

- 13 **CELL ENCAPSULATION TECHNOLOGY AS A USEFUL NON-DIETARY THERAPY FOR MATERNAL PHENYLKETONURIA** DONNA SANTILLAN, MARK SANTILLAN, STEPHEN HUNTER, University of Iowa, Iowa City, Iowa

OBJECTIVE: Our objective is to develop a novel non-dietary therapy for maternal phenylketonuria (PKU). Individuals with PKU cannot process phenylalanine (Phe) due to mutations within phenylalanine hydroxylase (PAH) rendering the enzyme partially or completely inactive. Increased Phe during pregnancy causes congenital malformations and mental retardation in children. Our therapy utilizes cell encapsulation technology to lower maternal Phe levels to protect offspring from the effects of maternal PKU disease.

STUDY DESIGN: In our approach, cells are engineered to overexpress PAH, encapsulated within polymeric microspheres, and transplanted into affected individuals. Encapsulated cells are protected from the immune system due to the small pore size, that prevents the immune system from reaching the cells. However, nutrients and amino acids are able to diffuse into spheres. Cells were transiently and stably transfected to overexpress PAH. RT-PCR and immunoblotting confirmed the presence of PAH mRNA and protein, respectively. Cells were cultured and timed measurements of the Phe concentration in the media were performed using tandem mass spectrometry to document enzyme activity.

RESULTS: Cells overexpressing PAH have successfully been produced. Both non-encapsulated and encapsulated transiently transfected cells significantly reduced the Phe concentration in media by approximately 50% in comparison to media alone. Stable PAH cell line clones significantly decreased Phe in media by up to 68% versus media alone.

CONCLUSION: Encapsulation of PAH expressing cells is a potential new therapy for maternal PKU. Currently, we demonstrate that we have generated cell lines with stable overexpression of PAH. Further, these cells significantly reduce the Phe concentration in cell culture media. In vivo, we expect that stable PAH over-expressing cell lines should provide a longer therapeutic effect than transiently transfected cells. Studies using PKU model mice will be important in determining the ability of our therapy to reduce the physical and mental abnormalities due to maternal PKU.

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- 14 **THE ROLE OF ESTROGEN RECEPTOR-B (ESR2) ON FETOPLACENTAL VASCULAR FUNCTION** EMILY SU¹, ZHI-HONG LIN¹, RANA ZEINE², SCOTT REIERSTAD¹, JOY INNES¹, BETH PLUNKETT¹, PING YIN¹, SERDAR BULUN¹, ¹Northwestern University, Obstetrics and Gynecology, Chicago, Illinois, ²Northwestern University, Pathology, Chicago, Illinois

OBJECTIVE: Adequate fetoplacental blood flow is required for optimal fetal growth, and these vessels appear to be reduced in caliber in growth-restricted pregnancies. Regulation of fetoplacental vascular function is likely mediated by endothelial cell-derived vasoactive mediators, as these vessels lack innervation. Estrogen and its receptors, estrogen receptor- α and estrogen receptor- β (ESR2), promote overall vascular health by producing various vasodilators. Our prior data has demonstrated that of the estrogen receptors, placental villous endothelial cells solely express ESR2. Thus, we sought to determine the role of ESR2 in mediating fetoplacental vascular function

STUDY DESIGN: Immunohistochemistry was performed on placental sections from growth-restricted pregnancies and gestational age-matched controls. Placental villous endothelial cells were isolated and cultured from term, uncomplicated pregnancies and treated with vehicle, estradiol, or diarylpropionitrile (DPN), an ESR2-specific agonist. RNA interference was utilized to knock-down ESR2. Real-time PCR and western blotting were performed, and cell culture supernatant were analyzed for prostanoid production via enzyme immunoassay.

RESULTS: Immunohistochemistry demonstrated increased ESR2 expression within villous endothelial cells of growth-restricted pregnancies. Within cultured primary villous endothelial cells from uncomplicated pregnancies, neither estradiol nor DPN affected cyclooxygenase-2 (COX-2) or prostanoid levels. RNA interference of ESR2 led to a concomitant decrease in COX-2 mRNA ($p < 0.05$) and protein levels, which was associated with diminished thromboxane levels ($p \leq 0.003$).

CONCLUSION: ESR2 expression patterns influence COX-2 and prostanoid levels within placental villous endothelial cells. Our data suggest that ESR2 may act via a ligand-independent mechanism to promote thromboxane production. Thus, elevated ESR2 expression within endothelial cells of growth-restricted pregnancies may be a key pathologic event, leading to increased COX-2 activity, thromboxane production, and vasoconstriction.

