EFFECTS OF IGFS ON BETA-CELL PROLIFERATION IN OVINE MODELS EXPOSED TO INTRAUTERINE GROWTH RESTRICTION

By

ELIZABETH TITLAYO OGUNBUNMI

_____

A Thesis Submitted to The Honors College

In Partial Fulfillment of the Bachelors degree With Honors in

Physiology

THE UNIVERSITY OF ARIZONA

MAY 2019

Approved by:

_____

Dr. Sean Limesand

Department of Animal and Comparative Biomedical Sciences

Elizabeth Ogunbunmi
May 1, 2019
Honors Thesis

Effects of IGFs on Ovine Models Exposed to Intrauterine Growth Restriction

Abstract

An intrauterine growth restricted fetus refers to a fetus that has failed to reach its genetic growth potential due to maternal or placental limitations that lower the supply of oxygen and nutrients (Barry & Anthony, 2008). The fetus is sensitive to nutrient deficiencies and responds by altering organ growth and development, specifically nutrient sensing tissues like insulin producing, pancreatic beta cells. Insulin-like growth factor-1 (IGF-1) modulates beta-cell proliferation, and insulin-like growth factor binding proteins (IGFBPs) regulate IGFs via transport and distribution (Duan & Xu, 2005). Previous reports have shown that IGF-1 is lower and IGFBP-2 is higher in islets from IUGR fetal sheep (Chen, Rozance, Hay Jr., & Limesand, 2012). We hypothesize that decreases in IGF-1 signaling lower beta-cell proliferation rates. We propose two possible mechanisms: IGFBP2 is up-regulated to activate IGF-1 or IGFBP2 to prevents IGF-1 activity.

In the present study, we investigated the interaction of IGF-1 and IGFBP-2 on the regulation of beta cell proliferation in pancreatic islets of IUGR and control fetuses. Isolated islets were cultured in media containing IGF-1, IGFBP-2, IGF-1 + IGFBP-2, and T_3 and compared to media without supplemented hormones. Proliferation was measured by incubating the islets with 5-Ethynyl-2'-deoxyuridine (EdU). After 48 h in culture the islets were prepared for histology. Beta cells were immunostained with guinea pig anti-porcine insulin polyclonal antibody, Edu with the Click-It assay to measure proliferation, and nuclei with DAPI.

Fluorescent images were taken with a digital camera. A thorough understanding of beta-cell physiology prenatally can possibly predict the onset of diabetes in adult patients.

Introduction

Diabetes mellitus (DM) is a disorder of impaired glucose homeostasis associated with metabolic changes and complications in remote organs. The worldwide prevalence of DM continues to rise in recent years. It was estimated that in 2016 one in every 11 adults will suffer from diabetes, with over 500 million diabetic patients worldwide (Liu, Tan, & Liu, 2017). There have been implications of prenatal origins in several adult diseases. Thus, there is a growing need to better understand islet physiology in a compromised intrauterine environment and its effect on the development of disease in adult life.

One possible risk factor for the development of DM is intrauterine growth restriction (IUGR). Intrauterine growth restriction (IUGR) refers to infants that failed to reach their genetic growth potential due to restrictive factors *in utero* (Barry & Anthony, 2008). IUGR can be induced by maternal: obesity, diabetes, hypertension or insufficient nutrients. Godfrey and Barker (2000), focused on maternal, placental and fetal communication, providing insight into how environmental effects have been shown to affect growth rates, trajectory and cell allocation in preimplantation embryos . Failure of the maternoplacental capacity to meet nutrition requirements of the fetus, results in a range of fetal adaptations and developmental changes (Godfrey & Barker, 2000). These changes can lead to permanent physiological alterations in the fetus to extend survival, possibly causing health issues long term.

One organ that can be affected by PI and IUGR is the pancreas, leading to decreased beta-cell proliferation, and beta-cell mass. Beta-cells regulate glucose homeostasis by secreting insulin, a hormone that mediates glucose uptake. Decreased beta-cell mass indicates less insulin-

secretion capabilities; thus, the total amount of insulin released depends on the absolute number of beta-cells that are functioning (Chen, Cohrs, Stertmann, Bozsak, & Spier, 2017). Obesity and pregnancy can lead to failure to adapt to physiological changes, and the subsequent development of hyperglycemia or diabetes (Bouwens & Rooman, 2005). The role of IGF-1 signaling in beta-cell mitosis, the effect of IUGR on beta-cell proliferation, and the relationship between IGF-1 and IGFBP-2 in response to IUGR will be reviewed in this study.

