that beta-catenin and LDH are elevated in preeclampsia placenta samples compared to control and negative control samples.

showed an increase of both beta-catenin and LDH in the villi trophoblast of the human placenta.

Discussion

Expression of PKM2 in cancer cells and tissue is well documented. Since the 1920's, when Otto Warburg observed that cancer cells use more glucose and produce more lactate than normal cells, researchers have been trying to understand the role of cancer metabolism in driving the process of cell transformation. Recently, PKM2 has emerged as a meditator of some of the metabolic processes observed in transformed cancer cells. Like cancer cells, developing embryonic tissues express high levels of PKM2 to support rapid growth and development (Christofk, Vander Heiden, Harris, et al., 2008). In this study we found that PKM2 expression is elevated in preeclampsia placentas, specifically in nuclear extracts, when compared to control placentas. Immunohistochemistry and western blot analysis showed a significant increase in PKM2 expression. Our immunohistochemistry findings indicate that PKM2 expression is elevated in preeclampsia and IUGR whole placental samples when compared to control full-term placenta samples. Elevated expression of PKM2 in the diseased human placenta indicates that PKM2 could play a role in placenta disease states. To further understand PKM2 in the human placenta, we extracted proteins from the cytoplasm and nucleus of human placenta samples to quantify expression of PKM2. Our data indicates PKM2 expression is elevated in the nuclear extracts of both IUGR ($p < 0.05$) and preeclampsia ($p < 0.008$) diseased placenta samples when compared to controls. Cytosolic PKM2 expression was not statistically different between the disease states and control, but preeclampsia PKM2 was greater than IUGR PKM2 ($p < 0.02$). Increased nuclear PKM2 expression indicates that PKM2 moves to the nucleus and

could be used as a transcriptional regulator as has been observed in cancer cells. To try and understand the pathway PKM2 regulates in human placentas, we looked at expression of p-PKM2, p-ERK, and ERK in our placenta samples. We hypothesized that cancer PKM2 and placental PKM2 would share the same mechanism in altering metabolism toward using glycolysis primarily for the production of anabolic intermediates instead of making pyruvate for energy production. Cytosolic p-PKM2 was elevated in preeclampsia ($p < 0.03$) but not in IUGR. Nuclear p-PKM2 was not statistically different in preeclampsia, and was lower in IUGR ($p < 0.05$) when compared to control nuclear p-PKM2. Both p-ERK and ERK were elevated in the cytoplasmic preeclampsia (p-ERK $p < 0.02$, ERK $p < 0.02$) and IUGR (p-ERK $p < 0.05$, ERK $p < 0.008$) compared to controls. Nuclear p-ERK and ERK expression were not statistically different from controls. These results are unexpected as we assumed that PKM2, p-PKM2, ERK, and p-ERK would be elevated in the cytoplasm and nucleus.

Because PKM2 is a metabolic enzyme that has been shown to have nuclear gene transcription activity in cancer cells, we looked at expression of beta-catenin and LDH. Studies have shown that phospho-PKM2 enters the nucleus and binds to beta-catenin and DNA, combined with c-Myc, other cancer promoting proteins are transcribed. Immunohistochemistry showed an increase in beta-catenin and LDH in preeclampsia diseased placenta when compared to control. Our findings indicate that PKM2 is expressed in human placenta samples, and PKM2 expression is elevated in preeclampsia placenta samples. We also show that expression of cancer related proteins are also altered in diseased human placentas. This suggests the mechanisms and pathways involved in cancer metabolism could be related to placental metabolism, specifically in preeclampsia affected placentas.

It is not clear if PKM2 activity in human placentas is a primary or secondary regulator of placenta metabolism. Several prior reports have suggested that elevated PKM2 expression is a secondary effect to hypoxia in cancer, and hypoxia in diseased placenta may be driving the elevated PKM2 levels we have observed. Perhaps elevated PKM2 expression in PE placentas could be a mechanism to prevent decreased placental and fetal growth when blood flow is decreased as observed during IUGR. This could be done by shunting glycolytic intermediates to help produce nucleic acids, amino acids and lipids to support growth. The PKM2 expression in placental tissues is not well understood, but could provide clues to placental metabolism and treatment of placental diseases. Future studies are needed to look at these pathways in greater detail to understand the mechanisms and causes of placental diseases and their correlation to PKM2.

CHAPTER 2: Decreased Expression of Phosphorylated Placental Heat Shock Protein 27 in Human and Ovine Intrauterine Growth Restriction (IUGR)

Brigham L. Bahr, Henry L. Galan, Juan A. Arroyo

Department of Physiology and Developmental Biology,
Brigham Young University, Provo, UT 84602, USA

Abstract

Introduction: Intrauterine growth restriction (IUGR) has been documented to increase placental apoptosis at term. HSP27 has been shown to be involved in the control of apoptosis. Our objective is to determine the expression of phosphorylated HSP27 (p-HSP27) in human IUGR, and to determine the role of HSP27 during gestation in an ovine hyperthermia induced model of IUGR.

Methods: Human placenta tissue samples were collected at term to quantify p-HSP27. Pregnant sheep were placed in hyperthermic (HT) conditions to induce IUGR. Placental tissues were collected at 55 (early), 95 (mid-gestation) and 130 (near-term) days gestational age (dGA) to determined phosphorylated and total HSP27 across the development of IUGR.

Results: Phosphorylated HSP27 was significantly reduced in human placenta IUGR compared to controls at term. HSP27 was increased throughout gestation during the development of IUGR in the sheep. P-HSP27 was increased in early gestation (55 dGA), and decreased near term (130 dGA). The near term decrease was localized to the trophoblast cells of the placenta.

Discussion and Conclusion: We conclude that decreased p-HSP27 at term is present when placental apoptosis is increased during IUGR. This could be a factor leading to the decreased placental weight observed during IUGR.

Introduction

Intrauterine growth restriction (IUGR) is a common, complex obstetric disease associated with long-term adverse conditions for the newborn and adult (Arroyo, Anthony, & Galan, 2008). Placental insufficiency is the most common cause of IUGR; combined with numerous placental abnormalities findings that include, but are not limited to hypoxia, apoptosis, aberrant trophoblast invasion of spiral arteries and increased placental resistance to blood flow (Allaire et al., 2000; Arroyo et al., 2008; DiFederico et al., 1999; Galan et al., 2005; Ishihara et al., 2002; Mori, Trudinger, Mori, Reed, & Takeda, 1997; Reynolds & Redmer, 1995). Normal placentation and placental development are critical for a successful pregnancy and mediate important steps necessary for fetal development (Arroyo et al., 2008). Although apoptosis is a component of normal development and differentiation in most tissues, increased apoptosis is a common feature of the trophoblast cells in the IUGR placenta at term (Arroyo et al., 2008). Abnormal regulation of apoptosis in different tissues has been implicated in the onset and progression of a broad range of diseases (Hetts, 1998).

Heat shock proteins (HSP) are a family of proteins that respond to environmental and physiological stress (Parsell & Lindquist, 1993; Parsell, Taulien, & Lindquist, 1993). We know heat shock protein 27 (HSP27) is involved in the inhibition of apoptosis in several cells (Aiko et al., 2002; Gorman, Szegezdi, Quigney, & Samali, 2005; Paul et al., 2002; Whitlock, Lindsey, Agarwal, Crosson, & Ma, 2005). HSP27 functions in vitro and in vivo (Bryantsev, Chechenova, & Shelden, 2007). It acts as a chaperone and increases cell survival by affecting both upstream signalling and downstream effector events in the apoptotic pathway (Tezel & Wax, 2000). Studies show HSP27 inhibits Caspase 3 and 9, which are associated with apoptosis (Garrido et al., 1999; Pandey et al., 2000). Understanding phosphorylation of HSP27 is important in determining its mechanism of

action because phosphorylation regulates HSP27's functions (Dai et al., 2008; W. Huang et al., 2007). Elevated HSP27 has been documented in several human diseases including cancer, where inhibition of apoptosis is vital for cancer proliferation (Tezel & Wax, 2000). In the placenta, HSP27 is localized in trophoblast cells (Shin et al., 2011). During rat gestation HSP27 is induced in late gestation and labor suggesting a role for this molecule during pregnancy. In human IUGR, Wataba et al. demonstrated an increase in HSP27 protein expression in the cytotrophoblast and syncytiotrophoblast layer of the placenta at term. This suggests that these cells respond locally to certain stress (Wataba et al., 2004). An increase in p-HSP27 was also observed in placentas from preeclamptic placentas (Geisler et al., 2004; Webster, Pitzer, Roberts, Brockman, & Myatt, 2007), The role of p-HSP27 during human IUGR at term and the expression of this protein throughout gestation in the hyperthermia induced IUGR sheep model is still unknown.

The objective of this study was four-fold: 1) to determine levels of placental p-HSP27 during human IUGR, 2) to determine placental apoptosis during early gestation IUGR, 3) to determine gestational age related changes in the phosphorylation of HSP27 in the placenta of normal ovine pregnancy, and 4) to assess the effect of a pathologic pregnancy condition such as IUGR on these proteins. We hypothesize that IUGR placentas, compared to control placentas, will show increased apoptosis early in gestation and a decreased phosphorylation of HSP27 at mid-gestation and term when apoptosis is maximal.

Materials and Methods

Human Placental Tissues

Frozen human term placental samples for control and IUGR were purchased from the Research Centre for Women's and Infants' Health BioBank, Ontario, Canada.

