Title: Prenatal Androgen induced Lean PCOS impairs Mitochondria and mRNA profiles in Oocytes

Authors: Neil. R. Chappell, Beth Zhou, Amy K. Schutt, William E. Gibbons and Chellakkan S. Blesson*

Reproductive Endocrinology and Infertility Division, Department of Obstetrics and Gynecology, Baylor College of Medicine and Family Fertility Center, Texas Children’s Hospital, Houston 77030, Texas, USA.

Running Title: Lean PCOS impairs oocyte mitochondria

*Address all correspondence and requests for reprints to:

Chellakkan S. Blesson M.Phil., Ph.D.
Reproductive Endocrinology and Infertility Division,
Department of Obstetrics and Gynecology
Baylor College of Medicine
One Baylor Plaza
MS: BCM610
Houston, Texas 77030
USA.
Phone: +1 832-826-7462, Fax: +1 832-825-7910.
E. mail: selvanes@bcm.edu

Key Words: PCOS, Mitochondria, Oocytes, Glucose intolerance

Word Count: 3469
Abstract

Polycystic ovary syndrome (PCOS) is the most common ovulatory defect in women. Although most PCOS patients are obese, a subset of PCOS women are lean but show similar risks for adverse fertility outcomes. A lean PCOS mouse model was created using prenatal androgen administration. This developmentally programmed mouse model was used for this study. Our objective was to investigate if mitochondrial structure and functions were compromised in oocytes obtained from lean PCOS mouse. The lean PCOS mouse model was validated by performing glucose tolerance test, HbA1c levels, body weight and estrous cycle analyses. Oocytes were isolated and were used to investigate inner mitochondrial membrane potential, oxidative stress, lipid peroxidation, ATP production, mtDNA copy number, transcript abundance and electron microscopy. Our results demonstrate that lean PCOS mice has similar weight to that of the controls but exhibited glucose intolerance and hyperinsulinemia along with dysregulated estrus cycle. Analysis of their oocytes show impaired inner mitochondrial membrane function, elevated reactive oxygen species (ROS) and increased RNA transcript abundance. Electron microscopy of the oocytes showed impaired mitochondrial ultrastructure. In conclusion, the lean PCOS mouse model shows a decreased oocyte quality related to impaired mitochondrial ultrastructure and function.
Introduction

Polycystic ovary syndrome (PCOS) is the most common ovulatory disorder in the world, affecting 5-10% of women, translating to ~100 million women worldwide (1). PCOS is associated with increased obstetric risks including preeclampsia, gestational diabetes, preterm delivery and higher infant mortality (2). PCOS patients have higher miscarriage rates (3, 4), higher risk of ovarian hyperstimulation (OHSS) and increased risks in offspring born after assisted reproduction technology (ART) (5). Further, there are longstanding implications in chronic disease states such as hypertension, diabetes, depression, stroke and some cancers (2).

PCOS is a broad and complex disease, comprised of several aberrations in physiology leading to variable phenotypes. Diagnosis of PCOS is based on the Rotterdam criteria: polycystic ovarian morphology, irregular menses and clinical or laboratory evidence of hyperandrogenemia (6). At least two of these three criteria will qualify a patient for the diagnosis of PCOS provided routine workup excludes other etiologies (6). Up to 80% of PCOS patients are obese, and obesity is associated with a wide range of adverse events (7-9), many of which are closely associated with outcomes seen in PCOS literature (10, 11). This makes obesity the quintessential confounder in the study of PCOS. Recently, a subpopulation of PCOS patients that is not obese, coined “lean PCOS”, has elicited interest among researchers (12-17). This group comprises 20-30% of the PCOS population and provides the opportunity to look at PCOS independent of obesity.

Mouse models have long served as a useful platform to study PCOS, and several models have been described, including a “lean PCOS” mouse model via administration of androgens prenatally during the critical window of fetal oogenesis (18-23). This model has since been well characterized metabolically, and exhibits a PCOS-like phenotype with hyperandrogenemia, increased LH activity, irregular cyclicity and abnormal glucose/insulin tolerance while maintaining the same weight and BMI as the control population (18, 20, 21). Despite the strong foundational data describing the metabolic components of this model, research on the reproductive system of these animals, in particular the oocyte itself, remains sparse. Prior studies have shown impaired
mitochondrial function with hyperandrogenic environments in the oocyte to have negative effects on reproductive outcomes (24-29). PCOS women by their nature are hyperandrogenic and thus may undergo these alterations (30-32). Our hypothesis is that prenatal androgen administration results in a lean PCOS phenotype with impaired mitochondrial structure and function in the oocytes.