Literature Review

IGF-1 is a small peptide protein that circulates via the vascular system mostly protein bound. It activates signaling pathways that regulate cellular growth, and its receptors are ubiquitous in all cell types (Clemmons, 2004). It is important for inducing mitogenic responses and is mediated by actions of growth hormone (GH) when it is synthesized by the liver. IGF-1 that is synthesized in peripheral tissues is regulated by GH and factors made in the respective cell type. The IGF-1 receptor allows IGF-1 to stimulate coordinated and proportional cell growth, and its expression is also regulated by GH. IGF-1 stimulates beta-cell proliferation and neogenesis from ducts and has an antiapoptotic effect (Bouwens and Rooman, 2005).

An important property of IGF-1 is its ability to prevent apoptosis, by inhibiting activation of downstream signaling proteins in the apoptotic pathway. IGF-1 is a potent activator of DNA synthesis, in vitro experiments have shown to enhance DNA transcription and protein production, and cell size (Clemmons, 2004). In addition, it can mediate metabolic effects such as: carbohydrate and fatty acid translocation, and glucose uptake. IGF-1 is an integral regulator of the cell cycle and is needed for progression from G1 to S phases. Insulin-like growth factor binding proteins (IGFBP) are a class of proteins that regulate IGFs, by either inhibiting or enhancing its activities. In extracellular fluids, IGFs are present in a complex with an IGF-

binding protein (IGFBP); IGFBPs are traditionally thought to function as carrier proteins and regulate circulating IGF turnover, transport, and distribution. There are many ways in which IGFBPs can do this: prevent IGF association with receptor, transporting IGFs to respective receptor, product of the interaction between IGFs and IGFBPs or cleavage of IGFBP that increase free IGF, therefore increasing metabolic availability (ALPCO, 2019). In a diseased state actions of IGF-1 and IGFBP-2 can be compromised, effecting the aforementioned metabolic roles of these enzymes.

Intrauterine development is sensitive to changes caused by the environment and can influence fetal growth rates and organ development. These influences can lead to adaptations in the fetus, that permanently change its structure and metabolism. The ability for beta-cells to adapt to an increased demand can be influenced by intrinsic genetic factors and metabolic state of the intrauterine environment (Kaiser & Leibowitz, 2009). One of the consequences of IUGR is decreased fetal birth weight. Complications in IUGR pregnancies include decreased placental mass that limits fetal growth and diminishes the amount of fetal pancreatic endocrine tissue by lowering mitotic activity (Limesand et al., 2005). This indicates that stress from placental insufficiency inhibits beta-cell proliferation, and because beta-cells have a central role in the regulation of glucose tolerance the reduction in mass is hypothesized to promote the risk for developing DM (Boehmer, Limesand, & Rozance, 2017).

IUGR fetal pancreases have smaller islets, fewer beta-cells, and lower insulin secretion. Limesand et al., (2005) showed that pancreatic insulin contents were reduced by 76% and pancreatic beta-cell mass was reduced by 58% in IUGR fetuses compared to control fetuses. The reduction in beta-cell mass was due to a reduction in the number of beta-cells undergoing mitosis. In a subsequent study, Chen et al., (2012) showed that in isolated islets of IUGR sheep

fetuses, there was greater IGFBP-2 mRNA, which can slow beta-cell proliferation by antagonizing IGF-1. Furthermore IGF-1 mRNA expression was reduced compared to controls, indicating that decreased IGF-1 signaling may be responsible for the slower proliferation rates.