Animal Care and Tissue Preparation

The Brigham Young University Animal Care and Use Committee approved this study. For these experiments we utilized a hyperthermia (HT)-induced ovine model of IUGR that has multiple features similar to the IUGR disease process seen in humans (Bell, McBride, Slepetis, Early, & Currie, 1989; Bell, Wilkening, & Meschia, 1987; Galan et al., 2005; Teng, Tjoa, Fennessey, Wilkening, & Battaglia, 2002). 28 sheep were studied; 14 sheep were placed in HT conditions beginning at 40 days of gestation (dGA; term 147 dGA) to induce IUGR and 14 sheep were placed in thermoneutral conditions as controls. Animal distribution is as follows: 4 animals per HT and control group representing early gestation (55 dGA), 5 animals per HT and control group representing mid-gestation (95 dGA), and 5 animals per HT and control group representing near-term (130 dGA). At necropsy (55, 95 or 130 dGA), the fetal and placental weights were recorded. Placental tissues were frozen in liquid nitrogen for protein analysis. Whole placentomes were sectioned, fixed in 4% paraformaldehyde, and sent for paraffin embedding for histochemical studies.

Terminal Deoxynucleotidyl Transferase Mediated dUTP Nick End Labeling (TUNEL)

TUNEL was performed on paraffin-embedded whole placentome sections. The TUNEL protocol was followed as previously shown in our lab (Arroyo et al., 2010b). In summary, slides were dewaxed and the equilibration buffer added directly to the tissue slide for 10 s. This was followed by incubation with the TdT enzyme for 1 hr at 37°C. Following the enzyme treatment, the anti-digoxigenin conjugate was incubated on the slide for 30 min. 4', 6-diamidino-2-phenylindole, dihydrochloride (DAPI) was used for nuclear staining in our slides. Slides were viewed using fluorescein excitation and emission filters. For apoptotic cells, the percent apoptosis was calculated in the

placentomes as the number of TUNEL positive cells divided by the total number of cells in 20 to 30 fields.

DNA Fragmentation Analysis

The DNA degradation protocol was followed as suggested by the manufacturer (R&D Systems). In summary, 0.1 g of grounded frozen cotyledon tissue was re-suspended in 200 μL of Sample Buffer for each sample. 20 μL of 10X Tissue Buffer was added and samples were incubated at 50° C for 12-18 hr. 100 μL of Lysis Solution was added to 100 μL of the tissue suspension and samples were mixed. Next, 700 μL of Extraction Solution and 400 μL of Extraction Buffer were added to the samples. Samples were mixed on a vortex and centrifuged at 12,000 x g for 5 min. The upper (aqueous) layer was transferred to a new microcentrifuge tube. Sodium Acetate was added to the aqueous DNA samples at 0.1 volume of the aqueous layer. To the total volume in the microcentrifuge tube, an equal volume of 2-propanol was added and mixed. Samples were centrifuged at 12,000 x g for 10 min. and the supernatants were removed and discarded without disturbing the DNA pellet. Pellets were washed with 1 mL of 70% ethanol and centrifuged at 12,000 x g for 5 min. once more. Supernatants were removed and DNA pellets were dried by inverting the tube on a laboratory tissue. DNA pellets were re-suspended in 100 μL of DNase-free Water and quantified in a spectrophotometer. To 0.1 μg/μL of DNA, 2 μL of Gel Loading Buffer was added and samples were loaded onto a 1.5% TreviGel 500 gel. The gel was stained for 15 min. in 0.5 μg/mL ethidium bromide and DNA was visualized using an UV transilluminator.

Western Blot Analysis

Human placental control and IUGR samples were purchased from the Research Centre for Women's and Infants' Health BioBank (N= 10 each). Western blotting was

performed as previously shown by Arroyo et al (Arroyo, Brown, & Galan, 2009). In summary, human placenta and cotyledon tissues were homogenized in lysis buffer. Protein tissue lysates (50μg) were separated on 4-12% Bis-Tris gel SDS-PAGE and transferred to a nitrocellulose membrane. Membranes were incubated in 5% BSA with an antibody against p-HSP27 (rabbit, Cell Signaling, Danvers, MA), or HSP27 (rabbit, Abcam, Cambridge, MA). Membranes were incubated with a secondary horseradish peroxidase (HRP) conjugated antibody in 5% milk for 1 hr at room temperature. An ECL substrate was added to the membranes and the emission of light was detected using x-ray film. To determine loading consistencies, each membrane was stripped of antibodies and re-probed with a primary antibody against beta-actin (mouse, Abcam, Cambridge, MA), and a secondary HRP conjugated antibody in 5% milk for 30 min at room temperature. Presence of these proteins was confirmed and quantified.

Immunohistochemistry

Immunohistochemistry (IHC) was performed on paraffin-embedded whole placentome sections. Protocol was followed as previously done in our laboratory (Arroyo et al., 2010b). In summary, slides were de-waxed followed with antigen retrieval. Slides were blocked with Sniper and incubated for one hour with a rabbit polyclonal primary antibody against Pan-Cytokeratin (Sigma, Saint Louis, MO) for trophoblast localization, anti-p-HSP27 (rabbit, Cell Signaling, Danvers, MA) antibody, or with a universal IgG negative control (Biocare Medical; Concord, CA). Slides were incubated with Mach 2 universal stain polymer followed by color development with diaminobenzidine (DAB; brown color). Hematoxylin was used for nuclear counterstaining. Slides were mounted using Permount mounting media.

Statistical Analysis

Comparisons of the following end-points were made between control and IUGR pregnancies: TUNEL positive cells, p-HSP27. All data was analyzed for normality and treatment effects were determined using the Mann-Whitney test with $p<0.05$ considered significant. A Kruskal-Wallis test was used to compare differences within groups using Dunns post hoc test with $p<0.05$ considered significant.

Results

Phospho-HSP27 (p-HSP27) in Human IUGR Placenta

Previous published results showed increased HSP27 in the human IUGR placenta at term (Wataba et al., 2004). We wanted to investigate phosphorylated HSP27 (p-HSP27) in the human placenta during IUGR. A characteristic western blot image for HSP27 and p-HSP27 is depicted in Figure 2.1A and B. Placental p-HSP27 is significantly decreased (1.7-fold, $p<0.007$) in the IUGR placenta at term (Figure 2.1B).

Fetal and Placental Weights

We utilized an IUGR model in sheep to study the expression of HSP27 during the development of IUGR. Table 2.1 shows the fetal and placental weights for control and IUGR groups at each gestational age in the study. As previously shown in our laboratory, we found a 1.8-fold reduction in fetal weight at 130 dGA ($p<0.008$) with no fetal weight differences at 55 and 95 dGA (Arroyo, Teng, Battaglia, & Galan, 2009). Placental weight was also reduced at 95 dGA ($p<0.003$) and 130 dGA ($p<0.004$) in the IUGR placenta, while showing a trend for decreased placental weight at 55 dGA ($p<0.053$) (Arroyo, Teng, et al., 2009).

TUNEL and DNA Degradation

To study development of HSP27 throughout gestation, we first investigated trophoblast apoptosis early in gestation with the IUGR sheep model. The TUNEL assay showed a significant increase in apoptosis (2-fold) of the villi in the hyperthermia, early gestation sheep (Figure 2.2). A representative picture for TUNEL positive early gestation apoptotic cells is shown in Figure 2.2 A-C. To confirm our TUNEL results we performed DNA degradation assays. A representative picture of our DNA degradation assay is shown in Figure 2.3. We observed increased DNA degradation in the IUGR placenta compared to controls.

Placental HSP27 Protein During IUGR Development

Next we investigated the HSP27 protein changes with the development of IUGR. Placental HSP27 was significantly increased (1.5-fold; $p<0.02$) early in gestation during the development of IUGR (Figure 2.5A). This increase was higher by mid-gestation (2.0-fold; $p<0.02$), and showed a 1.4-fold increase ($p<0.057$) in HSP27 near-term. When looking at the ontogeny across gestation, there was not a significant difference in HSP27 from early to mid-gestation in the control placenta (Figure 2.5B). In contrast HSP27 protein expression was significantly increased by near-term as compared to controls (Figure 2.5B). Ontogeny during IUGR showed only a significant increase (1.6-fold; $p<0.05$) from early to near term for the placental HSP27 (Figure 2.5C).

