Chicken Gonads

Martin Andres Estermann, Lic[1], Craig Allen Smith, PhD[2].
[1]MONASH UNIVERSITY, Melbourne, Australia, [2]Monash University, Clayton, Australia.

During early embryogenesis, the undifferentiated gonad is bipotential and subsequently commits to an ovarian or testicular fate. In birds, double dose of the Z-linked gene *DMRT1* is required for testicular differentiation in male embryos (genetically ZZ). In female birds, estrogen plays a key role in ovarian differentiation. 17β-estradiol (E2) induces gonadal feminization when applied to male embryos (ZZ). Conversely, inhibition of estrogen synthesis with the drug fadrozole (FAD) results in testicular development in genetically female embryos (ZW). However, activation of male markers in sex-reversed ZW embryos is typically delayed, raising the possibility that FAD-treated embryos may transition through an undifferentiated state before masculinization. Recently, PAX2 was identified as a marker of undifferentiated supporting cells in the chicken embryo, being downregulated in both sexes at the onset of gonadal sex determination. To investigate the supporting cell differentiation process in estrogen-mediated sex reversal, we injected 1 mg of fadrozole in 100µl of PBS or vehicle into embryonic day 3.5 (E3.5) chicken eggs. Eggs were incubated until E9.5, genotypically sexed (ZZ or ZW) and processed for qRT-PCR and immunofluorescence. Quantitative RT-PCR confirmed that sex reversal had occurred in FAD-treated females, showing a reduction of pre-granulosa cell markers *aromatase* ($P<0.005$) and *FOXL2* ($P<0.05$), compared to the control. Interestingly, *PAX2* mRNA expression was up-regulated ($P<0.05$) in sex-reversed females, suggesting an increase in undifferentiated supporting cells (n=6). To confirm this observation, immunofluorescence was used to detect aromatase, SOX9 (male marker) and PAX2. In FAD-treated females, both $SOX9^+$ (male) and $aromatase^+$ (female) cells co-existed in the same gonad, but in separated defined regions. Aromatase positive cells were located in the most apical region of the gonad whereas SOX9 positive cells were detected in the basal region. We detected an increase in PAX2 positive cells in the gonadal medulla between the SOX9 and aromatase positive supporting cells. No SOX9 or PAX2 positive cells were detected in control female gonads (n=3). For feminization experiments 100µl of a 1mg/ml solution of E2 or vehicle (Oil) was injected into E3.5 chicken eggs. No significant increase in PAX2 was detected by qRT-PCR ($p>0.05$, n=6) and no PAX2 positive cells were detected in E2 treated gonads at E9.5. These results suggest that in fadrozole-mediated masculinization (but not in estrogen-induced feminization) there is an increase in undifferentiated supporting cells. The absence of both estrogens (feminizing) and elevated DMRT1 (masculinizing) could explain why the supporting cells remain in an undifferentiated state in ZW (genetically female) embryos. Further research is required to evaluate the fate of these undifferentiated cells in gonadal sex differentiation.

Reproductive Endocrinology

OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

Genetic Sex Effects of Polycystic Ovary Syndrome Reveal Distinct Metabolic Etiology

Ky'Era Actkins, BS[1], Digna R. Velez Edwards, PhD[2], Melinda Aldrich, PhD[2], Lea K. Davis, PhD[2].
[1]Meharry Medical College, Nashville, TN, USA, [2]Vanderbilt University Medical Center, Nashville, TN, USA.