Powell-Braxton et al., (1993) showed that IGF-1 is critical in pre- and postnatal development using mice models that had an inactive IGF-1 gene. They observed a profound effect of IGF-1 on embryonic and postnatal size. Heterozygous mice carrying one allele for IGF-1 knockout were 10-20% smaller than wild-type mice; and homozygous, carrying two alleles, mice were <60% body weight of wild type mice. The decreased body size was attributed to the reduction in organ size, muscle and bone mass (Powell-Braxton et al., 1993). This study provided evidence of the actions of IGF-1 concerning fetal growth. However, conflicting evidence was found concerning the effects of IGF-1 on beta-cell proliferation (Kulkarni, 2002; Lu et al., 2004) in mice models. Kulkarni (2002) used IGF-1 receptor knockouts in mice which demonstrated progressive glucose intolerance and, in some subjects overt diabetes within 10 months. Lu et al., (2004) used IGF-1 gene-deficient mice, in which pancreatic islets were enlarged and number of islets per unit of pancreatic tissue were elevated; they hypothesized that the IGF-1 deficiency activated pro-islet growth factors. The conflicting evidence demonstrates the need for testing in ovine models that mimic human gestation more closely than mice or rat models.

The most relevant portion of the Limesand et al. (2005) study is *Fetal and Pancreatic Characteristics of Control and IUGR Animals*. This section explains the relationship between fetal and pancreatic weight, and insulin secretion and insulin mRNA. This finding is used as a basis for other studies (Limesand, Rozance, Zerbe, Hutton, & Hay Jr., 2006; Chen, Rozance, Hay Jr. & Limesand, 2012) in understanding the relationship between reduction of fetal pancreatic

weight and decreased beta-cell proliferation. Limesand et al. (2005) findings suggested that reduced beta-cell mass and insulin availability during fetal development might define a mechanism in which the fetus adapts its somatic growth rate to nutrient content available. If this mechanism is not corrected after birth, strenuous demands for insulin production on a smaller starting population of beta-cells in IUGR offspring suggests susceptibility to diabetes.

Limesand et al. (2006) compare insulin secretion of control islets and IUGR islets. The researchers found that IUGR islets had a lower insulin response to glucose challenges and basal conditions. IUGR beta-cells secreted more insulin per islet, demonstrating an enhanced response to glucose stimulation; however due to decreased IUGR beta-cell mass, and subsequent decrease in insulin content, IUGR beta-cells were incapable of responding appropriately overall. This study demonstrates that decreased insulin response, was due to beta-cell mediated insulin biosynthesis and storage, not beta-cell responsiveness. Thus, presenting evidence that IUGR may be altering beta-cell signaling that leads to decreased beta-cell proliferation overall.

Locally expressed IGFBPs can inhibit and/or potentiate IGF activities (Duan & Xu, 2005). IGFs and receptors are needed to modulate regulation of protein turnover and potent mitogenic and differentiating effects on cells; IGFs are critical for fetoplacental growth during gestation and growth mediated effects are specifically mediated by IGF-1 and the type I IGF receptor (Luigi et al., 2005). Dysregulated actions of IGF-1 due to irregular expression of IGFs, dysfunctional IGF receptor or post-receptor signaling mechanisms can lead to abnormal fetal growth (Luigi et al., 2005). Luigi et al., (2005) observed reductions in IGF-1 receptor in IUGR mice placenta and hypothesized that IGF-1 signaling is disrupted due to subexpression of proteins also needed for signaling.

The IGF system is an evolutionarily conserved signaling pathway, thus IGF signaling system plays a fundamental role in regulating growth and differentiation in utero as well as in maintaining homeostasis in adults (Duan & Xu, 2005). IGF-I mRNA expression is diminished in IUGR islets, and likely regulates pancreatic beta-cell development, expansion and survival through local trophic mechanisms. Elevated levels of IGF-I are associated with increased rates of beta-cell mitosis and regeneration, and low levels are associated with decreased beta-cell mass and pancreas development (Rozance et al., 2012). The presence of IGFBP-2 inhibits actions of IGF-I, suggesting that IGFBP-2 may regulate specific tissue and organ growth (Duan & Xu, 2005).

Research Methodology

Animal Preparation. Studies were conducted in 4 pregnant Columbia-Rambouillet crossbred ewes carrying a singleton fetus (Agricultural Research Center Farm, AZ). Ewes were fed Standard-Bread Alfalfa Pellets (Sacate Pellet Mills, AZ) and provided water and salt *ad libitum*. Indwelling vascular catheters were surgically placed into ewes and fetuses for arterial blood sampling and intravenous infusions at 124 ± 1 days gestational age (dGA). Animal husbandry was performed as previously reported (Chen et al. 2014; Macko et al. 2016). All animal procedures were approved by the Institutional Animal Care and Use Committee and performed at the University of Arizona.