Placental p-HSP27 Protein During IUGR Development

Because activity of HSP27 is associated with its level of phosphorylation, we investigated phosphorylated HSP27 across gestation during the development of IUGR. Phosphorylated HSP27 (p-HSP27) was significantly increased (1.2-fold; $p<0.008$) at early gestation during the development of IUGR (Figure 2.5D). There was no

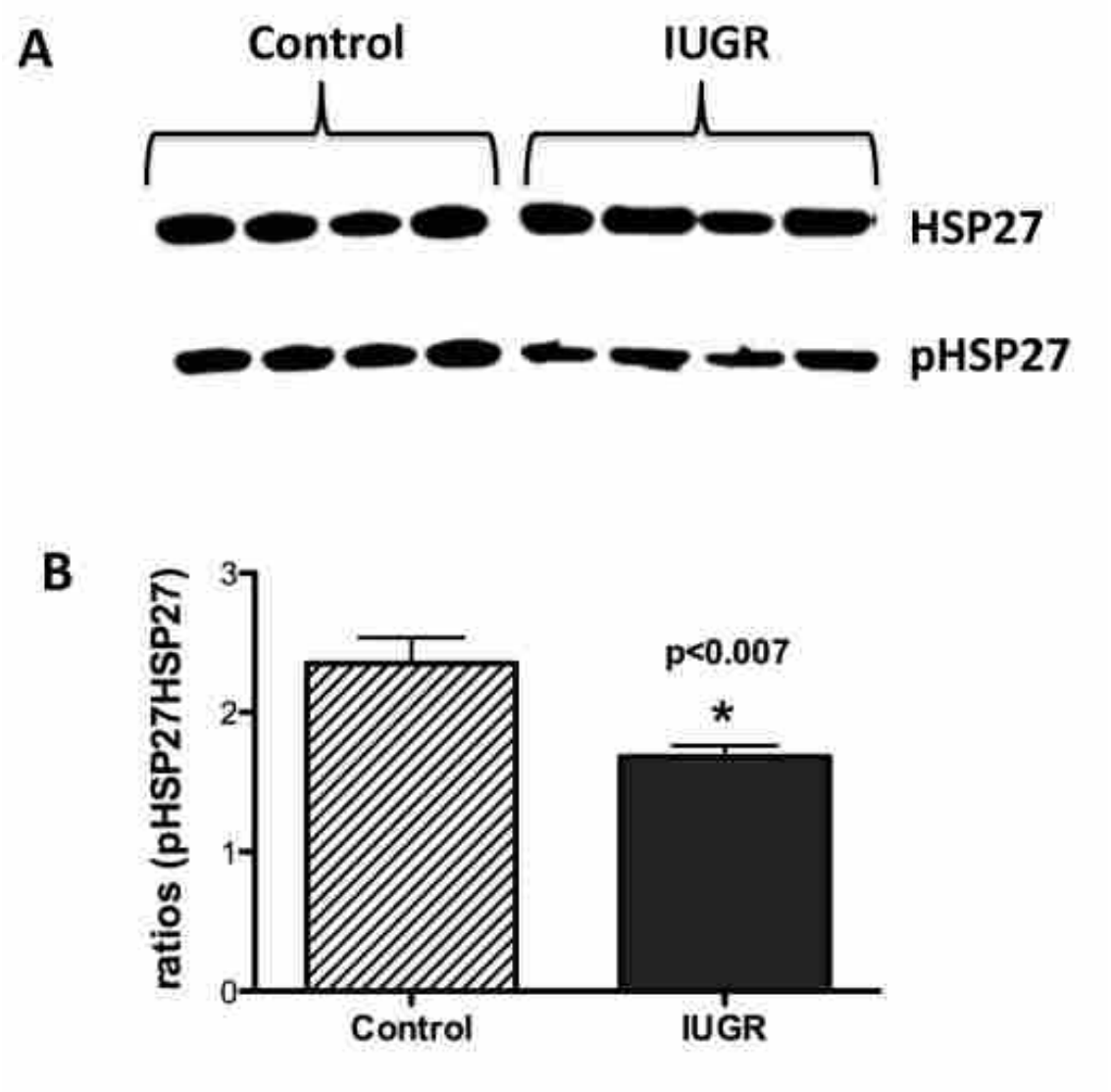

Figure 2.1: Phosphorylated HSP27 During Human IUGR. A characteristic western for phosphorylated HSP27 is shown in A. Placenta p-HSP27 protein is decreased in the human IUGR placenta as compared to controls tissues (B).

Table 2.1: Decreased Fetal and Placental Weights for Ovine Animal Models. Fetal and placental weights of control and IUGR animals demonstrate that IUGR animals have lower fetal and placental weights compared to control animals. IUGR fetal weights are statistically lower only at near-term. IUGR animal placentas are lower in weight at early, mid, and near-term points compared to control animal placentas.

Table 1. Fetal and Placental weights in control and IUGR animals.

Gestational age	Control	IUGR	p-Value
Fetal			
Early	32 ± 2.6g	29 ± 2.3g	0.22
Mid-gestation	715 ± 11g	682 ± 205g	0.79
Near-term	2914 ± 201g	1718 ± 433g	<0.008
Placental			
Early	181 ± 20g	135 ± 3g	≤ 0.053
Mid-gestation	440 ± 50g	186 ± 18g	<0.003
Near-term	349 ± 21g	169 ± 43g	≤ 0.004

Table adapted from "Placental TonEBP/NFAT5 Osmolyte Regulation in an Ovine Model of Intrauterine Growth Restriction (IUGR) " by Arroyo et al.

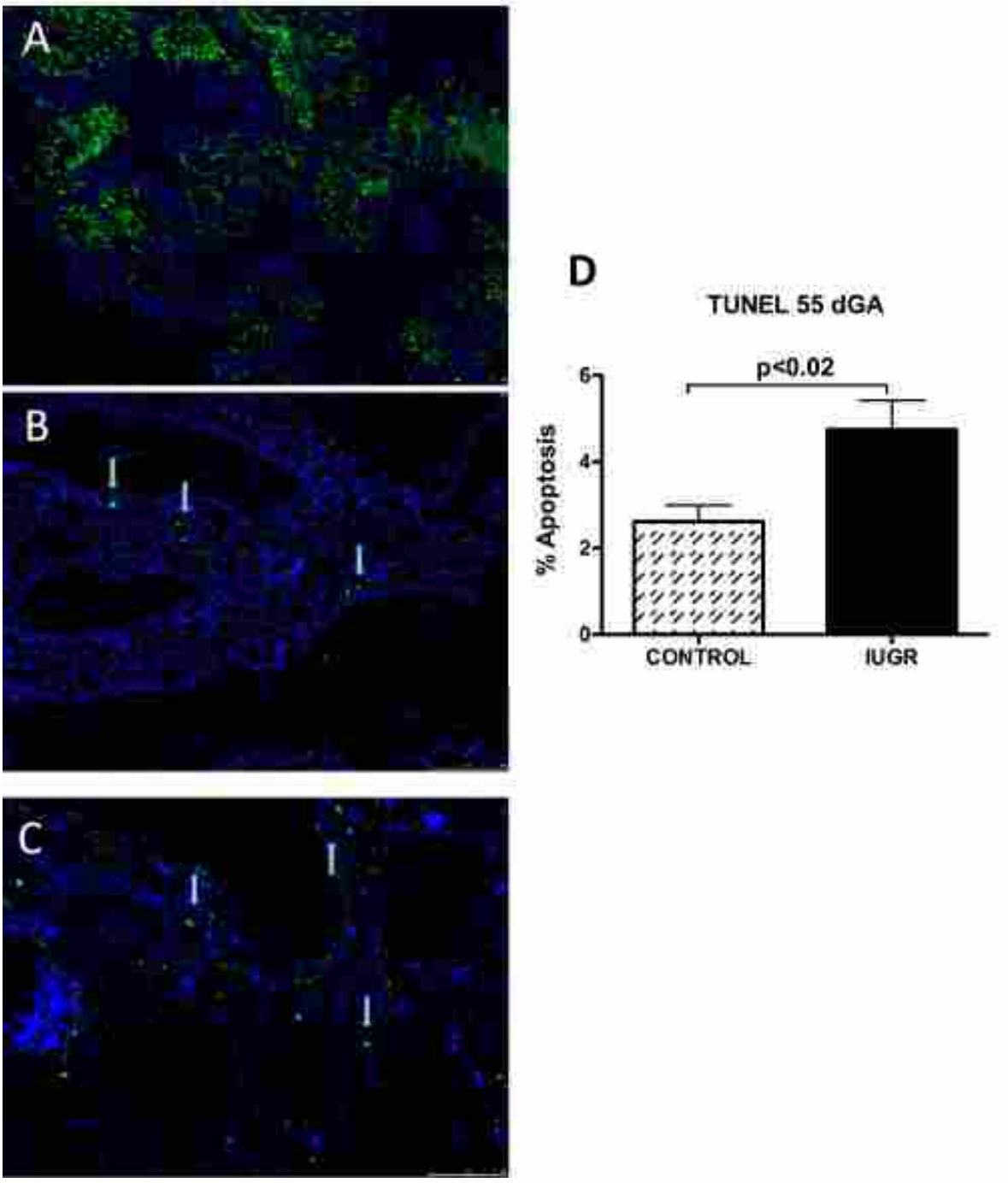

Figure 2.2: TUNEL Assays in Early Placentas During IUGR. A) TUNEL positive control samples. B) Early placenta TUNEL assay in control animals. C) Early placenta TUNEL assay in the IUGR placenta. D) Quantification of apoptotic cells showing increased apoptosis in the early placenta during IUGR as compared to controls.

