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

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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.

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

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Primary Ovarian Insufficiency (POI) is a female fertility disorder which affects 1% of women before 40 years of age and manifests with amenorrhea, elevation of serum gonadotrophins and low estrogens. POI has a strong genetic component with incomplete penetrance. Several candidate genes have been described so far, however, its etiopathogenesis is mostly unknown. In order to discover the POI-related causative mechanisms, microarray transcriptome analysis in human granulosa cells (hGCs) stimulated with recombinant human BMP15 (rhBMP15) and next generation sequencing analysis (NGS) on the identified differentially expressed genes in a selected group of patients with POI were conducted on NGS Illumina platform. In the present study, we obtained 19 differentially expressed genes upon rhBMP15 stimulation in hGCs. **Results:** showed that all identified genes were upregulated and associated to pluripotency, inhibition of apoptosis, cell proliferation, BMP signaling and apoptosis. Moreover, we identified nine POI patients bearing six rare variants in 5 of the BMP15-induced genes (*SAMD11*, *SMAD6, ID1, USP35*, *GPCR137C*). The BMP15-induced transcriptome analysis in hGCs contributed the understanding of BMP15 role as transcriptional regulator, through the activation of transcriptional repressors, by inducing pathways inhibiting the ovarian follicle maturation, thus possibly maintaining an undifferentiated state of hGCs. These findings lead to the identification of novel candidate genes for POI.

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DHT Differentially Regulates T Helper Cell Related Cytokines and MicroRNAs In Visceral and Subcutaneous Adipose Tissue of Female Mice

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Hyperandrogenemic, insulin resistant polycystic ovarian syndrome (PCOS) patients often have low-grade inflammation due to elevated circulating pro-inflammatory markers. As up to 60% of PCOS patients are obese, whether this low-grade inflammatory state is due to increased adiposity or other factors such as hyperandrogenemia is unknown. Moreover, the systemic inflammation of obesity is correlated with recruitment of pro-inflammatory immune cell populations to WAT. We hypothesized that short-term administration of the potent androgen, dihydrotestosterone (DHT), to female mice would increase pro-inflammatory cytokines and microRNA (miR) associated with pro-inflammatory cytokines and immune cell populations in WAT. Sexually mature, normally-cycling female C57/Bl6 mice received a daily sc injection of oil (0 g; n=7) or DHT (27.5 g; n=7) beginning at estrus. Females had vaginal cytology daily. After three cycles or 12-16 days if mice became acyclic, mice were euthanized for collection of blood and WAT. Serum was analyzed for DHT and testosterone (TEST) by LC-MS/MS. TaqMan^{TM} Array Mouse Immune Response PCR assays (Thermofisher Scientific) were used to measure transcript expression levels in vWAT and scWAT. Ingenuity Pathway Analysis (IPA) (Qiagen) was used to analyze relationships between different transcript levels in each treatment group for each tissue. DHT mice had 17 fold higher serum DHT levels than oil mice but there was no difference in serum TEST between treatment groups. DHT mice had a significantly longer estrous cycle length then oil mice. Short-term administration of DHT significantly upregulated 23% (21 of 92) of transcripts in scWAT and downregulated 49% (45 of 92) of transcripts in vWAT. The top four canonical pathways identified by IPA in WAT were: T helper cell 1 (Th1), Th1 & T helper 2 activation, Helper T cell differentiation, and Altered B & T cell signaling. Based on the Th1 pathway derived from IPA, the following miRs (both -3p and 5p) downstream of Th1 activation targets were selected for qPCR in vWAT and scWAT: miR21, miR146a, miR29a, and miR155. Interestingly, miR-21a-5p, miR-146a-5p, and miR-155-5p were significantly upregulated in scWAT from DHT mice. No miRs were different between treatment groups in vWAT. We demonstrate for the first time that short-term DHT administration may cause immunosuppression in vWAT and inflammation, possibly mediated by miRs, in scWAT of female mice.

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Dietary Coconut Oil Mitigates Hyperandrogenemia in Obese Female Pigs Due to Suppression of Androgen Steroidogenesis in the Adrenal Cortex and