Necropsy and Islet Isolation. Ewes and fetuses (control and IUGR) were euthanized with an overdose of sodium pentobarbital (86 mg/kg, Euthasol; Virbac Animal Health, Fort Worth, TX). The fetal pancreas was collected for islet isolation (Limesand et al. 2006). Islets were purified over a histopaque gradient after the fetal pancreas was digested in Krebs-Ringer Buffer (KRB) with Collagenase V (0.425 mg/mL; Sigma) and DNase I (2 μU/mL; Roche Diagnostics,

Mannheim, Germany) as described previously (Limesand et al. 2006; Chen et al. 2014; Rozance et al. 2015).

Isolated islets were washed in KRB containing 0.5% BSA (KRB- BSA). Islets were cultured 2 hours a base media of RPMI-1640 medium (Sigma-Aldrich) supplemented with 5% fetal bovine serum, 2.8 mmol/L glucose, and penicillin–streptomycin to provide adequate nutrients and prevent microbial growth (50 U and 50 mg; Sigma-Aldrich) at 37°C with 95% O2- 5% CO2 (Limesand et al. 2006; Green et al. 2012).

Experimental Design. Five treatments were prepared for each islet isolation: base media (BM) alone, BM + IGF-1 (Sigma-Aldrich), BM + IGFBP-2 (Creative Biomart), BM + IGF-1+ IGFBP-2, and BM + T_3 (Sigma-Aldrich). Dilutions were prepared based off of recommendations from the manufacturers of each treatment. For cellular proliferation, all islet cultures included 10μM 5-ethynyl-2´-deoxyuridine (EdU, Molecular Probes, Eugene, OR) for 48 hours. BM + IGF-1+ IGFBP-2 was prepared using 9.980 mL of RPMI, 10 μl IGF-1 (10 μg/mL stock) and 10 μl IGFBP-2 (100 μg/ mL stock). 5% FBS + IGF-1 was prepared using 9.990 mL of RPMI, 10 μl IGF-1 (10 μg/mL stock) as a control model. 5% FBS + IGFBP-2 was prepared using 9.990 mL RPML, 10 μl IGFBP-2 (100 μg/mL stock). 5% FBS + T_3 was prepared using 9.995 mL of RPMI, 5 μl of T_3 (20 μg/ mL stock). Each treatment had a final volume of 10 mL and split equally into two conical tubes, so each tube had a final volume of 5 mL. Islets were picked equally and placed into Petri dishes containing 5 mL of each treatment, respectively. The islets were oxygenated for 6 minutes then incubated for 24 hrs at 37°C. After 24 hours, the islets were placed into fresh media in newly labeled Petri dishes using the second vial of 5 mL treatment media and incubated for another 24 hours; this was done to prevent nutrients from being a

limiting factor and discourage microbe formation. After another 24 hours, the islets were removed from the treatment, and prepared for histology.

Histology. Islets (50-100) were fixed in 4% paraformaldehyde, then snap frozen in Optimal Cutting Temperature compound (OCT, Sakura Finetek USA, Inc., Torrance, CA) and stored at -80°C. Embedded islets were sectioned with a Microm HM 520 cryostat (Southeast Pathology Instrument Service, Charleston, SC) at a thickness of 10μm. Sections with an interval distance of ≥100-μm apart were placed onto Superfrost Plus microscope slides (Fisher Scientific, Pittsburg, PA). Insulin positive cells were immunostained using a guinea pig anti-porcine insulin polyclonal antibody (1:500, Dako, Carpinteria, CA, USA) and detected with donkey anti-guinea pig IgG antibody conjugated to Alexa Fluor® 594 (1:500, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, 1μg/mL, Sigma-Aldrich, St. Louis, MO). Proliferating β-cells were stained for EdU (Click-iT EdU Alexa Fluor 488 HCS Assay) and co-stained with anti-insulin and DAPI as described above. Fluorescent images were visualized on the Leica DM5500 microscope system at 20x magnification and digitally captured with an ORCA-Flash4.0 LT Digital CMOS Camera C11440 (Hamamatsu Phototonics K.K, Japan) using HCImage Live software (Hamamatsu Phototonics K.K, Japan). Morphometric analysis was performed with ImagePro 6.3 software (Media Cybernetics, Silver Spring, MD).