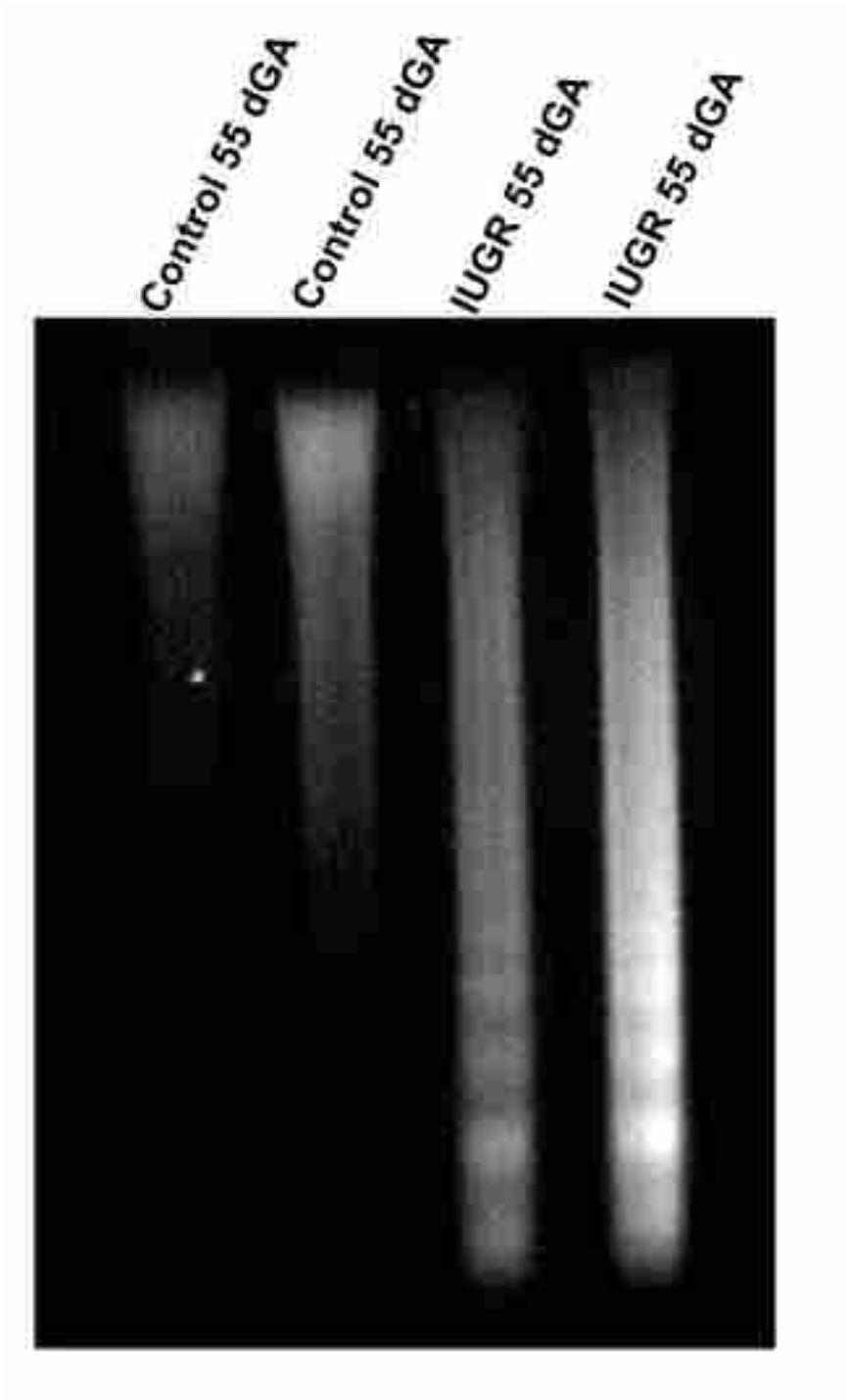

Figure 2.3: DNA Degradation in the Early Placenta During IUGR. DNA degradation analysis showed increased apoptosis in the IUGR placenta as compared to controls.

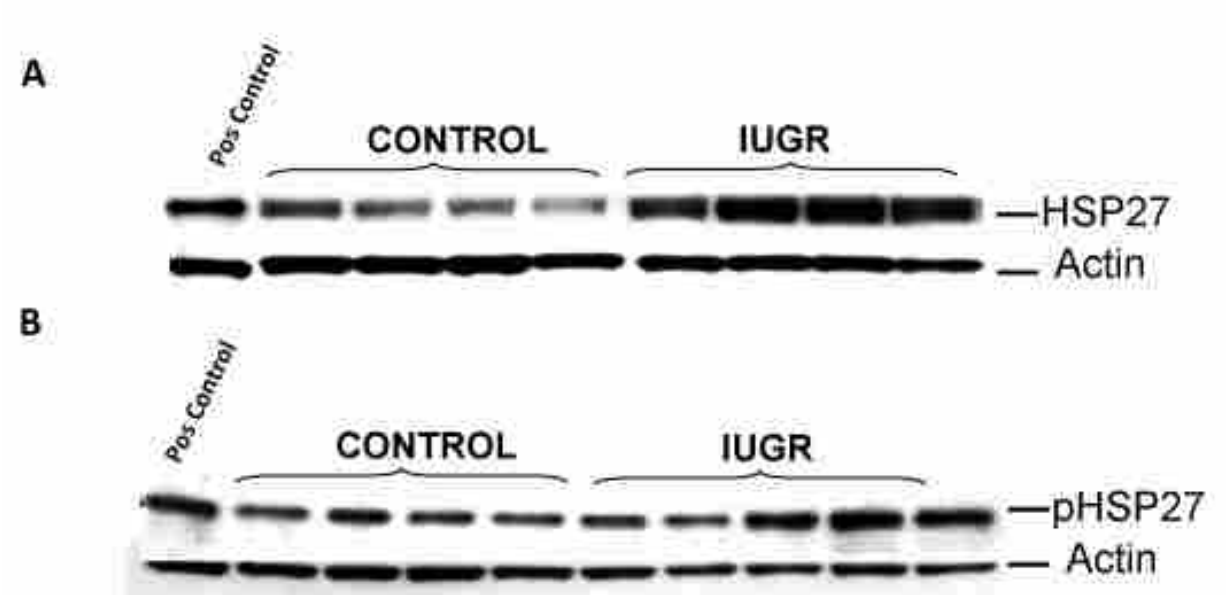

Figure 2.4: Representative Western Blot for HSP27. A) Representative western blot for HSP27 protein in the early placenta from control and IUGR animals. B) Representative western blot for phosphorylated HSP27 protein in the early placenta in control and IUGR samples.

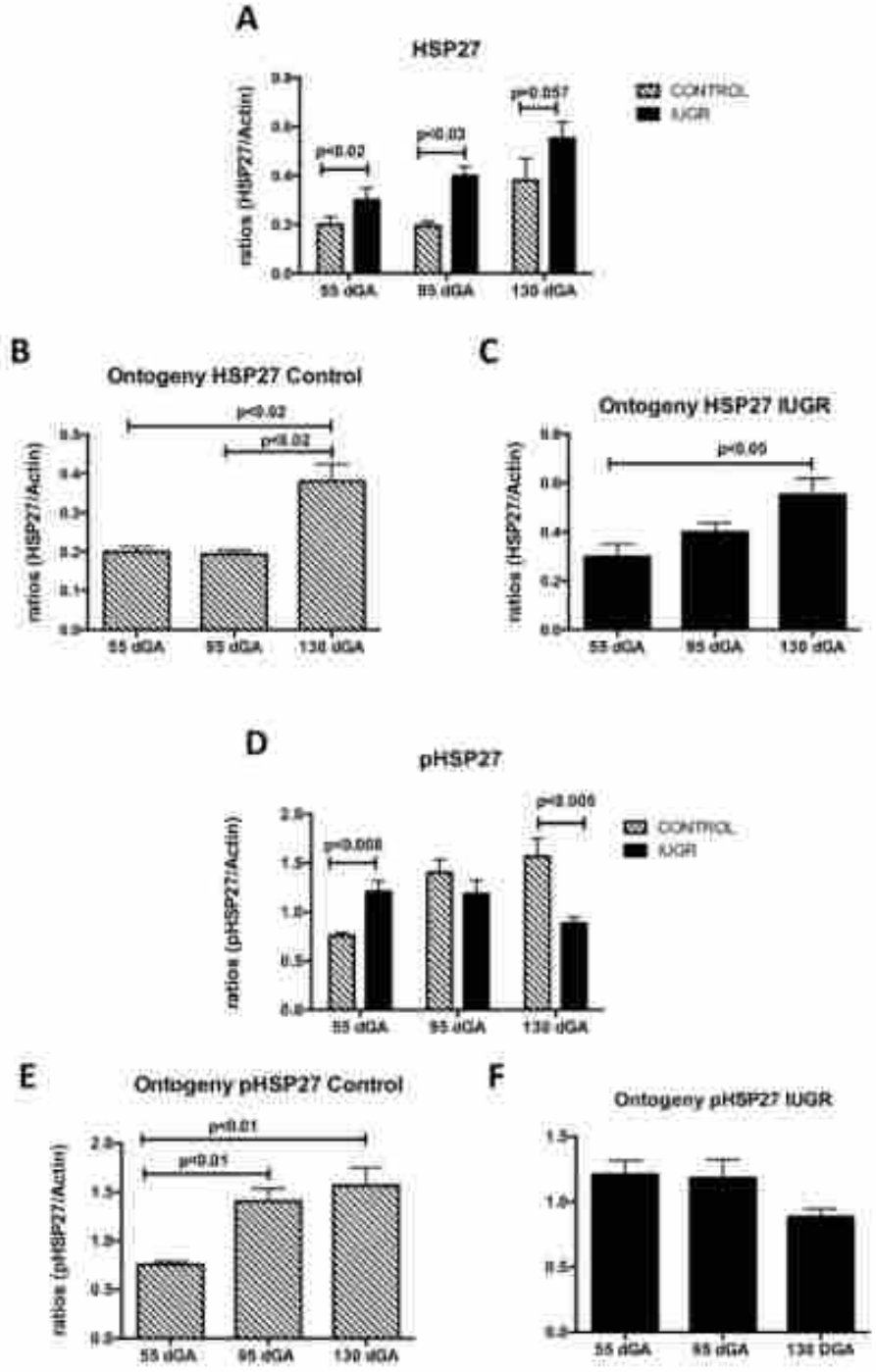

Figure 2.5: HSP27 and p-HSP27 Proteins Across Gestation During IUGR. HSP27 is increased across gestation in the placenta of IUGR animals as compared to controls (A). Ontogeny of HSP27 in the control animals showed this protein to be increased in the placenta at term as compared to early and mid-gestation placenta (B). Similarly HSP27 protein is increased also at term during IUGR (C).
p-HSP27 protein is increased early in gestation and decreased near-term during IUGR in sheep (D). Ontogeny for p-HSP27 showed an increase in this protein at mid and near-term gestation in the placentas of the control animals (E). During IUGR ontogeny studies did not show any differences for placenta p-HSP27 across gestation (F).