**Materials and Methods**

All experiments were approved through the Institutional Animal Care and Use Committee of Baylor College of Medicine (AN-7156). To create the lean PCOS mouse model, 8 week old C57/Bl6 female mice (n=26) were mated with males of proven fertility. Copulatory plugs were visualized to confirm mating on the following morning, which was considered Day 0.5. Pregnant dams were injected with 250 µg of dihydrotestosterone (DHT, Sigma-Aldrich, MO, USA) prepared in sesame oil (Texas Lab Supply, Lubbock, TX, USA) or vehicle only on 16.5, 17.5 and 18.5 days post coitus. All male pups were culled at weaning, and female pups (n=30 controls and n=33 DHT) were used for the experiments. One pup each from different dams were used for each experiment and sisters were not used in the same experiments. From within each litter, pups were randomly chosen for different experiments.

Pups were weighed and measured from snout to anus in millimeters at 3, 4, 8, and 12 weeks up to euthanasia at 16 weeks to calculate their body mass index (BMI). Test for estrous cyclicity was performed at 3 months of age by observing the vaginal cytology using vaginal smears as previously described (33-35) for approximately 21 days, or 4 consecutive cycles. Glucose tolerance test (GTT) was performed 12 weeks old mice as in previously published mouse models (21, 36). Hemoglobin A1c levels were analyzed at the time of euthanasia at 16 weeks using the A1cNow+ test kit (PTS Diagnostics, Indianapolis, IN, USA) following the manufacturer’s instructions. Due to the sub-fertile nature of the animal model, in order to study the oocyte, it was necessary to perform superovulation to retrieve oocytes. The protocol is detailed in the Supplemental Materials.

Mitochondrial function was evaluated on oocytes by single cell imaging experiments using fluorescent probes and the images were analyzed using Image J software. Inner
mitochondrial membrane (IMM) potential was measured using JC-1 dye (Affymetrix, Santa Clara, CA), reactive oxygen species (ROS) formation was measured using CellRox Green (ThermoFisher, Waltham, MA), and lipid peroxidation was measured by using BODIPY (Life Technologies, Carlsbad, CA). Single cell imaging of oocytes for IMM, ROS and lipid peroxidation were performed using 2-5 oocytes/study/group from 5-8 mice.

ATP concentration was measured pooling 5-15 oocytes/mouse, using a luciferase assay kit (ThermoFisher, Waltham, MA). RNA transcript abundance was measured using qPCR. RNA was isolated and cDNA was amplified using 3-5 oocytes collected from each mouse. Genes were amplified using specific primers using cDNA library as templates. Genomic DNA was isolated and amplified to measure mitochondrial DNA copy number. During euthanasia, ovaries from 4 month old unstimulated mice in diestrus were collected and processed for transmission electron microscopy (TEM). The 'n' in each study corresponds to the number of mice used in each experiment. Detailed protocols utilized are described in the Supplemental Materials. Student’s t test, chi square and ANOVA were used where appropriate for all above measurements. p < 0.05 was considered statistically significant. The data were analyzed using GraphPad Prism version 6.0 (San Francisco, CA, USA).

Results:

Control and PCOS mouse showed similar body weights and BMI

The lean PCOS mouse model exhibited similar weights when compared to controls throughout the experimental period (Figure 1A). They did not show any difference at 16 weeks at the time of euthanasia (Control 22.48 ± 0.47 grams vs. Lean PCOS 23.67 ± 0.29 grams at 16 weeks, p=0.19). Further, there was no difference in body mass index (BMI) at 12 weeks between the two groups (Control 0.25 ± 0.007 g/cm² vs. Lean PCOS 0.26 ± 0.004 g/cm², p=0.22 Figure 1B).

Lean PCOS mice had irregular cycles
Lean PCOS mice displayed irregular cycles when compared to controls. The mean cycle length for the lean PCOS mice was longer than that of the controls, indicating a longer estrus cycles (Control 4.44 ± 0.15 days, n=12, vs. Lean PCOS 5.12 ± 0.25 days, p<0.05; Figure 1C). All control mice had regular estrus cycles (defined as 3-5 days per cycle) however, a significant proportion of lean PCOS mice had did not have regular cycles (Control 100% regular cycles vs. Lean PCOS 35% regular cycles, n=17, p<0.001; Figure 1D). The percentage of time of spent in the diestrus was calculated for each mouse, (days in diestrus/number of days monitored). For the control population, mice spent a similar time in diestrus with a small variation (37.2% ±2.6), while lean PCOS mice showed significant variations (37.1% ± 3.8) (Figure 1E) with days ranging from 2-10 as opposed to 3-5 days in controls (Figure 1F). Serum obtained at 4 months was used to measure anti-Mullerian hormone (AMH) levels using standard ELISA kits, and no differences were noted between the control group and the lean PCOS group (Control 68.4 ± 7.74 ng/ml vs. Lean PCOS 55.08 ± 7.8 ng/ml for AMH).