Analysis. The percentage of insulin positive cells are calculated as the number of cells positive for insulin divided by the total cell nuclei (minimum 10,000 per isolation). The percentage of proliferating β-cells are calculated as the number of cells positive for both EdU and insulin divided by the total number of insulin positive cells. Analyzing the stains with this

method will provide the approximation of cells that produce insulin and are proliferating, for each respective treatment.

Results and Discussion

Treatments	18-23-1 %proliferation (control)	18-26-1 %proliferation (control)	18-22-1 %proliferation (IUGR)	18-24-1 %proliferation (IUGR)
BM	2.56	1.497	3.172	-
IGF-1	2.712	1.176	4.732	2.230
IGFBP-2	2.79	.9879	-	-
IGF-1 + BP2	2.428	2.76	7.032	3.2467
T3	2.8	.9969	-	-

There were 5 treatments prepared: base media (BM) to test the effects of IGF-1 and IGFBP-2, together and respectively, on beta-cell regeneration. Islets were collected, equally distributed and incubated in each treatment for 48 hours; and underwent a media change at 24 hours. The islets were then embedded, sectioned and immunostained with fluorescents for analysis. In the study a representative count was obtained. However, there was not enough data collected to offer distinction between control groups. Across all animals it is suggested that IGF-1 did not have an effect. The analysis of the islets led us to conclude that there may not have been an effect on beta-cell proliferation by IGF-1 and IGFBP2 activities. Where the experiment may need careful consideration are times the islets are collected relative to each other (IUGR and controls), which occurred at separate times in this study. Also, a larger cohort of animals is needed to offer statistical validity to this study. The limitations that can be fixed are increase number of animals, as well as reducing time lapse between the collection and staining of each treatment group. The cellular processes that may have affected outcomes include: beta-cells

produced enough of their own IGF-1 that exogenous IGF-1 had no effect and/or down-regulation of IGF-1 receptor complexes.

Conclusion

The evidence concerning the pathophysiology of DM include, decreased beta-cell mass, dysregulation of growth factors and dysregulation of insulin biosynthesis and storage, are important variables to consider that have variable impacts on pancreatic islet health. Although we did not successfully characterize the IGF-1/IGFBP-2 actions, this study remains relevant, due to the relationship between IGF-1 and IGFBP-2 never explored before in ovine models. The closely mirrored gestation environment between ovine and human models leaves room for future studies that want to further investigate processes that can lead to a better understanding of beta-cell physiology in humans.

References

ALPCO. (2019). Role of IGF-1 in the Growth Hormone/IGF Axis. https://www.alpco.com/role-igf-1-growth-hormoneigf-axis.

Akarsu, S., Bagirzade, M., Omeroglu, S., & Buke, B. (2017). Placental Vascularization and apoptosis in Type-1 and gestations DM. (2016). *Journal of Maternal-Fetal &Neonatal Medicine*, 30, 1045-1050.

Barry, J. S., & Anthony, R. V. (2008). The Pregnant Sheep as a Model for Human Pregnancy. Theriogenology, 69(1), 55–67. http://doi.org/10.1016/j.theriogenology.2007.09.021

Boehmer, B. H., Limesand, S. W., & Rozance, P. (2017). The impact of IUGR on pancreatic islet development and β-cell function. *Journal of Endocrinology*, 235(2), R63-R76. doi: 10.1530/JOE-17-0076.

Bouwens, Luc, & Rooman, Ilse. (2005). Regulation of Pancreatic Beta-Cell Mass. *Physiology Review*, 85, 1255-1270.

Camacho, L. E., Chen X., Hay Jr., W. W., & Limesand, S. W. (2017). Enhanced insulin secretion and insulin sensitivity in young lambs with placental insufficiency-induced intrauterine growth restriction. *American Journal of Physiology*, 313(2), R101-R109. doi: 10.1152/ajpregu.00068.2017.

