following 30 minutes of supine rest. Medical records, testicular size, questionaries, and detailed history of strength training and AAS use were obtained in a structured interview. Serum INSL3 and testosterone were measured using liquid chromatography mass spectrometry. **Results:** Serum INSL3 was markedly suppressed among current AAS users compared with former AAS users and controls, P < 0.001. Additionally, former AAS users also displayed lower serum INSL3 concentrations than controls, mean (SD), 0.43 (0.31) versus 0.60 (0.22) μg/L, P = 0.006 and the difference remained significant in a multivariate linear regression, (B) (95%CI), -0.17 (-0.28;-0.55) μg/L, P=0.004, adjusted for plasma LH, plasma sexual hormone-binding globulin, age, body fat %, smoking and use of other illicit drugs. Longer accumulated duration of AAS use (log2) was associated with reduced serum INSL3 levels in former AAS users, (B) (95%CI), -0.08 (-0.14;-0.01), P=0.022, suggesting a dose-response relation between AAS use and suppression of serum INSL3. We evaluated the association between INSL3 and total testosterone levels and they were not associated among former users and controls in a multivariate linear regression, P=0.821. We noted recovery of serum inhibin B levels among former AAS users reaching the mean plasma level of controls after elapsed duration since AAS cessation of ≈ 21 months; (B) (95%CI), 2.2 (0.7; 3.7) months, P = 0.006. In contrast, we did not note any recovery of serum INSL3, P = 0.541, or total testosterone, P = 0.861, among former AAS users. **Conclusions:** Serum INSL3 is decreased years following AAS cessation in former AAS users, independently of plasma testosterone, suggesting persistent impaired Leydig cell function, which should be investigated further.

Reproductive Endocrinology

MALE REPRODUCTIVE HEALTH

Use of a Lab-Ordering Pathway to Improve Adherence to Endocrine Society (ES) Guidelines for Early AM Timing and Testosterone Assay Method for Establishing Testosterone Deficiency

Emily Gammoh, MD[1], Alexandra Clark, MD[2], Erika Hoffman, MD[2], R Harsha Rao, MD, FRCP[2].
[1]University of Pittsburgh Medical Center, Pittsburgh, PA, USA, [2]VA Pittsburgh Healthcare System, Pittsburgh, PA, USA.

Introduction: Primary care providers (PCPs) prescribe the bulk of testosterone replacement therapy (TRT) at our institution, but our data show they are less likely than endocrinologists (ENDOs) to follow ES Guidelines for diagnosis of hypogonadism, based on two unequivocally low early AM [T] levels by tandem mass spectrometry (LC/MS/MS, PCP:213/783[27%]; ENDO:104/164[63%];p<0.0001). **Methods:** A lab ordering pathway was promulgated to direct PCPs to order 8AM T based on whether it was for monitoring therapy or diagnosing hypogonadism. The former leads to an order for Total T by electrochemiluminescence immunoassay (ECLIA), whereas the latter presents a choice between "*Initial (diagnostic)*" T or "*Confirmatory*" T. Ordering an initial diagnostic T requires a symptom to be chosen from a list of Low Libido, Loss of Early Morning Erections (EME), Fatigue, Erectile Dysfunction, or Other. Low libido or EME loss leads to an order for 8AM total T by LCMSMS, with a pop up reminder to instruct the patient to present at 8AM. Choosing any other symptom triggers a warning of non-specific symptoms, and an option to order 8AM total T by ECLIA, with the same pop up warning regarding timing. Ordering a confirmatory T requires a low initial 8AM T. **Objective:** To compare adherence by PCPs to ES guidelines based on timing and assay method for diagnosis of hypogonadism in the 6 months before (PRE) and 6 months after (POST) the date the pathway was promulgated. **Results:** There were 678 PRE and 884 POST lab orders for a diagnostic T for suspected hypogonadism. Although, adherence to 8AM timing was similar before and after promulgation (PRE 362/678[53.4%]; POST 452/884[51.1%]), there was a significant increase in the use of the accurate assay LC/MS/MS (PRE 39/678 [5.7%]; POST 105/884[11.9%];p<0.001). The 6.3% increase in LC/MS/MS use was reflected in a proportionate 7.3% reduction in ECLIA use (PRE 323/678 [47.6%]; POST (347/884 [39.3%]). Of note, all 105 patients in the POST cohort had a specific symptom (Loss of libido/EME) to justify the LC/MS/MS assay, whereas there was no such justification in the 39 in the PRE cohort. **Conclusions:** Promulgating a lab ordering pathway induced more appropriate use of LC/MS/MS in patients with specific symptoms associated with a high pre-test probability of hypogonadism. Although encouraging, it remains to be determined whether the more appropriate use of LC/MS/MS assays impacted testosterone prescribing practice. On the other hand, the lab ordering pathway did not improve adherence to early AM timing, despite the inclusion of pop up reminders to instruct patients to report early for blood draw. It is unclear whether that is attributable to PCPs not following through with the reminders, or to patients not following instruction due to ignorance, non-compliance, or practical problems, such as transportation and/or wait times at the lab. The lack of adherence to early AM timing has major implications for TRT.