Females with polycystic ovary syndrome (PCOS) have an increased risk of developing metabolic disorders such as insulin resistance, obesity, and type 2 diabetes (T2D). High-risk groups or individuals with a family history are more likely to have a greater genetic susceptibility to these diseases, which drastically increases their risk of developing other chronic health conditions. In this study, we systematically evaluated the bidirectional genetic burden of PCOS and its comorbidities among females and males. First, we analyzed the pleiotropic effects of the PCOS polygenic risk score (PRS), a measurement of genetic liability to PCOS, across 1,857 medical conditions recorded in the Vanderbilt University Medical Center electronic health record. We conducted a phenome-wide association study (PheWAS) adjusted for median age, sex, and genetic ancestry. In the European sex-combined model (n = 72,824), we observed that PCOS PRS was significantly (Bonferroni corrected $p < 7.86e-06$) associated with T2D (OR = 1.11, p = 8.75e-08) and hypertension (OR = 1.06, p = 1.13e-07) in addition to polycystic ovaries (OR = 1.11, p = 1.91e-07). In the sex-stratified model, we found that males (n = 32,022) with a higher PRS for PCOS were more likely to develop cardiovascular diseases (CVD) compared to females (n = 40,802) who had higher odds of developing T2D. Although we were underpowered to detect any phenome-wide significant effects in our African descent sample (n=15,283), uterine leiomyoma (OR = 1.24, p = 3.79e-03), osteoarthritis (OR = 1.21, p = 3.94e-03), and benign neoplasm of uterus (OR = 1.24, p = 4.00e-03) were the top three nominal significant results ($p < 0.05$) in females (n = 9,418). To understand the genetic relationships observed in the PheWAS, we used LD score regression to determine the genetic correlation between the phenotypes. We found that PCOS was positively correlated with T2D (rg = 31%), systolic blood pressure (rg = 12%), and pulse pressure (rg = 15%). However, we found no significant associations between the genetic risk of CVD and PCOS diagnosis in the European or African descent samples. Our findings show that the genetic architecture of PCOS has distinct metabolic sex differences, but the genetic risk of those comorbidities is not predictive of a PCOS diagnosis. This suggests that other drivers are contributing to the endocrine and cardiovascular comorbid signatures that underlie PCOS.

Reproductive Endocrinology

OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

Gonadotropin Dependent Neuregulin1 Signaling Regulates Luteal Cell Survival

Jennifer Jones, B.Sc.[1], Saswati Banerjee, Ph.D., M.Sc.[1], Winston E. Thompson, PhD[2], INDRAJIT CHOWDHURY, PhD, MS,[1].
[1]Morehouse School of Medicine, Atlanta, GA, USA, [2]Morehouse Schl of Medical, Atlanta, GA, USA.

The formation of a functional corpus luteum (CL) is an absolute requirement for reproductive success and is induced

by the mid-cycle surge of luteinizing hormone (LH). The CL is a transient ovarian endocrine structure that maintains pregnancy in primate during the first trimester and in rodents during the entire pregnancy by producing steroid hormone progesterone (P4). CL growth and differentiation are tightly regulated by both survival and cell death signals, including endocrine (LH), intra-ovarian regulators, and cell-cell interactions. Neuregulin-1 (NRG1) is a member of the epidermal growth factor-like factor family that mediates it's effect through the erythroblastoma (ErbB) family. However, the detailed mechanisms associated with the interplay of NRG1 and its receptors in CL function is not known. Therefore, we examined the role and action of NRG1 and its receptors in the gonadotropin signaling pathway that impacts CL functions. Immunocolocalization of NRG1 and ErbB2/3 in pregnant rat CL on day 14 and 21 suggest that both NRG1 and ErbB2/3 are differentially expressed in CL. Moreover, both NRG1 and ErbB2/3 are highly expressed in rat CL on day 14 compared to day 21. Furthermore, *in vitro* studies revealed that rat luteal cells (LCs) treated with exogenous tumor necrosis factor-α (TNFα, an inflammatory cytokine) promoted apoptosis in LCs in a dose and time-dependent manner. However, the effects of TNFα was attenuated in presence of exogenous NRG1. Under these experimental conditions, immunoblot analysis indicated that exogenous TNFα treatment in the presence of NRG1 inhibits apoptosis through increased levels of the anti-apoptotic proteins Bcl2 and Bclxl, and activation of ErbB2-ErbB3-PI3K-Akt signaling pathway. Collectively, these studies provide new insights on the NRG1-mediated anti-apoptotic mechanism in LCs through ErbB3-ErbB2-PI3K-Akt→Bcl/Bcl-xL pathway and may have important clinical implications.

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Reproductive Endocrinology

OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

Hepatic Dysregulation of Bile Acid Homeostasis in Hyperandrogenemic Female Mouse Model of Polycystic Ovary Syndrome

Alexandra M. Huffman, MS, Samar Rezq, PhD, Jelina Basnet, MS, Licy L. Yanes Cardozo, MD, Damian G. Romero, PhD.
University of Mississippi Medical Center, Jackson, MS, USA.