Chunguang Chen, Christian M. Cohrs, Julia Stertmann, Robert Bozsak, Stephan Speier. (2017). Human beta cell mass and function in diabetes: Recent advances in knowledge and technologies to understand disease pathogenesis. *Molecular Metabolism*, 6, Issue 9, 943-957

Chen X., Green A. S., Macko A. R., Yates D. T., Kelly A. C., Limesand S. W. (2014). Enhanced insulin secretion responsiveness and islet adrenergic desensitization after chronic norepinephrine suppression is discontinued in fetal sheep. *Am J Physiol Endocrinol Metab.*, 306, E58–E64.

Chen, X. Kelly, A. C., Yates, D. T., Macko, A. R., Lynch, R. M., & Limesand, S. W. (2017). Islet Adaptations in Fetal Sheep Persist Following Chronic Exposure to High Norepinephrine. *Journal of Endocrinology*, 232(2), 285-295. doi:10.1530/JOE-16-0445.

Chen, Xiachuan, Rozance, Paul J., Hay Jr., William W & Limesand, Sean. (2012). Insulin-like growth factor and fibroblast expression profiles in growth restricted fetal sheep pancreas. *Experimental Biology and Medicine*, 237(5), 524-529. doi:10.1258/ebm.2012.011375.

Clemmons, David R. (2004). The relative roles of growth hormone and IGF-1 in controlling insulin sensitivity. *The Journal of Clinical Investigation*, 113, 25-27. doi: 10.1172/JCI200420660.

Duan, C., & Xu, Q. (2005). Roles of insulin-like growth factor (IGF) binding proteins in regulating IGF actions. *General and Comparative* Endocrinology, 142(1–2), 44-52. doi.org/10.1016/j.ygcen.2004.12.022.

Godfrey, Keith M. & Barker, David J. P. (2000). Feta nutrition and adult disease. *The American Journal of Clinical Nutritionists*, 71, 1344S-1352S.

Green, A. S. , Chen, X., Macko, A. R., Anderson, M. J., Kelly, A. C., Hart, N. J., Lynch, R. M., & Limesand, S. W. (2012). Chronic pulsatile hyperglycemia reduces insulin secretion and increases accumulation of reactive oxygen species in fetal sheep islets. *Journal of Endocrinology*, 212, 327–342.

Haeften, T. W., & Twickler, Th. B. (2004). Insulin-like growth factors and pancreas beta cells. *European Journal of Clinical Investigation*, 34, 249-255.

Kaiser, N., & Leibowitz, G. (2009). Failure of beta-cell adaptation in type 2 diabetes: Lessons from animal models. *Frontiers in Bioscience*, 14, 1099-1115.

Kelly, A. C., Bidwell, C. A., McCarthey, F. M., Taska, D. J., Anderson, M. J., Camacho, L. E., & Limesand, S. W. (2017). RNA Sequencing Exposes Adaptive and Immune Responses T Intrauterine Growth Restriction in Fetal Sheep Islets. *Endocrinology*, 158(4), 743-755.

Kulkarni, R. N. (2002). Receptors for insulin and inslun-like growth factor-1 and insulin receptor substrate-1 mediate pathways that regulate islet function. *Bioch Soc Trans*, 30(2), 317-322.

Limesand, Sean W., Jensen, J., Hutton, John C., & Hay Jr, William. (2005). Diminished β-cell replication contributes to reduced β-cell mass in fetal sheep with intrauterine growth restriction. *Am J Physiol Regul Integr Comp Physiol*, 288, 1297-1305. doi: 10.1152/ajpregu.00494.2004.

Limesand, Sean W., Rozance, Paul J., Zerbe, Gary O., Hutton, John C., & Hay Jr., William W. (2006). Attenuated Insulin Release and Storage in Fetal Sheep Pancreatic Islets with Intrauterine Growth Restriction. *Endocrinology*, 147(3), 1488-1497. doi:10.1210/en.2005-0900.

Liu, Y., Tan, Q., & Liu, F. (2017). Differentially methylated circulating DNA: A novel biomarker to monitor beta cell death. *Journals of Diabetes and Its Complications*, 32, 349-353.

