### *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