Lean PCOS mice were glucose intolerance but normal Hemoglobin A1c levels

Glucose values were consistently higher at 30 minutes (Control 13.5 ± 0.5 mmol/l, vs. Lean PCOS 15.5 ± 0.5 mmol/l, p<0.01), 60 minutes (Control 11.3 ± 0.3 mmol/l vs. Lean PCOS 13.2 ± 0.9 mmol/l, p<0.01), 120 minutes (Control 8.5 ± 0.3 mmol/l vs. Lean PCOS 10.2 ± 0.2 mmol/l, p<0.01) and 180 (Control 7.8 ± 0.2 mmol/l vs. Lean PCOS 9.7 ± 0.1 mmol/l, p<0.01) minutes during GTT (Figure 2A). Interestingly fasting glucose did not show any difference between the groups (Figure 2A). The lean PCOS mice exhibited glucose intolerance when compared to controls with an increase in the GTT area under the curve (AUC) demonstrating an overall increase in glucose intolerance in the lean PCOS group when compared to the controls (Figure 2B, Control 1891 ± 53.81 mmol/l*180 minutes vs. Lean PCOS 2121 ± 39.56 mmol/l*180 minutes, p<0.01). The lean PCOS group showed evidence of hyperinsulinemia at the 30 minutes (Figure 2C, Control 70.134 ± 9.68 pmol/l vs. Lean PCOS 114.7 ± 14.71 pmol/l, p<0.05) with a higher AUC compared to controls (Figure 2D, Control 10843 ± 1484 pmol/l*180 minutes vs. Lean PCOS 16462 ± 1261 pmol/l*180 minutes, p<0.05). However, there were no differences in the insulin levels in any other time points during the GTT. Further, there
was no difference in hemoglobin A1c between the lean PCOS group and controls (Control 4.3 ± 0.12% vs. Lean PCOS 4.46 ± 0.11%).

*Inner mitochondrial membrane potential was compromised in lean PCOS oocytes*

Changes in IMM potential were assessed in live oocytes obtained from controls and lean PCOS mouse using JC-1, a dye that fluoresces red when the inner mitochondrial membrane is charged and green when depolarized. JC-1 is a marker for overall mitochondrial health and function as this membrane potential is essential for the electron transport chain to be able to produce ATP (29, 37). Our analysis showed that the lean model exhibited a lower red to green ratio in oocytes collected, indicating a lower IMM potential (Control 1.62 ± 0.1 vs. Lean PCOS 1.23 ± 0.09, p<0.05; Figure 3A and 3B).

*Oocytes from lean PCOS mice exhibited increased ROS*

ROS was measured using CellRox Green which fluoresces when oxidized by reactive oxygen species (ROS), and thus the higher the fluorescent signal measured in relative fluorescent units (RFUs), the higher the amount of ROS present. In the lean PCOS model, we observed higher RFUs compared to controls indicating a higher concentration of ROS (control 9.62 ± 1.58 RFUs vs. lean PCOS 18.46 ± 3.25 RFUs, p<0.05; Figure 4A and 4B).

*Lipid peroxidation was similar between the control and PCOS oocytes*

Lipid peroxidation was measured using BODIPY, a dye used to measure lipid peroxidation. Mitochondria are a major site of lipid metabolism, and peroxidation of lipids in mitochondria has been used as a marker for mitochondrial function (25). We measured mitochondrial lipid peroxidation in oocytes using a fluorescent dye, BODIPY. We observed no differences between the control and lean PCOS oocytes (Control 13.8 ± 1.51 RFUs vs. Lean PCOS 15.41 ± 1.61 RFUs; Figure 5A and 5B).