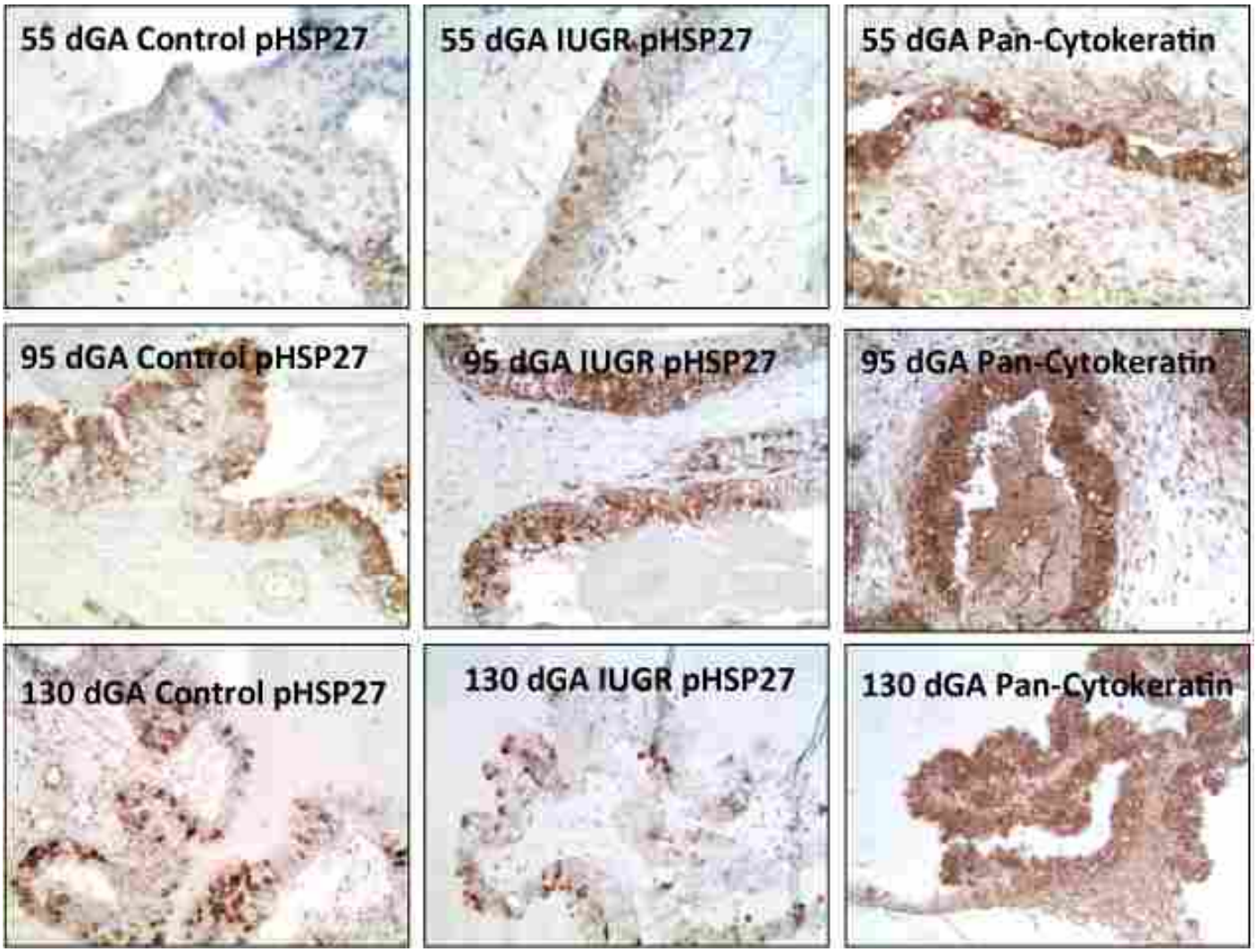

Figure 2.6: Immunohistochemistry for Placental p-HSP27 in Control and IUGR Animals Across Gestation. Left panels show p-HSP27 protein throughout gestation in the placenta of control animals. Center panels show p-HSP27 across gestation in the IUGR placenta. Right panels show trophoblast localization within the sheep placenta.

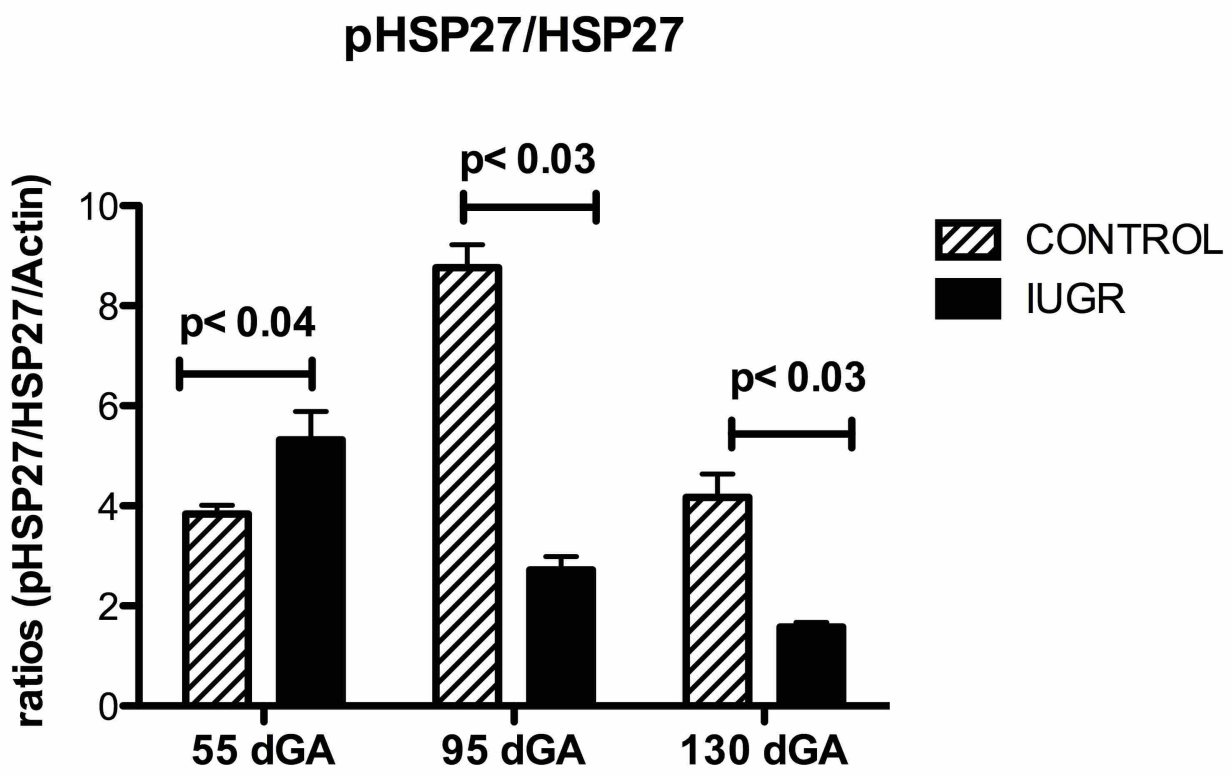

Figure 2.7: Ratio of p-HSP27/HSP27 Across Gestation During IUGR. Phosphorylation of HSP27 is increased early in gestation during IUGR. In contrast, there was a significant decrease in placental HSP27 phosphorylation at mid and near–term gestation during IUGR in the sheep.

difference in phosphorylation between control and IUGR placentas at mid-gestation (Figure 2.5D). In contrast, p-HSP27 was significantly decreased (1.8-fold; $p<0.005$) near-term in the IUGR placenta (Figure 2.5D). The ontogeny expression of pHSP27 showed a significant increase in activation (1.8-fold; $p<0.01$) for this protein from early to mid-gestation in the control placental samples (Figure 2.5E). This increase was maintained (2.0-fold; $p<0.01$) when comparing the early and near-term control samples (Figure 2.5E). In contrast, there were no significant differences in p-HSP27 across gestation in the IUGR placental samples (Figure 2.5F).

Phospho-HSP27 Localization

Immunohistochemistry (IHC) was performed to determine pHSP27 localization to the trophoblasts and that the changes observed were occurring in these cells. An antibody to pan-cytokeratin was used to identify the trophoblast cells within the cotyledon of the placentome (Figure 2.6). Using sections from placentome tissue, IHC showed that pHSP27 protein is localized to the trophoblast cells of the placenta throughout gestation in the cotyledon of the sheep (Figure 2.6). IHC showed an increase in pHSP27 at 55 dGA (early gestation) and a decrease in pHSP27 at 130 (near-term) in the IUGR cotyledon (Figure 2.6); similar to the results obtain by western blot.