Lu, Y., Herrera, P., Guo, Y., Sun, D., Tang, Z., LeRoith, D., & Liu, J. (2004). Pancreatic-Specific Inactivation of IGF-1 Gene Causes Enlarged Pancreatic Islets and Significant Resistance to Diabetes. *Diabetes*, 53, 3131-3141.

Luigi Laviola, Sebastio Perrini, Gaetana Belsanti, Annalisa Natalicchio, Carmela Montrone, Anna Leonardini, Antonella Vimercati, Marco Scioscia, Luigi Selvaggi, Riccardo Giorgino, Pantaleo Greco, Francesco Giorgino. (2005). Intrauterine Growth Restriction in Humans Is Associated with Abnormalities in Placental Insulin-Like Growth Factor Signaling, *Endocrinology*, Volume 146(3), 1498–1505, https://doi.org/10.1210/en.2004-1332

Powell-Braxton, L., Hollingshead, P., Warburton, C., Dowd, M., Pitts-Meek, S., Dalton, D., Gillet, N., & Stewart, T. A. (1993). IGF-1 is required for normal embryonic growth in mice. *Genes and Development*, 7, 2609-2617.

Macko, A. R., Yates, D. T., Chen, X., Shelton, L. A., Kelly, A. C., Davis, M. A., Camacho, L. E., Anderson, M. J., & Limesand, S. W. (2016). Adrenal Demedullation and Oxygen Supplementation Independently Increase Glucose-Stimulated Insulin Concentrations in Fetal Sheep With Intrauterine Growth Restriction. *Endocrinology*, 157, 2104–2115.

Rechler, Matthew M. (1993). Insulin-like Growth Factor Binding Proteins. *Vitamins and Hormones*, 47, 1-114.

Rashid, C. S., Lien, Y. C., Jaekle-Santos, L. J., Won, K. J., & Simmons, R. A. (2018). Transcriptomic Analysis Reveals Novel Mechanisms Mediating Islet Dysfunction in the Intrauterine Growth-Restricted Rat. *Endocrinology*, 159(2), 1035-1049. doi: 10.1210/en.2017-00888.

Rozance, J., Anderson, M. J., Martinez, M., Fahy, A., Macko, A. R., Kailey, J., Seedford, G. J., Abman, S. H., Hay Jr., W. W., & Limesand S.W. (2015). Placental Insufficiency Decreases Pancreatic Vascularity and Disrupts Hepatocyte Growth Factor Signaling in the Pancreatic Islet Endothelial Cell in Fetal Sheep. *Diabetes*, 64(2), 555-564.

Wang, P., Fiaschi-Taesdvh, N. M., Vasavada, R. C., Scott, D. K., García-Ocaña, A., & Stewart, A. (2015). Diabetes mellitus- advances and challenges in human β-cell proliferation. Nature Reviews Endocrinology, 11, 201-212.

The Effects of IGFs on Ovine Models Exposed to Intrauterine Growth Restriction

Abstract

The intrauterine environment is sensitive to environmental stimuli, which can negatively impact fetal growth and organ development. Placental Insufficiency (PI) is a complication of pregnancy that occurs when the placenta is unable to provide appropriate oxygen and nutrients to the developing fetus. PI is the primary cause for intrauterine growth restriction (IUGR), which refers to infants that have failed to reach their genetic growth potential. Characteristic of type 2 diabetes is decreased insulin secretion, reflective of beta-cell dysfunction. Insulin-like growth factor-1 (IGF-1) is crucial for beta-cell proliferation and pancreatic development; and is associated with elevated beta-cell mitosis. IGF binding proteins (IGFBPs) regulate various IGFs via transport, turnover and distribution. In PI- IUGR fetuses, decreased plasma IGF-1 concentration and increased IGFBP-2 mRNA in isolated islets have been observed. The aim of this study is to determine the role of IGFBP-2 and the relationship of IGF-1 and IGFBP-2 in fetal ovine islets. Control islets and IUGR islets were split into treatment groups: control, IGF-1, IGFBP-2, IGF-1+ IGFBP-2, and T_3. Fluorescent images were taken for morphometric analysis. The results suggested that IGFs and IGFBP had no effect on beta-cell proliferation, however more animal models need to be analyzed for statistical validity.