*mtDNA copy number and ATP levels showed no differences*

Mitochondrial DNA copy number and ATP production were assessed in oocytes obtained from control and lean PCOS groups. Mitochondrial DNA copy number was
assessed by qPCR of a mitochondrial gene (mtCo1) compared with a somatic reference gene (tubulin). Our data shows that there were no difference noted in mtDNA copy number between the groups (control 1127 ± 707 vs. lean PCOS 1161 ± 572). ATP contents were measured from oocyte lysates and there was no difference between the groups (Control 21.08 ± 4.26 vs. lean PCOS 22.15 ± 4.66).

**Lean PCOS oocytes showed increased transcript abundance**

RNA transcript abundance was quantified using qPCR for nuclear and mitochondrial genes. Interestingly all the statistically significant differences showed increased abundance in lean PCOS group (Figure 6). qPCR was performed for 39 genes known to be involved in PCOS and reproduction. Our results showed that 5 nuclear genes and 7 mitochondrial genes were upregulated. Of the nuclear genes that were investigated, there was a significant increase (p<0.05) in gene transcripts involved in centrosome formation (Spast), folliculogenesis (Bmp15, Zp1, Zp3, Igfr1) and cell adhesion (Itgav6, Dnm1). Mitochondrial genes that were upregulated (p<0.05) included genes involved with complex 1 of the electron transport chain (mtNd1, mtNd6), and complex IV (mtCo1, mtCo2, mtCo3). The mitochondrial genes mtNd2, mtNd3, mtNd4, mtCytb, mtAtp8, and mtRnr2 in addition to the nuclear genes Opa1, Mfn2, Ect2, Atrx, Cep70, Tacc1, Pcm1, Gdf9, Nobox, Zmic1, Kat2b, Ppp2r1a, and Ppp2ca all showed a tendency to be more abundant, however did not reach statistical significance. Mitochondrial genes mtNd4l, mtNd5, mtAtp6, and mtRnr1 as well as nuclear genes Gja1 and Sfrp4 showed similar expression in both controls and lean PCOS groups, and Xrcc1 showed a trend in lower abundance that did not reach statistical significance (Data not shown). Complete list of primers used and their details are given in supplementary file.

**Lean PCOS oocytes showed compromised mitochondrial ultrastructure**

TEM images of mouse oocytes showed that mitochondria in the lean PCOS group were structurally abnormal when compared to the controls. The controls had well rounded mitochondria with cristae at equal intervals. Further, they also had normal looking electron dense materials inside the mitochondria. Almost all the mitochondria observed had uniform shape with normal ultrastructure. Interestingly, almost all the mitochondria in oocytes from lean PCOS group showed aberrant ultrastructure with swollen cristae.
Further, they were also severely vacuolated without any electron dense contents (Figure 7A and 7B).

**Discussion:**

The lean PCOS mouse model demonstrated characteristics consistent with lean PCOS patients seen in clinical practice, with metabolic abnormalities, irregular cyclicity and evidence of ovulatory dysfunction with no increase in body weight or BMI. Although we noted a slight increase (1.2 grams, ~5%) in the body weight of lean PCOS mice at 4 months, it did not reach statistical significance (p=0.19). This increase may have some possible impact, despite lack of statistical difference. Lean PCOS mice showed glucose intolerance and hyperinsulinemia during the GTT, suggesting compromised glucose metabolism as seen in the majority of lean PCOS patients (2, 38). Interestingly, there was no difference in hemoglobin A1c levels compared to controls, indicating a lack of persistent hyperglycemia in these mice. Rather, they showed compensatory hyperinsulinemia indicating that higher insulin secretion was able to maintain glucose homeostasis. Such compensatory increase in insulin levels are often observed in patients with metabolic diseases; however, if unchecked will ultimately progress to worsening insulin resistance and lead to pancreatic failure (39). Earlier studies have shown that this model has elevated testosterone levels and impaired estrogen feedback (18).

To the best of our knowledge, our study presents evidence for the first time that prenatal androgen administration affects oocyte mitochondrial structure and function. The critical window of oocyte development in a mouse begins around day 16-18 of in utero fetal development (the period which the DHT was administered in this model) and continues into neonatal life. Importantly, administration of DHT after this decisive window does not carry the same effects (40). In contrast, our study clearly shows that administration of DHT during this window of fetal development affects the mitochondrial structure and function of oocytes during adulthood. The oocyte is the largest cell in the body and mitochondria are present in abundance (27). A primordial oocyte will only have a handful of mitochondria, though throughout folliculogenesis, that number will increase to over 100,000 (27). After ovulation, this abruptly ceases, and there will be no changes in
mitochondrial abundance until blastulation (27, 41). Therefore, a healthy amount of functional oocyte mitochondria are essential in the formation and maintenance of a strong energy reserve for the early processes of embryogenesis from fertilization through the first cell divisions (42). Thus, the implications of impaired mitochondrial function at the level of the oocyte are clear from our findings, and may help to explain the adverse outcomes seen in the PCOS population.