Discussion

HSP27 is a stress induced chaperone molecule known to regulate cytoskeletal stability, cell motility, protein folding, cellular stress, and prevention of apoptosis in different cells (Bolhuis & Richter-Landsberg, 2010; Dai et al., 2008). Cellular functions of HSP27 are controlled by phosphorylation of HSP27 (Dai et al., 2008). As previously noted, studies show increased HSP27 in IUGR placenta at term (Wataba et al., 2004). When we assessed pHSP27 expression, we found that phosphorylation of this molecule

is decreased in human IUGR placenta. This is important because decreased levels of HSP are correlated with increased placental apoptosis, which is observed during IUGR at term. This suggests that perhaps a decrease in phosphorylation of this molecule could be one of the factors preventing protection of apoptosis in the IUGR placenta. Interestingly, placental IUGR is characterized by increased activation of caspase 3, a molecule whose activation is prevented by HSP27 (Garrido et al., 1999; Pandey et al., 2000). Our HSP27 results suggest that a mechanism for increased placental apoptosis during IUGR could be due to decreased HSP27 phosphorylation, which could lead to increased activation of caspase 3 and trophoblast cell death.

As noted above, we used a hyperthermia-induced IUGR model in sheep to study HSP27 throughout the development of IUGR (Bell et al., 1989; Bell et al., 1987; Galan et al., 1999; Galan et al., 2001). Previously our laboratory showed a significant increase in placental apoptosis and a significant decrease in placental weight at mid (95 dGA) and near-term (130 dGA) gestational ages during hyperthermia induced IUGR in sheep (Arroyo et al., 2008). Before investigating HSP27 expression during the development of IUGR in sheep, we investigated if hyperthermia treatment could cause placental apoptosis during early gestation. TUNEL assay showed a significant increase in apoptosis during development of IUGR in the sheep, at 15 d of hyperthermia treatment. This was confirmed in our DNA degradation studies and suggests that apoptosis is an early event in the sheep hyperthermia model of IUGR and could explain the trend of decreased placental weight observed at early gestational age (Arroyo, Teng, et al., 2009). Increased apoptosis could be the beginning of placental functional changes that fail to meet fetal demands required for normal growth, which have previously described in this model (Anthony, Scheaffer, Wright, & Regnault, 2003).

HSP27 protein was significantly increased in all gestational ages studied during the development of IUGR. This suggests that this could be a compensatory mechanism of the trophoblast cells for increased apoptosis in the placenta during the development of IUGR. Increased HSP27 during gestation in this IUGR model is expected because heat shock proteins respond to stress in order protect cells and tissues against initial insults and avoid apoptosis (Huang, Min, Masters, Mivechi, & Moskophidis, 2007). Intersestingly, our near-term results are similar to those observed in human IUGR placenta at term, suggesting that HSP27 could be a key molecule involved in apoptosis control of the IUGR placenta.

Phospho-HSP27 was increased during early gestation in the IUGR placenta. This is interesting and unexpected because increased apoptosis was observed during early gestation in the development of IUGR. In contrast p-HSP27 was decreased near-term. This in not surprising because placental apoptosis is at a maximum during this gestational period, especially during IUGR. When we investigated the phosphorylation level of HSP27 to the total protein concentration, we observed an increase in HSP27 phosphorylation early in gestation. This suggests that the level of apoptosis perhaps is not sufficient to affect HSP27 phosphorylation during early gestation in the IUGR placenta. In contrast we observed a significant decrease in HSP27 phosphorylation at mid-gestation and near-term placenta during IUGR. These results are consistent with the previous published data of increased placental apoptosis at these gestational ages during IUGR development in the sheep. It is important to mention that the near-term results are similar to results observed during human IUGR, suggesting a role for HSP27 not only during hyperthermia induced IUGR but also during human IUGR.

The present study suggests a role for phosphorylated HSP27 in placental trophoblast apoptosis during IUGR pregnancies. We chose to study p-HSP27 for two reasons. First, because p-HSP27 is involved in the prevention of apoptosis and is expressed in trophoblast cells, and second because IUGR is characterized by an increase in apoptosis of trophoblast cells. In this study we showed that apoptosis in the placenta is increased early in gestation during the development of IUGR. In addition, we showed that placental HSP27 protein expression is increased across gestation in the IUGR sheep model. In contrast we observed a decrease in p-HSP27 at mid-gestation and near-term ovine model of IUGR. Thus we speculate that a possible mechanism for the increased apoptosis observed in the placenta of treated animals is secondary to a decrease in phosphorylation of HSP27 in the placenta of treated animals as compared to controls. While ovine and human placental structure and placentation differ vastly, we observed similar results in HSP27 phosphorylation between the human disease and our ovine model of IUGR. To our knowledge this is the first report to show a decrease in p-HSP27 associated with an increase in placental apoptosis during IUGR in animal or human studies. Further mechanistic studies are needed to determine the role of other apoptosis molecules associated with increased placental apoptosis during IUGR.

REFERENCES

Aiko, K., Tsujisawa, T., Koseki, T., Hashimoto, S., Morimoto, Y., Amagasa, T., & Nishihara, T. (2002). Involvement of cytochrome c and caspases in apoptotic cell death of human submandibular gland ductal cells induced by concanamycin A. *Cell Signal., 14*(8), 717-722.

Alexander, J. M., & Wilson, K. L. (2013). Hypertensive emergencies of pregnancy. *Obstet Gynecol Clin North Am, 40*(1), 89-101. doi: 10.1016/j.ogc.2012.11.008

Allaire, A. D., Ballenger, K. A., Wells, S. R., McMahon, M. J., & Lessey, B. A. (2000). Placental apoptosis in preeclampsia. *Obstet.Gynecol., 96*(2), 271-276.

Anthony, R. V., Scheaffer, A. N., Wright, C. D., & Regnault, T. R. (2003). Ruminant models of prenatal growth restriction. *Reprod Suppl, 61*, 183-194.

Arroyo, J. A., Anthony, R. V., & Galan, H. L. (2008). Decreased placental X-linked inhibitor of apoptosis protein in an ovine model of intrauterine growth restriction. *Am.J.Obstet.Gynecol., 199*(1), 80-88.

Arroyo, J. A., Brown, L. D., & Galan, H. L. (2009). Placental mammalian target of rapamycin and related signaling pathways in an ovine model of intrauterine growth restriction. *Am J Obstet Gynecol, 201*(6), 616 e611-617. doi: S0002-9378(09)00818-7 [pii] 10.1016/j.ajog.2009.07.031

Arroyo, J. A., Li, C., Schlabritz-Loutsevitch, N., McDonald, T., Nathanielsz, P., & Galan, H. L. (2010a). Increased placental XIAP and caspase 3 is associated with increased placental apoptosis in a baboon model of maternal nutrient reduction. *American Journal of Obstetrics and Gynecology, 203*(4). doi: 10.1016/j.ajog.2010.05.021

Arroyo, J. A., Li, C., Schlabritz-Loutsevitch, N., McDonald, T., Nathanielsz, P., & Galan, H. L. (2010b). Increased placental XIAP and caspase 3 is associated with increased placental apoptosis in a baboon model of maternal nutrient reduction. *Am J Obstet Gynecol, 203*(4), 364 e313-368. doi: 10.1016/j.ajog.2010.05.021

Arroyo, J. A., Teng, C., Battaglia, F. C., & Galan, H. L. (2009). Determination of the NFAT5/TonEBP transcription factor in the human and ovine placenta. *Syst Biol Reprod Med, 55*(4), 164-170. doi: 10.3109/19396360902846401

Barker, D. J. (1993a). Fetal origins of coronary heart disease. *Br.Heart J., 69*(3), 195-196.

Barker, D. J. (1993b). The intrauterine origins of cardiovascular disease. *Acta Paediatr Suppl, 82 Suppl 391*, 93-99.

Barker, D. J. (2004). The developmental origins of adult disease. *J.Am.Coll.Nutr., 23*(6 Suppl), 588S-595S.

Barker, D. J., Gluckman, P. D., Godfrey, K. M., Harding, J. E., Owens, J. A., & Robinson, J. S. (1993). Fetal nutrition and cardiovascular disease in adult life. *Lancet, 341*(8850), 938-941.

Bax, B. E., & Bloxam, D. L. (1997). Energy metabolism and glycolysis in human placental trophoblast cells during differentiation. *Biochim Biophys Acta, 1319*(2-3), 283-292.

Bell, A. W., McBride, B. W., Slepetis, R., Early, R. J., & Currie, W. B. (1989). Chronic heat stress and prenatal development in sheep: I. Conceptus growth and maternal plasma hormones and metabolites. *J.Anim Sci., 67*(12), 3289-3299.

Bell, A. W., Wilkening, R. B., & Meschia, G. (1987). Some aspects of placental function in chronically heat-stressed ewes. *J Dev Physiol, 9*(1), 17-29.

Bolhuis, S., & Richter-Landsberg, C. (2010). Effect of proteasome inhibition by MG-132 on HSP27 oligomerization, phosphorylation, and aggresome formation in the OLN-93 oligodendroglia cell line. *J Neurochem, 114*(4), 960-971. doi: 10.1111/j.1471-4159.2010.06600.x

Brar, H. S., & Rutherford, S. E. (1988). Classification of intrauterine growth retardation. *Semin.Perinatol., 12*(1), 2-10.

