at each phase of the menstrual cycle. However, microbiome differences amongst the three phases of the menstrual cycle were not significant in any of the treatment groups: T+CD (0.176), C+WSD (0.107) and T+WSD group (p=0.278).

CONCLUSIONS: Treatment with chronic T, in both the absence and presence of WSD consumption, alters the community structure and function of the gut and lower reproductive tract microbiomes throughout the menstrual cycle. All variation between phases of the menstrual cycle was lost in each of the treatment cohorts (T+CD, C+WSD, and T+WSD), suggesting a potential interaction between the menstrual phase microbiome, hyperandrogenism, diet, and reproductive outcomes.

IMPACT STATEMENT: In primates, WSD feeding in combination with hyperandrogenemia is associated with menstrual cycle-specific alterations to the cervico-vaginal microbiome. These differences may directly contribute to the variation in fertility outcomes in PCOS patients. Given the independent involvement of WSD feeding, which is modifiable, there is the potential to modulate PCOS reproductive outcomes with beneficial dietary change.

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EVIDENCE FOR DIFFERENTIAL EFFECTS OF Δ9-THC (Δ9-TETRAHYDROCANNABINOL) ON PRE- VERSUS POST-IMPLANTATION EMBRYONIC STEM CELLS. Abigail Anne Armstrong, M.D.,[1] Gurugowtham Ulaganathan, B.S.,[2] Roxane Verdikt, PhD,[2] Patrick Allard, PhD[2] [1]University of California, Los Angeles; [2]University of California at Los Angeles.

OBJECTIVE: Recreational marijuana use is becoming increasingly widespread. With potential implications for fertility and embryonic development, we set out to evaluate the impact of THC exposure on the pre- and post-implantation embryo through embryonic stem cell proliferation, mitochondrial function and metabolism.

MATERIALS AND METHODS: Mouse embryonic stem cells (ESCs) can be differentiated into epiblast-like cells (EpiLCs) using known in vitro methods via growth factors and cytokines. ESCs recapitulate important features of pre-implantation stem cells; EpiLCs model post-implantation features. ESCs and EpiLCs were exposed to a range of Δ9-THC concentrations: 0-100μM over 48 hours. Cell proliferation and viability were assessed via Trypan blue and Countess II FL Automated Cell Counter. The cells were exposed to WST-1 and analyzed by ELISA reader to measure wavelength absorption as a proxy for oxidative phosphorylation. The live cells were stained with a MitoTracker dye to label the mitochondria. Changes in membrane potential were measured whereby increases in membrane potential represented mitochondrial disruption and early stages of cell apoptosis. Comparative analyses between the untreated versus treated cells were performed with unpaired t-tests.

RESULTS: In response to 10nM and 100nM Δ9-THC concentrations, ESCs demonstrated a biphasic dose-dependent proliferation response: low concentration THC increased proliferation, whereas high-dose THC increased apoptosis. These effects were not observed in EpiLCs. We demonstrated these biphasic results are due in part to differential effects on membrane potential and oxidative phosphorylation.

CONCLUSIONS: Compared to untreated cells, Δ9-THC at low doses significantly increases proliferation in ESCs but not EpiLCs. We demonstrate early evidence for metabolic differences underlying this unique proliferation dependence. Given ESCs represent pre-implantation cells, while EpiLCs model post-implantation cells, these data suggest an intriguing hypothesis that implantation may trigger a shift in endocannabinoid metabolism. Future work will be addressed toward clarifying these effects.

IMPACT STATEMENT: Although the impact of Δ9-THC on early developmental differentiation and programming is unknown, it is possible that Δ9-THC exposure may disrupt the metabolism and epigenetic machinery in germ cells. Given that cannabis is the most widely illicit drug used in the world, with increasing consumption in women of reproductive age, our work is relevant to understanding the basic action of cannabis on embryonic stem cells. Future research should investigate the transgenerational effect of marijuana use from *in utero* exposure.

SUPPORT: None

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TESTOSTERONE TREATMENT NEGATIVELY IMPACTS THE REPRODUCTIVE POTENTIAL OF FIRST GENERATION FEMALE OFFSPRING CONCEIVED BY IN VITRO FERTILIZATION. Amanda R. Schwartz, MD,[1] Min Xu, PhD,[1] Cynthia Dela Cruz, PhD,[2] Daniel Pfau, PhD,[2] Vasantha Padmanabhan, MS, PhD,[2] Ariella Shikanov, PhD,[2] Molly B. Moravek, MD, MPH[1] [1]University of Michigan, Ann Arbor; [2]University of Michigan, Ann Arbor, MI.