One of the main functions of mitochondria is to generate energy in the form of ATP, which is done via the electron transport chain (ETC) (43). The ETC is responsible for pumping protons across the IMM to generate a transmembrane gradient which is then utilized by an ATPase enzyme to create ATP (37). As the membrane potential across the IMM decreases, the ETC’s function is compromised, and this has been shown to be a marker for poor mitochondrial function (37). Further, mitochondria are also integral in reactive oxygen species (ROS) formation and scavenging and regulation of apoptotic pathways (44). While a certain amount of ROS is considered normal in the oocyte, increased ROS is linked to poor reproductive outcomes via developmental arrest, apoptosis, and physical DNA damage (29, 43, 45, 46). Oocytes obtained from lean PCOS mice displayed compromised IMM potentials and increased ROS formation. Formation of ROS is tightly coupled to mitochondria; in fact, the mitochondrial genome is more susceptible to DNA damage from ROS than the nuclear genome (47). This is thought to act as a buffering mechanism whereby excessive amounts of ROS can result in mitochondrial DNA damage which triggers apoptotic pathways before nuclear damage can accumulate to a dangerous degree (47). Mechanisms by which the mitochondria control ROS formation have been postulated to involve scavenger activity, increased antioxidant production, and alterations in fission/fusion balance of mitochondrial number (43, 47, 48).

The lack of difference in mtDNA copy number and ATP concentration despite mitochondrial damage may be due to compensatory mechanisms in PCOS oocytes. Our recent study in androgen programmed neonatal ovaries showed similar mitochondrial damage possibly due to increased oxidative stress with increased basal ATP production (49). It is possible that persistent exposure to androgens later in life
may trigger alterations in mitochondrial replication that could result in differences in ATP concentration or mitochondrial copy number.

The persistence of the RNA transcripts seen in the lean PCOS mouse has exciting implications. Unlike most cells whose mRNA transcripts lasts for only a few hours (50), an oocyte transcribes mRNAs to last several days (50) from fertilization to blastulation and implantation (50). The longevity of these mRNA transcripts in this unique environment is thought be due to changes in the adenylation of the 3' tail of the mRNA, increased expression of binding proteins, inhibition of degradation pathways, and through the influence of miRNAs (50). Therefore, studies of RNA expression that would typically quantify gene expression in other cell lines, are more appropriately considered to be measuring quantities of RNA transcripts created prior to ovulation in the case of the oocyte (50). The majority of the genes tested in this lean model showed increased abundance of RNA transcripts in oocytes obtained from lean PCOS group suggesting a global difference in either transcription or stabilization of these transcripts (50). The mechanism for global increase in RNA transcript DHT exposed oocytes is not known. The stabilization and prolongation of mRNA half-life can be achieved by various mechanisms (50), or there may be altered expression of miRNA transcripts, which have been reported in PCOS cohorts (51). And finally, there may be increased storage of mRNA transcripts in PCOS. In fact, studies have shown increased expression of the gene GATA6 in PCOS, which is involved in mRNA storage in the oocyte (52).

KEGG analysis of the regulated genes indicate that oxidative phosphorylation (especially in Complex I and Complex IV of the ETC) is the major pathway involved in the dysregulation. One of the main functions of the mitochondria is energy generation via the ETC, and during folliculogenesis, the number of mitochondria increase exponentially. Therefore, decreased mRNA transcript abundance of proteins involved in the ETC may indicate compromised ETC function in these oocytes. Precisely how the programming affect the growth and maturation of the oocytes and embryos that may arise from these oocytes if fertilized is not yet understood. Although the mechanisms remain unknown, a prior study in neuronal tissue of mice exposed to androgen (DHEA) has shown impairment in Complex I of mitochondrial electron transport chain (26).
Other pathways involved included ovarian steroidogenesis, meiosis, and signaling via the AMPK and PI3kAkt pathways. Multiple genes involved in folliculogenesis including Bmp15 and Igfr1 were also increased, highlighting the dysregulation in oocyte maturation that is seen in PCOS. Interestingly, KEGG analysis also highlighted pathways involved in Parkinson’s disease and depression, the latter of which is well known to be associated with PCOS, but the role of these RNA transcripts in the oocyte with these disorders is unknown at this time (2).