Bryantsev, A. L., Chechenova, M. B., & Shelden, E. A. (2007). Recruitment of phosphorylated small heat shock protein Hsp27 to nuclear speckles without stress. *Exp Cell Res, 313*(1), 195-209. doi: 10.1016/j.yexcr.2006.10.004

Cairns, Rob A., Harris, Isaac S., & Mak, Tak W. (2011). Regulation of cancer cell metabolism. *Nature Reviews Cancer, 11*(2), 85-95. doi: 10.1038/nrc2981

Cetin, I., & Antonazzo, P. (2009). The role of the placenta in intrauterine growth restriction (IUGR). *Z Geburtshilfe Neonatol, 213*(3), 84-88. doi: 10.1055/s-0029-1224143

Challis, J. R., Lye, S. J., Gibb, W., Whittle, W., Patel, F., & Alfaidy, N. (2001). Understanding preterm labor. *Ann N Y Acad Sci, 943*, 225-234.

Christofk, H. R., Vander Heiden, M. G., Harris, M. H., Ramanathan, A., Gerszten, R. E., Wei, R., . . . Cantley, L. C. (2008). The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature, 452*(7184), 230-U274. doi: 10.1038/nature06734

Christofk, H. R., Vander Heiden, M. G., Wu, N., Asara, J. M., & Cantley, L. C. (2008). Pyruvate kinase M2 is a phosphotyrosine-binding protein. *Nature, 452*(7184), 181-U127. doi: 10.1038/nature06667

Dai, S., Jia, Y., Wu, S. L., Isenberg, J. S., Ridnour, L. A., Bandle, R. W., . . . Karger, B. L. (2008). Comprehensive characterization of heat shock protein 27 phosphorylation in human endothelial cells stimulated by the microbial dithiole thiolutin. *J Proteome Res, 7*(10), 4384-4395. doi: 10.1021/pr800376w

Delpisheh, A., Brabin, L., Attia, E., & Brabin, B. J. (2008). Pregnancy late in life: a hospital-based study of birth outcomes. *J Womens Health (Larchmt), 17*(6), 965-970. doi: 10.1089/jwh.2008.0514

DiFederico, E., Genbacev, O., & Fisher, S. J. (1999). Preeclampsia is associated with widespread apoptosis of placental cytotrophoblasts within the uterine wall. *Am.J.Pathol., 155*(1), 293-301.

Dombrauckas, J. D., Santarsiero, B. D., & Mesecar, A. D. (2005). Structural basis for tumor pyruvate kinase M2 allosteric regulation and catalysis. *Biochemistry, 44*(27), 9417-9429. doi: 10.1021/bi0474923

Galan, H. L., Anthony, R. V., Rigano, S., Parker, T. A., de Vrijer, B., Ferrazzi, E., . . . Regnault, T. R. (2005). Fetal hypertension and abnormal Doppler velocimetry in an ovine model of intrauterine growth restriction. *Am J Obstet Gynecol, 192*(1), 272-279.

Galan, H. L., Hussey, M. J., Barbera, A., Ferrazzi, E., Chung, M., Hobbins, J. C., & Battaglia, F. C. (1999). Relationship of fetal growth to duration of heat stress in an ovine model of placental insufficiency. *Am J Obstet Gynecol, 180*(5), 1278-1282.

Galan, H. L., Regnault, T. R., Le Cras, T. D., Tyson, R. W., Anthony, R. V., Wilkening, R. B., & Abman, S. H. (2001). Cotyledon and binucleate cell nitric oxide synthase expression in an ovine model of fetal growth restriction. *J Appl Physiol, 90*(6), 2420-2426.

Garrido, C., Bruey, J. M., Fromentin, A., Hammann, A., Arrigo, A. P., & Solary, E. (1999). HSP27 inhibits cytochrome c-dependent activation of procaspase-9. *FASEB J., 13*(14), 2061-2070.

Geisler, J. P., Manahan, K. J., Geisler, H. E., Tammela, J. E., Rose, S. L., Hiett, A. K., . . . Zhou, Z. (2004). Heat shock protein 27 in the placentas of women with and without severe preeclampsia. *Clin.Exp.Obstet.Gynecol., 31*(1), 12-14.

Goldenberg, R. L., & Rouse, D. J. (1998). Prevention of premature birth. *N Engl J Med, 339*(5), 313-320. doi: 10.1056/NEJM199807303390506

Gorman, A. M., Szegezdi, E., Quigney, D. J., & Samali, A. (2005). Hsp27 inhibits 6-hydroxydopamine-induced cytochrome c release and apoptosis in PC12 cells. *Biochem.Biophys.Res.Commun., 327*(3), 801-810.

Gray, P. H., O'Callaghan, M. J., Harvey, J. M., Burke, C. J., & Payton, D. J. (1999). Placental pathology and neurodevelopment of the infant with intrauterine growth restriction. *Dev.Med.Child Neurol., 41*(1), 16-20.

Heiden, M. G. V., Cantley, L. C., & Thompson, C. B. (2009). Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation. *Science, 324*(5930), 1029-1033. doi: 10.1126/science.1160809

Hetts, S. W. (1998). To die or not to die: an overview of apoptosis and its role in disease. *JAMA, 279*(4), 300-307.

Holemans, K., Van, Bree R., Verhaeghe, J., Aerts, L., & Van Assche, F. A. (1993). In vivo glucose utilization by individual tissues in virgin and pregnant offspring of severely diabetic rats. *Diabetes, 42*(4), 530-536.

Huang, L., Min, J. N., Masters, S., Mivechi, N. F., & Moskophidis, D. (2007). Insights into function and regulation of small heat shock protein 25 (HSPB1) in a mouse model with targeted gene disruption. *Genesis, 45*(8), 487-501. doi: 10.1002/dvg.20319

Huang, W., Fileta, J. B., Filippopoulos, T., Ray, A., Dobberfuhl, A., & Grosskreutz, C. L. (2007). Hsp27 phosphorylation in experimental glaucoma. *Invest Ophthalmol Vis Sci, 48*(9), 4129-4135. doi: 10.1167/iovs.06-0606

Hung, T. H., Skepper, J. N., Charnock-Jones, D. S., & Burton, G. J. (2002). Hypoxia-reoxygenation: a potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia. *Circ Res, 90*(12), 1274-1281.

Ishihara, N., Matsuo, H., Murakoshi, H., Laoag-Fernandez, J. B., Samoto, T., & Maruo, T. (2002). Increased apoptosis in the syncytiotrophoblast in human term placentas complicated by either preeclampsia or intrauterine growth retardation. *Am.J.Obstet.Gynecol., 186*(1), 158-166.

Jurica, M. S., Mesecar, A., Heath, P. J., Shi, W. X., Nowak, T., & Stoddard, B. L. (1998). The allosteric regulation of pyruvate kinase by fructose-1,6-bisphosphate. *Structure, 6*(2), 195-210. doi: 10.1016/s0969-2126(98)00021-5

Krebs, C., Macara, L. M., Leiser, R., Bowman, A. W., Greer, I. A., & Kingdom, J. C. (1996). Intrauterine growth restriction with absent end-diastolic flow velocity in the umbilical artery is associated with maldevelopment of the placental terminal villous tree. *Am.J.Obstet.Gynecol., 175*(6), 1534-1542.

Leung, D. N., Smith, S. C., To, K. F., Sahota, D. S., & Baker, P. N. (2001). Increased placental apoptosis in pregnancies complicated by preeclampsia. *American Journal of Obstetrics and Gynecology, 184*(6), 1249-1250. doi: 10.1067/mob.2001.112906

Levy, R., & Nelson, D. M. (2000). To be, or not to be, that is the question. Apoptosis in human trophoblast. *Placenta, 21*(1), 1-13.

Lindheimer, M. D., Taler, S. J., & Cunningham, F. G. (2010). Hypertension in pregnancy. *Journal of the American Society of Hypertension, 4*(2), 68-78. doi: 10.1016/j.jash.2010.03.002

Mayhew, T. M., Ohadike, C., Baker, P. N., Crocker, I. P., Mitchell, C., & Ong, S. S. (2003). Stereological investigation of placental morphology in pregnancies complicated by pre-eclampsia with and without intrauterine growth restriction. *Placenta, 24*(2-3), 219-226.

Mazurek, S. (2011). Pyruvate kinase type M2: A key regulator of the metabolic budget system in tumor cells. *International Journal of Biochemistry & Cell Biology, 43*(7), 969-980. doi: 10.1016/j.biocel.2010.02.005

Mori, A., Trudinger, B., Mori, R., Reed, V., & Takeda, Y. (1997). The fetal aortic pressure pulse waveform in normal and compromised pregnancy. *Br J Obstet Gynaecol, 104*(11), 1255-1261.

Pandey, P., Farber, R., Nakazawa, A., Kumar, S., Bharti, A., Nalin, C., . . . Kharbanda, S. (2000). Hsp27 functions as a negative regulator of cytochrome c-dependent activation of procaspase-3. *Oncogene, 19*(16), 1975-1981.

Parsell, D. A., & Lindquist, S. (1993). The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. *Annu.Rev.Genet., 27*, 437-496.

Parsell, D. A., Taulien, J., & Lindquist, S. (1993). The role of heat-shock proteins in thermotolerance. *Philos.Trans.R.Soc.Lond B Biol.Sci., 339*(1289), 279-285.

Paul, C., Manero, F., Gonin, S., Kretz-Remy, C., Virot, S., & Arrigo, A. P. (2002). Hsp27 as a negative regulator of cytochrome C release. *Mol.Cell Biol., 22*(3), 816-834.