Reproductive Endocrinology

OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

A Novel Mouse Model for Studying the Effects of Cyp17 Overexpression in a Temporal- and Spatial-Specific Manner

CHRISTIAN SECCHI, PhD, MARTINA BELLI, PhD, DWAYNE STUPACK, PhD, SHUNICHI SHIMASAKI, PhD.
UC San Diego, La Jolla, CA, USA.

Background: Cyp17 plays a key role in theca cells (TCs) to produce androgens, which, in turn, are converted to estrogens in granulosa cells. Intrinsic alterations in ovarian steroidogenesis contribute to excessive ovarian androgen production that characterizes polycystic ovary disease (PCOS)[1,2]. Hyperandrogenism has been associated with higher levels of Cyp17 in TCs, and correlate with increased numbers of antral follicles[3]. While androgen excess is one of the hallmark features of PCOS, its putative role in the follicular development and function remains poorly known. Most efforts have used androgen administration or Cyp19 blockade approach to study how androgens prolong folliculogenesis[4]. Although some insights have been made, it is not clear if these

models accurately address the cascade of effects that follow ovarian hyperandrogenism. **Aim:** Here, we aim to study the specific effects of hyperandrogenemia on ovarian morphology, follicle function and fertility with a new transgenic (TG) mouse model expressing elevated Cyp17 levels exclusively in TCs. **Methods:** We generated a breeding line of triple TG mice using a combination of the Tet-dependent expression system and the Cre/LoxP gene control system. Specifically, we used Cyp17 promoter-iCre mice crossed with trans-activator mice (R26-STOP-rtTA-IRES-EGFP transgene, Jackson Lab) and with a responder mouse carrying the TRE-Cyp17 transgene. Cyp17 promoter-iCre mice were used to ensure rtTA/EGFP is expressed specifically in TCs of secondary follicles. After the DNA segment between the two LoxP sites is excised by Cyp17iCre specifically in TCs, the R26-STOP-rtTA gene remains activated in all daughter TCs. Only upon treatment with Doxycycline (DOX) can suppression be relieved and active transcription of TRE-Cyp17 be induced in a dose-dependent manner. **Results:** Cyp17 mRNA expression levels in TCs of TG mice treated with 20, 100 or 200 mg/Kg DOX compared with corresponding untreated control mice showed a modulation in a dose-dependent manner (P=0.01 ANOVA). Confocal and RNAscope analysis validated (i) the effective combination of the Cyp17iCre/rtTA expression system visualizing the rtTA/EGFP specifically expressed in ovarian TCs and (ii) the DOX-induced increase of Cyp17 expression compared with the WT mice. DOX treated TG females were acyclic, being mostly arrested in diestrus. Analysis of estrous cycle stages revealed that treated TG females spent significantly more time in diestrus than control females (P=0.007, ANOVA). **Conclusions:** Our new *in vivo* model is the first that analyzes androgen impact independent of any extraovarian source of androgen, complementing current clinical efforts to study the occurrences of TCs elevated androgen levels in normal and PCOS women. 1 Rosenfield, R. L. *et al. Endocr Rev* (2016)2 Azziz, R. *et al. Nat Rev Dis Primers* (2016)3 Comim, F. V., *et al. Hum Reprod* (2013)4 Stener-Victorin, E. *et al. Endocr Rev* (2020)

Reproductive Endocrinology

OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

Abnormalities in Microarchitecture and Reduced Mechanical Bone Strength in a Rat Model of Polycystic Ovary Syndrome