Finally, as these changes in the oocyte mitochondria are transferred to the offspring, this may explain the inheritance pattern seen offspring of PCOS patients (53). PCOS is well known to be hereditary, though numerous studies investigating the genetic contributions to the disease have been unable to identify strong gene candidates to explain this pattern (54-56). Other alternative explanations may include alterations in mitochondrial DNA, increased cellular stress resulting in apoptosis, or perhaps via direct effects to the mitochondria itself (26, 29, 42). Furthermore, it stands to reason that the propagation of these mitochondria from a fertilized egg to a blastocyst and ultimately a live born offspring should result in abnormal mitochondrial function throughout the body. Recent studies in a rat PCOS model have noted mitochondrial dysfunction in the pancreas (57) and kidney (58), and human studies have now reported altered mitochondrial function in skeletal muscle (59) as well as follicular fluid and cumulus cells of PCOS patients (46).

In conclusion, our findings indicate that prenatal androgen programming affects mitochondrial function and structure in oocytes. It is likely that the prenatal administration of androgens during the window of fetal oogenesis affects the mitochondria in the fetal oocyte along with dysregulated transcript abundance.

**Declaration of Interest:** The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
**Funding:** This work was supported by training grants by the Department of Obstetrics and Gynecology, Baylor College of Medicine (N.C. and B.Z.) and R-01 research grant (Grant # DK114689) for CSB from National Institutes of Health.

**Acknowledgements:**

The authors would like to acknowledge the Department of Obstetrics and Gynecology at the Baylor College of Medicine for their support in this project. The authors would also like to acknowledge Dr. Ignatia Van den Veyver and Dr. JoAnne Richards for their input as well as the Baylor College of Medicine Integrative Microscopy Core and the Animal Facility for their assistance.

**References:**

1 Goodarzi MO, Dumesic DA, Chazenbalk G & Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. *Nat Rev Endocrinol* 2011 7 219-231. (doi.org//10.1038/nrendo.2010.217).

2 Palomba S, Santagni S, Falbo A & La Sala GB. Complications and challenges associated with polycystic ovary syndrome: current perspectives. *Int J Womens Health* 2015 7 745-763. (doi.org//10.2147/IJWH.S70314).

3 Liu L, Tong X, Jiang L, Li TC, Zhou F & Zhang S. A comparison of the miscarriage rate between women with and without polycystic ovarian syndrome undergoing IVF treatment. *Eur J Obstet Gynecol Reprod Biol* 2014 176 178-182. (doi.org//10.1016/j.ejogrb.2014.02.041).

4 Luo L, Gu F, Jie H, Ding C, Zhao Q, Wang Q & Zhou C. Early miscarriage rate in lean polycystic ovary syndrome women after euploid embryo transfer - a matched-pair study. *Reprod Biomed Online* 2017 (doi.org//10.1016/j.rbmo.2017.07.010).

5 Grigorescu V, Zhang Y, Kissin DM, Sauber-Schatz E, Sunderam M, Kirby RS, Diop H, McKane P & Jamieson DJ. Maternal characteristics and pregnancy outcomes after assisted reproductive technology by infertility diagnosis: ovulatory dysfunction versus tubal obstruction. *Fertil Steril* 2014 101 1019-1025. (doi.org//10.1016/j.fertnstert.2013.12.030).

6 Rotterdam EA-SPCWG. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004 81 19-25.
7 Broughton DE & Moley KH. Obesity and female infertility: potential mediators of obesity’s impact. *Fertil Steril* 2017 **107** 840-847. (doi.org/10.1016/j.fertnstert.2017.01.017).

8 Practice Committee of the American Society for Reproductive M. Obesity and reproduction: a committee opinion. *Fertil Steril* 2015 **104** 1116-1126. (doi.org/10.1016/j.fertnstert.2015.08.018).

9 Shah DK, Missmer SA, Berry KF, Racowksky C & Ginsburg ES. Effect of obesity on oocyte and embryo quality in women undergoing in vitro fertilization. *Obstet Gynecol* 2011 **118** 63-70. (doi.org/10.1097/AOG.0b013e31821fd360).

10 Bailey AP, Hawkins LK, Missmer SA, Correia KF & Yanushpolsky EH. Effect of body mass index on in vitro fertilization outcomes in women with polycystic ovary syndrome. *Am J Obstet Gynecol* 2014 **211** 163 e161-166. (doi.org/10.1016/j.ajog.2014.03.035).