OBJECTIVE: The objective of this study was to examine in vitro fertilization (IVF) outcomes of first generation offspring conceived from oocytes with long-term testosterone (T) exposure versus control. We hypothesized that there would be no difference in outcomes.

MATERIALS AND METHODS: C57BL/6N female mice were implanted with silastic tubing with either 10 mg of T enthanate in ethanol (n = 9) or ethanol alone (n = 10) at 10 weeks. At 12-weeks post implantation, mice underwent ovarian stimulation with 0.2 mL intraperitoneal CARD HyperOva followed 48 hours later by 7.5 international units (IU) of intraperitoneal human chorionic gonadotropin (hCG) with collection of oocytes from oviducts at 14 hours post hCG. Oocytes were fertilized and cultured to two-cell embryos, which were transferred into the oviducts of pseudopregnant recipient females to obtain first generation offspring. At 8 weeks, female offspring (n = 10) were stimulated by the same protocol with oocytes fertilized and cultured to blastocyst. Offspring were sacrificed for oocyte collection and terminal blood collected. For male offspring (n = 8), sperm was retrieved at 12-weeks and used to fertilize oocytes from 6-week female mice via a split fertilization method. Data were analyzed using Chi squared and unpaired t-tests with Prism 9.0.

RESULTS: Female offspring conceived with oocytes from T-treated mice had fewer oocytes retrieved (51.80 vs 63.00; p = 0.035), mature oocytes (22.80 vs 30.00; p = 0.050), 2 cell embryos (22.60 vs 30.00; p = 0.046), 4-8 cell embryos (22.40 vs 30.00; p = 0.043), morulas (22.20 vs 29.60; p = 0.036) and blastocysts (19.60 vs 27.00; p = 0.031) as compared to control offspring. There was no difference in individual ovarian weight (p = 0.061), maturity rate (p = 0.466), fertilization rate (p = 0.250) or hatching rate (p = 0.723). Female offspring from T-exposed oocytes had lower terminal antimullerian hormone (180.2 vs 236.6; p = 0.022) and progesterone (31.85 vs 56.05; p = 0.015) with no difference in terminal estradiol (54.42 vs 38.72; p = 0.110) or T levels (24.93 vs 32.06; p = 0.167). First generation male offspring from T-treated oocytes had no difference in fertilization rate (84.46 vs 87.55; p = 0.763), blastulation rate (71.71 vs 79.11; p = 0.558) or hatching rate (41.87 vs 46.15; p = 0.683) as compared to controls. Additionally, there was no difference in individual testis weight (p = 0.085), sperm concentration (p = 0.086) or sperm motility (p = 0.607) between male offspring from T-treated oocytes versus controls.

CONCLUSIONS: In a mouse model of gender-affirming hormone treatment, testosterone exposure had a detrimental impact on female offspring IVF outcomes with no change noted in male offspring.

IMPACT STATEMENT: Reproductive consequences of gender-affirming testosterone in transmasculine people are currently unknown with our mouse model suggesting a detrimental impact on female offspring. Further research is needed to determine whether these results are translatable to humans and whether effects are duration dependent or reversible with a period of testosterone cessation.

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***IN VIVO* AGE-RELATED CHANGES IN THE OVARY COINCIDE WITH WIDESPREAD REPERCUSSIONS ON GENE EXPRESSION UNIQUE TO THE SPECIFIC OVARIAN CELL POPULATIONS.** Mandy Katz-Jaffe, PhD,[1] Jennifer Russ, BS,[2] Mary E. Haywood, PhD,[2] William B. Schoolcraft, MD[3] [1]CCRM Genetics, Lone Tree, CO; [2]CCRM Research, Lone Tree, CO; [3]Colorado Center for Reproductive Medicine, Lone Tree, CO.

OBJECTIVE: Ovarian aging precedes that of any other mammalian organ and is the primary instigator of female age-related infertility. The biological mechanisms responsible for ovarian aging and subsequent reproductive