Fabio Vasconcellos Comim, MD, PhD[1], Lady Katerine Serrano Mujica, PhD[2], Fernanda Valente, PhD[2], Ligia G. Miyazato, PhD[2], Manuela W. Manta, MsC[2], Carolina D. Amaral, MsC[2], Melissa O. Premaor, PhD[1], Soraia Macari, PhD[1], Tarcilia Silva, PhD[1], Breno R. Barrioni, PhD[1], Bruna L. Carlos, PhD[3], Guilherme JA Silva, MsC[3], Antonio Carlos Shimano, PhD[3], Alfredo Q. Antoniazzi, PhD[2].
[1]UFMG, Belo Horizonte-MG, Brazil, [2]UFSM, Santa Maria, Brazil, [3]USP, Ribeirão Preto, Brazil.

Evidence from the literature is contentious about the impact of polycystic ovary syndrome (PCOS) on the skeleton, suggesting a possible negative role of this condition on non-obese women. We investigated this hypothesis employing a well-characterized testosterone propionate (TP) rodent model of PCOS to address the consequences of androgenization on bone microarchitecture, histology, and mechanical strength. For this study, Wistar rats (n= 38) were divided in 4 groups: 1) "Control OVX" (single dose of corn oil s.c. at day 5 of life and ovariectomy at day 100, n=9); 2) "Control SHAM" (n=9); 3) "Androgenized OVX"(single dose of TP 1.25 mg s.c. at day 5 of life and ovariectomy at day 100, n=10); and 4) "Androgenized SHAM" (n=10). Full characterization of estrous cycles and weight was performed during growth, and all animals were euthanized at day 180. Successful ovariectomy was confirmed by neglected levels of serum estradiol. Endpoints evaluated include bone micro CT (femur and spinal column), bone histology (number of osteoclasts and osteoblasts in the femur), and mechanical tests. The study was approved by the local Ethics Committee. At the end of the study (day 180), Androgenized OVX rats were heavier than the other three groups. MicroCT Analysis: Androgenized SHAM rats exhibited a significantly higher trabecular mass in the spine (BV/BT) (mean + SEM) 49.21 + 2.42 % versus Control SHAM 36.42 + 1.39 % (Student T-test p=0.001). Following ovariectomy, BV/BT in Androgenized OVX was 40.4 + 2.83 % against 20.34 + 1.85 % in Control OVX (Student T-test p=0.0003). Lumbar trabecular thickness(μm) was also higher in Androgenized OVX (p=0.0065) as well the Trabecular number (n/mm) (p=0.0003). A similar increase in trabecular mass was observed in the femur. Androgenized SHAM rats had a significant higher BV/BT (%), trabecular thickness(μm), and decreased trabecular separation ($p < 0.001$). However, a significant reduction in cortical bone (thickness) was noted (Student T-test p=0.001). A histological study of the distal femur of Androgenized SHAM rats also show a significantly increased number of osteoclasts and decreased number of osteoblasts than Control SHAM (0< 001). When submitted to the mechanical test, Androgenized Sham rats presented a decreased strength ($p<0.01$) in relation to its controls. After ovariectomy, there was a reduction in bone in all oophorectomized groups. However, differently than the vertebral bones, no differences regarding bone mechanical strength or stiffness as well microCT values, or bone histology parameters were noted in the femur of Control OVX or Androgenized OVX. Our results suggest that androgenization in a rodent model of PCOS leads, at the same time, to a generalized increase in trabecular (cancellous) bone mass (especially in the spine), associated with a reduced cortical bone mass and decreased strength of the femur.

Reproductive Endocrinology

OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

Analysis of BMP15-Induced Transcriptome in Human Granulosa Cells for the Identification of Novel Candidate Genes for Primary Ovarian Insufficiency

Raffaella Rossetti, PhD[1], Marco Fornili, PhD[2], Silvia Moleri, PhD[1], Ilaria Ferrari, PhD[1], Davide Gentilini, PhD[3], Alessio Paffoni, PhD[4], Edgardo Somigliana, MD, PhD[4], Elia Biganzoli, PhD[2], Anna Marozzi, MD, PhD[5], Francesco Brancati, MD, PhD[6], Costanzo G. Moretti, MD[7], Cristina Giuliani, PhD[8], Luca Persani, MD, PhD[9].
[1]IRCCS ISTITUTO AUXOLOGICO ITALIANO, Department of Endocrine and Metabolic Diseases and Lab of Endocrine and