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- 15 **PROGRAMMED REPRODUCTIVE DYSFUNCTION IN LOW BIRTHWEIGHT (LBW) OFFSPRING** MINA DESAI, ERIN KEEN-RHINEHART, GUANG HAN, MICHAEL G. ROSS, Harbor-UCLA Med. Ctr. (LABioMed), Dept. of Ob/Gyn, Torrance, California

OBJECTIVE: LBW offspring are at increased risk of programmed adult obesity, and obesity in women is associated with reproductive dysfunction. However, it is unknown whether the reproductive dysfunction is solely a result of obesity or attributable, in part, to a gestationally programmed mechanism. Food restriction (FR) in rat pregnancy results in LBW pups with hypoleptinemia. When nursed normally, FR females develop pre-pubescent hyperleptinemia and adult obesity. Leptin affects the hypothalamic-pituitary-gonadal (HPG) axis both centrally, influencing secretion of hypothalamic gonadotropin-releasing hormone (GnRH) and pituitary gonadotropins, and peripherally, regulating ovarian steroidogenesis. We hypothesized that programmed altered leptin impacts the HPG axis, leading to reproductive dysfunction in LBW female offspring.

STUDY DESIGN: Control dams received ad libitum food, whereas study dams were 50% FR from pregnancy day 10 to 21. All pups were nursed by Control dams and weaned at 3 weeks to ad libitum feed. At 1 day of age, female hypothalamic protein expression of GnRH and leptin receptor (ObRb), and ovarian ObRb were analyzed by Western Blot. Data were normalized to β -actin. At 10 months of age, reproductive cycling was assessed by daily examination of vaginal cytology.

RESULTS: At 1d of age, LBW pups showed significant downregulation of hypothalamic GnRH (0.5-fold). ObRb expression was significantly upregulated in both the hypothalamus (2-fold) and the ovary (1.6-fold). By 10 months of age, FR adult females exhibited early anovulation as evident by persistent estrus phase with absence of normal cycling.

CONCLUSION: The results suggest a marked dysfunction in basal hypothalamic reproductive index in the LBW newborns. It is likely that the early reduced leptin levels in LBW newborns alter growth, maturation and function of HPG axis which later lead to reproductive dysfunction.

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- 16 **17 OH PROGESTERONE BLUNTS THE HYPERTENSIVE RESPONSE ASSOCIATED WITH REDUCTIONS IN UTERINE PERFUSION PRESSURE IN PREGNANT RATS** EDWARD VELLON¹, SHARON KEISER¹, MARC PARRISH¹, WILLIAM BENNETT¹, KATHY COCKRELL², LILLIAN FOURNIER², JOEY GRANGER², JAMES MARTIN¹, BABBETTE LAMARCA², ¹University of Mississippi Medical Center, Obstetrics and Gynecology, Jackson, Mississippi, ²University of Mississippi Medical Center, Physiology, Jackson, Mississippi

OBJECTIVE: Reduction in uteroplacental perfusion (RUPP) is hypothesized to be the initiating factor for the development of hypertension during pregnancy. The objective of this study was to determine if 17OH Progesterone is a promising treatment for hypertension in response to placental ischemia in the pregnant rat. Arterial pressure (MAP) and endothelial factors were examined in a conscious, chronically instrumented rat model of RUPP with or without 17 OHP administration. Hypertension produced by RUPP is associated with endothelial dysfunction, enhanced ET-1 and ROS production, decreased NO synthesis, and a hypertensive shift in the pressure natriuresis relationship.

STUDY DESIGN: Sprague-Dawley rats were anesthetized on Day 14 of pregnancy and underwent either 1) examination under anesthesia; 2) RUPP: the lower abdominal aorta was isolated and clipped (0.203mm ID) above the iliac bifurcation; branches of both the right and left ovarian arteries were clipped (0.100mm ID) 3) injection of intraperitoneal 17OHP, or 4) RUPP + injection of intraperitoneal 17OHP. On day 18, carotid artery catheters were placed in each rat; on day 19 MAP, maternal and pup weight were measured. Blood, kidneys and placentas were collected and weighed.

RESULTS: MAP increased 23 mmHg in RUPP rats compared to control pregnant rats (126 \pm 2 vs 103 \pm 2 mmHg ($p < 0.03$)). However, RUPP rats receiving progesterone displayed a 16 mmHg increase in MAP (119 \pm 2 (P0.03). ET-1 increased 3 fold in the cortex from RUPP rats (2.5 \pm 0.49 relative units, n=6) compared to normal pregnant rats (-1.5 \pm 0.9 relative units, n=6) ($P < 0.02$) but was -0.6 relative units (n=4) in RUPP + 17 OHP. There was no change in maternal, pup or placental weights in response to 17 OHP administration in RUPP rats.

CONCLUSION: Administration of 17 OH Progesterone blunts the hypertension associated with RUPP in pregnant rats possibly via protecting against endothelial activation that is associated with placental ischemia.

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