11 Cardozo E, Pavone ME & Hirshfeld-Cytron JE. Metabolic syndrome and oocyte quality. *Trends Endocrinol Metab* 2011 **22** 103-109. (doi.org/10.1016/j.tem.2010.12.002).

12 Altuntas Y, Bilir M, Ucak S & Gundogdu S. Reactive hypoglycemia in lean young women with PCOS and correlations with insulin sensitivity and with beta cell function. *Eur J Obstet Gynecol Reprod Biol* 2005 **119** 198-205. (doi.org/10.1016/j.ejogrb.2004.07.038).

13 Caglar GS, Kahyaoglu I, Pabuccu R, Demirtas S & Seker R. Anti-Mullerian hormone and insulin resistance in classic phenotype lean PCOS. *Arch Gynecol Obstet* 2013 **288** 905-910. (doi.org/10.1007/s00404-013-2833-9).

14 Mario FM, do Amarante F, Toscani MK & Spritzer PM. Lean muscle mass in classic or ovulatory PCOS: association with central obesity and insulin resistance. *Exp Clin Endocrinol Diabetes* 2012 **120** 511-516. (doi.org/10.1055/s-0032-1309006).

15 Shumak SL. Even lean women with PCOS are insulin resistant. *BMJ* 2009 **338** b954. (doi.org/10.1136/bmj.b954).

16 Kriseman M, Mills C, Kovanci E, Sangi-Haghpeykar H & Gibbons W. Antimullerian hormone levels are inversely associated with body mass index (BMI) in women with polycystic ovary syndrome. *J Assist Reprod Genet* 2015 **32** 1313-1316. (doi.org/10.1007/s10815-015-0540-0).

17 McCormick B, Thomas M, Maxwell R, Williams D & Aubuchon M. Effects of polycystic ovarian syndrome on in vitro fertilization-embryo transfer outcomes are influenced by body mass index. *Fertil Steril* 2008 **90** 2304-2309. (doi.org/10.1016/j.fertnstert.2007.10.077).
18 Moore AM, Prescott M & Campbell RE. Estradiol negative and positive feedback in a prenatal androgen-induced mouse model of polycystic ovarian syndrome. *Endocrinology* 2013 **154** 796-806. (doi.org/10.1210/en.2012-1954).

19 Moore AM, Prescott M, Marshall CJ, Yip SH & Campbell RE. Enhancement of a robust arcuate GABAergic input to gonadotropin-releasing hormone neurons in a model of polycystic ovarian syndrome. *Proc Natl Acad Sci U S A* 2015 **112** 596-601. (doi.org/10.1073/pnas.1415038112).

20 Roland AV & Moenter SM. Reproductive neuroendocrine dysfunction in polycystic ovary syndrome: insight from animal models. *Front Neuroendocrinol* 2014 **35** 494-511. (doi.org/10.1016/j.yfrne.2014.04.002).

21 Roland AV, Nunemaker CS, Keller SR & Moenter SM. Prenatal androgen exposure programs metabolic dysfunction in female mice. *J Endocrinol* 2010 **207** 213-223. (doi.org/10.1677/JOE-10-0217).

22 Sullivan SD & Moenter SM. Prenatal androgens alter GABAergic drive to gonadotropin-releasing hormone neurons: implications for a common fertility disorder. *Proc Natl Acad Sci U S A* 2004 **101** 7129-7134. (doi.org/10.1073/pnas.0308058101).

23 Caldwell AS, Middleton LJ, Jimenez M, Desai R, McMahon AC, Allan CM, Handelsman DJ & Walters KA. Characterization of reproductive, metabolic, and endocrine features of polycystic ovary syndrome in female hyperandrogenic mouse models. *Endocrinology* 2014 **155** 3146-3159. (doi.org/10.1210/en.2014-1196).

24 Fragouli E, Spath K, Alfarawati S, Kaper F, Craig A, Michel CE, Kokocinski F, Cohen J, Munne S & Wells D. Altered levels of mitochondrial DNA are associated with female age, aneuploidy, and provide an independent measure of embryonic implantation potential. *PLoS Genet* 2015 **11** e1005241. (doi.org/10.1371/journal.pgen.1005241).

25 Huang Y, Yu Y, Gao J, Li R, Zhang C, Zhao H, Zhao Y & Qiao J. Impaired oocyte quality induced by dehydroepiandrosterone is partially rescued by metformin treatment. *PLoS One* 2015 **10** e0122370. (doi.org/10.1371/journal.pone.0122370).