Phipps, K., Barker, D. J., Hales, C. N., Fall, C. H., Osmond, C., & Clark, P. M. (1993). Fetal growth and impaired glucose tolerance in men and women. *Diabetologia, 36*(3), 225-228.

Pilling, E. L., Elder, C. J., & Gibson, A. T. (2008). Growth patterns in the growth-retarded premature infant. *Best Pract Res Clin Endocrinol Metab, 22*(3), 447-462. doi: S1521-690X(08)00038-9 [pii] 10.1016/j.beem.2008.03.002

Pollack, R. N., & Divon, M. Y. (1992). Intrauterine growth retardation: definition, classification, and etiology. *Clin Obstet Gynecol, 35*(1), 99-107.

Reusens-Billen, B., Remacle, C., & Hoet, J. J. (1989). The development of the fetal rat intestine and its reaction to maternal diabetes. II. Effect of mild and severe maternal diabetes. *Diabetes Res.Clin.Pract., 6*(3), 213-219.

Reynolds, L. P., & Redmer, D. A. (1995). Utero-placental vascular development and placental function. *J.Anim Sci., 73*(6), 1839-1851.

Roos, S., Jansson, N., Palmberg, I., Saljo, K., Powell, T. L., & Jansson, T. (2007). Mammalian target of rapamycin in the human placenta regulates leucine transport and is down-regulated in restricted fetal growth. *J.Physiol, 582*(Pt 1), 449-459.

Shin, J. K., Baek, J. C., Kang, M. Y., Park, J. K., Lee, S. A., Lee, J. H., . . . Paik, W. Y. (2011). Proteomic analysis reveals an elevated expression of heat shock protein 27 in preeclamptic placentas. *Gynecol Obstet Invest, 71*(3), 151-157. doi: 10.1159/000315162

Smith, S. C., Baker, P. N., & Symonds, E. M. (1997). Increased placental apoptosis in intrauterine growth restriction. *Am.J.Obstet.Gynecol., 177*(6), 1395-1401.

Tal, R., Shaish, A., Barshack, I., Polak-Charcon, S., Afek, A., Volkov, A., . . . Harats, D. (2010). Effects of hypoxia-inducible factor-1alpha overexpression in pregnant mice: possible implications for preeclampsia and intrauterine growth restriction. *Am J Pathol, 177*(6), 2950-2962. doi: 10.2353/ajpath.2010.090800

Teng, C. C., Tjoa, S., Fennessey, P. V., Wilkening, R. B., & Battaglia, F. C. (2002). Transplacental carbohydrate and sugar alcohol concentrations and their uptakes in ovine pregnancy. *Exp.Biol.Med.(Maywood.), 227*(3), 189-195.

Tezel, G., & Wax, M. B. (2000). The mechanisms of hsp27 antibody-mediated apoptosis in retinal neuronal cells. *J Neurosci, 20*(10), 3552-3562.

Tissot van Patot, M. C., Murray, A. J., Beckey, V., Cindrova-Davies, T., Johns, J., Zwerdlinger, L., . . . Serkova, N. J. (2010). Human placental metabolic adaptation to chronic hypoxia, high altitude: hypoxic preconditioning. *Am J Physiol Regul Integr Comp Physiol, 298*(1), R166-172. doi: 10.1152/ajpregu.00383.2009

Warburg, O. (1956). ORIGIN OF CANCER CELLS. *Science, 123*(3191), 309-314. doi: 10.1126/science.123.3191.309

Wataba, K., Saito, T., Takeuchi, M., Nakayama, M., Suehara, N., & Kudo, R. (2004). Changed expression of heat shock proteins in various pathological findings in placentas with intrauterine fetal growth restriction. *Med Electron Microsc, 37*(3), 170-176. doi: 10.1007/s00795-003-0244-x

Webster, R. P., Pitzer, B. A., Roberts, V. H., Brockman, D., & Myatt, L. (2007). Differences in the proteome profile in placenta from normal term and preeclamptic preterm pregnancies. *Proteomics Clin Appl, 1*(5), 446-456. doi: 10.1002/prca.200600745

Whitlock, N. A., Lindsey, K., Agarwal, N., Crosson, C. E., & Ma, J. X. (2005). Heat shock protein 27 delays Ca2+-induced cell death in a caspase-dependent and -independent manner in rat retinal ganglion cells. *Invest Ophthalmol.Vis.Sci., 46*(3), 1085-1091.

Xiong, X., Mayes, D., Demianczuk, N., Olson, D. M., Davidge, S. T., Newburn-Cook, C., & Saunders, L. D. (1999). Impact of pregnancy-induced hypertension on fetal growth. *American Journal of Obstetrics and Gynecology, 180*(1), 207-213. doi: 10.1016/s0002-9378(99)70176-6

Xiong, Y., Lei, Q. Y., Zhao, S., & Guan, K. L. (2011). Regulation of glycolysis and gluconeogenesis by acetylation of PKM and PEPCK. *Cold Spring Harbor symposia on quantitative biology, 76*, 285-289. doi: 10.1101/sqb.2011.76.010942

Yang, W. W., Xia, Y., Hawke, D., Li, X. J., Liang, J., Xing, D. M., . . . Lu, Z. M. (2012). PKM2 Phosphorylates Histone H3 and Promotes Gene Transcription and Tumorigenesis. *Cell, 150*(4), 685-696. doi: 10.1016/j.cell.2012.07.018

Yang, W. W., Xia, Y., Ji, H. T., Zheng, Y. H., Liang, J., Huang, W. H., . . . Lu, Z. M. (2011). Nuclear PKM2 regulates beta-catenin transactivation upon EGFR activation. *Nature, 480*(7375), 118-U289. doi: 10.1038/nature10598

Yang, W. W., Zheng, Y. H., Xia, Y., Ji, H. T., Chen, X. M., Guo, F., . . . Liu, Z. M. (2012). ERK1/2-dependent phosphorylation and nuclear translocation of PKM2 promotes the Warburg effect. *Nature Cell Biology, 14*(12), 1295-+. doi: 10.1038/ncb2629

CURRICULUM VITAE

Brigham L. Bahr

801-885-7281
brigham.bahr@gmail.com

EDUCATION

2014 - Master of Science in Physiology and Developmental Biology, Brigham Young University, Provo, UT, current GPA 3.69, Expected graduation April 2014
2007 - Bachelor of Science in Exercise Science, Brigham Young University, Provo, UT GPA 3.21, Graduated April 2007

TEACHING EXPERIENCE

Teaching Assistant, Brigham Young University 2012-2013.
Courses: Human Physiology Lab (PDBIO 305)

RESEARCH EXPERIENCE

Masters Research Assistant, Department of Physiology and Developmental Biology, Brigham Young University, Provo, UT, 2013 (research advisor David J. Bearss). Investigating metabolic activators of PKM2 and how they alter oncogene-mediated changes in tumor cell metabolism.
Masters Research Assistant, Department of Physiology and Developmental Biology, Brigham Young University, Provo, UT, 2014 (research advisor Juan A. Arroyo). Researching metabolism and stress related proteins in normal, preeclamptic, and intrauterine growth restriction human placentas.

PUBLICATIONS

Bahr BL, Galan HA, Arroyo JA. Decreased Expression of Phosphorylated Placental Heat Shock Protein 27 in Human and Ovine Intrauterine Growth Restriction (IUGR). Placenta, 2014 March

WORKS IN PROGRESS

Bahr BL, Price MD, Merrill D, Mejia C, Call L, Bearss D, Arroyo JA. Different Expression of Pyruvate Kinase M2 in Normal, Preeclamptic, and Intrauterine Growth Restriction Pregnancies. Brigham L. Bahr, Matthew D. Price, Daniel Merrill, Camilo Mejia, Lee Call, David Bearss, Juan A. Arroyo

POSTER PRESENTATIONS

A novel series of metabolic activators of PKM2 alter oncogene-mediated changes in tumor cell metabolism, Brigham L. Bahr, Jenny Stevens, Spenser Squire, Christopher Moreno, Lee T. Call, Bret J. Stephens, Alexis Mollard, Steven J.

Warner, David J. Bearss, American Association of Cancer Research Annual Meeting, 2013

WORK EXPERIENCE

Anesthesia Technician, Utah Valley Regional Medical Center, Provo, UT, 2010-2012
Operating Room Orderly, Utah Valley Regional Medical Center, Provo, UT, 2008-2012

PROFESSIONAL MEMBERSHIPS

American Association of Cancer Research, Associate Member, 2012-2013

AWARDS

Research Assistantship 2014, Advisor Juan A. Arroyo, Physiology and Developmental Biology, Brigham Young University, Provo, UT
Graduate Student Funding Scholarship, Fall 2012, Winter, Spring, Summer, Fall 2013, Winter 2014, Brigham Young University.
Research Assistantship 2013, Advisor David J. Bearss, Physiology and Developmental Biology, Brigham Young University, Provo, UT
Teaching Assistantship 2012-2013, Human Physiology Lab, Physiology and Developmental Biology, PDBIO 305
Orderly of the Year, Operating Room, Utah Valley Regional Medical Center, 2009

GRANTS

Research was supported in part by NIH grant 7R00HD055054-06

LANGUAGES

Proficient in Russian

VOLUNTEER EXPERIENCE

Boy Scouts of America, Webelos Den Leader, Provo Peak District, Provo, UT, Pack 749, August 2013-current
Give Kids A Smile, Patient Chaperone, Orem, UT, February 2013
Emergency Room Trauma Volunteer, Utah Valley Regional Medical Center, Provo, UT, Feb-Nov 2008