Introduction and Purpose: Polycystic Ovary Syndrome (PCOS) is recognized as the most common endocrine disorder in women of reproductive age. Notably, PCOS women with hyperandrogenism have a pronounced increased risk for cardio-metabolic comorbidities compared with healthy individuals. Bile acids are endocrine signaling molecules that modulate hepatic lipid, glucose, and energy metabolism by aiding in absorption of lipids. Alteration of bile acid homeostasis affects overall metabolic homeostasis and contributes to pathogenesis of an array of metabolic diseases, although the molecular mechanisms of this have not been studied in PCOS. **Methods:** Four-week old C57BL/6N female mice were implanted subcutaneously with dihydrotestosterone (DHT, 8.0 mg) or vehicle silastic tubes (n=8/grp). Weekly body weight, food intake, and body composition was assessed. Fasting serum was obtained and the oral glucose tolerance test (OGTT) was performed in the last week of treatment. Animals were euthanized on treatment day 90 and livers were harvested. Expression levels of mRNA were assessed using RT-qPCR. **Results:** DHT treated females had significantly higher liver mass (1,387 ± 51 vs 1,197 ± 29 g, $p<0.05$), increased lean mass (21.25 ± 0.27 vs 19.58 ± 0.23 g, $p<0.05$) and increased fat mass (4.83 ± 0.47 vs 3.59 ± 0.36 g, $p<0.05$) compared to the vehicle counterparts. These hyperandrogenemic females additionally showed altered glucose homeostasis, having increased fasting glucose (201.10 ± 11.11 vs 152.80 ± 9.23 mg/dL, $p<0.05$) and an increased area under the curve (209.2± 11.0 vs 160.8± 3.5 mg.min/dL, $p<0.05$) following OGTT. Hepatic expression of both classic (Cyp8b1, 1.4 ± 0.1-fold, $p<0.05$) and alternative (Cyp7b1, 2.0 ± 0.3-fold, $p<0.05$) bile acid synthesis cytochrome P450 enzyme genes were significantly upregulated in DHT treated animals. Additionally, expression of sulfotransferase Sult2a2 was completely abolished in DHT treated animals compared with vehicle animals, indicating the possibility of androgen regulation of the sulfonation of bile acids marked for elimination. Liver expression of both the bile acid receptor G-protein coupled bile acid receptor 1 and the androgen receptor were both significantly downregulated (Gpbar1: 0.68 ± 0.08-fold, AR: 0.46 ± 0.04-fold, $p<0.05$) in DHT treated animals. **Conclusions:** Bile acid synthesis, transport, and elimination are tightly controlled processes in the liver to maintain a constant bile acid pool and limit reabsorption. Together, our results highlight the potential role of androgens in DHT-treated female mice in the dysregulation of bile acid homeostasis and its potential contribution to influence metabolic dysfunction. (Supported by NIH grants NIGMS P20GM-121334 to LLYC and DGR, and NIH NIDDK R21DK-113500 to DGR and the Mississippi Center of Excellence in Perinatal Research.)

Reproductive Endocrinology

OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

Involvement of BMP-15 in Glucocorticoid Actions on Ovarian Steroidogenesis by Rat Granulosa Cells

Chiaki Kashino, MD, Toru Hasegawa, MD, PhD, Yasuhiro Nakano, MD, Nahoko Iwata, PhD, Koichiro Yamamoto, MD, Yasuhiko Kamada, MD, PhD, Hisashi Masuyama, MD, PhD, Fumio Otsuka, MD, PhD.
Okayama University Grad School, Okayama, Japan.

Glucocorticoid receptor (GR) are known to be expressed in the ovary and glucocorticoids are shown to exert direct effects on granulosa cell functions. In the clinical setting, menstrual abnormality, amenorrhea and hypermenorrhea can be shown in patients with glucocorticoid excess. On the other hand, glucocorticoids can also be used for the treatment of PCOS with hyperandrogenism. However, the effects of glucocorticoids on the reproductive system have not been fully elucidated. In the present study, we investigated the