26 Safiulina D, Peet N, Seppet E, Zharkovsky A & Kaasik A. Dehydroepiandrosterone inhibits complex I of the mitochondrial respiratory chain and is neurotoxic in vitro and in vivo at high concentrations. *Toxicol Sci* 2006 **93** 348-356. (doi.org/10.1093/toxsci/kfl064).

27 St John JC, Facucho-Oliveira J, Jiang Y, Kelly R & Salah R. Mitochondrial DNA transmission, replication and inheritance: a journey from the gamete through the embryo and into offspring and embryonic stem cells. *Hum Reprod Update* 2010 **16** 488-509. (doi.org/10.1093/humupd/dmq002).

28 Tarumi W, Tsukamoto S, Okutsu Y, Takahashi N, Horiuchi T, Itoh MT & Ishizuka B. Androstenedione induces abnormalities in morphology and function of developing
oocytes, which impairs oocyte meiotic competence. *Fertil Steril* 2012 97 469-476. (doi.org//10.1016/j.fertnstert.2011.11.040).

29 Van Blerkom J. Mitochondrial function in the human oocyte and embryo and their role in developmental competence. *Mitochondrion* 2011 11 797-813. (doi.org//10.1016/j.mito.2010.09.012).

30 Daan NM, Koster MP, Steegers-Theunissen RP, Eijkemans MJ & Fauser BC. Endocrine and cardiometabolic cord blood characteristics of offspring born to mothers with and without polycystic ovary syndrome. *Fertil Steril* 2017 107 261-268 e263. (doi.org//10.1016/j.fertnstert.2016.09.042).

31 Dumesic DA, Schramm RD, Peterson E, Paprocki AM, Zhou R & Abbott DH. Impaired developmental competence of oocytes in adult prenatally androgenized female rhesus monkeys undergoing gonadotropin stimulation for in vitro fertilization. *J Clin Endocrinol Metab* 2002 87 1111-1119. (doi.org//10.1210/jcem.87.3.8287).

32 Xita N & Tsatsoulis A. Review: fetal programming of polycystic ovary syndrome by androgen excess: evidence from experimental, clinical, and genetic association studies. *J Clin Endocrinol Metab* 2006 91 1660-1666. (doi.org//10.1210/jcem.2005-2757).

33 Byers SL, Wiles MV, Dunn SL & Taft RA. Mouse estrous cycle identification tool and images. *PLoS One* 2012 7 e35538. (doi.org//10.1371/journal.pone.0035538).

34 Nelson JF, Felicio LS, Randall PK, Sims C & Finch CE. A longitudinal study of estrous cyclicity in aging C57BL/6J mice: I. Cycle frequency, length and vaginal cytology. *Biol Reprod* 1982 27 327-339.

35 Caligioni CS. Assessing reproductive status/stages in mice. *Curr Protoc Neurosci* 2009 Appendix 4 Appendix 4I. (doi.org//10.1002/0471142301.nsa04is48).

36 Andrikopoulos S, Blair AR, Deluca N, Fam BC & Proietto J. Evaluating the glucose tolerance test in mice. *Am J Physiol Endocrinol Metab* 2008 295 E1323-1332. (doi.org//10.1152/ajpendo.90617.2008).

37 Seidler EA & Moley KH. Metabolic Determinants of Mitochondrial Function in Oocytes. *Semin Reprod Med* 2015 33 396-400. (doi.org//10.1055/s-0035-1567822).

38 Norman RJ, Dewailly D, Legro RS & Hickey TE. Polycystic ovary syndrome. *Lancet* 2007 370 685-697. (doi.org//10.1016/S0140-6736(07)61345-2).

39 Morciano A, Romani F, Sagnella F, Scarinci E, Palla C, Moro F, Tropea A, Policola C, Della Casa S, Guido M, et al. Assessment of insulin resistance in lean women with polycystic ovary syndrome. *Fertil Steril* 2014 102 250-256 e253. (doi.org//10.1016/j.fertnstert.2014.04.004).

40 Walters KA. Role of androgens in normal and pathological ovarian function. *Reproduction* 2015 149 R193-218. (doi.org//10.1530/REP-14-0517).
41 Meldrum DR, Casper RF, Diez-Juan A, Simon C, Domar AD & Frydman R. Aging and the environment affect gamete and embryo potential: can we intervene? *Fertil Steril* 2016 **105** 548-559. (doi.org/10.1016/j.fertnstert.2016.01.013).

42 Babayev E & Seli E. Oocyte mitochondrial function and reproduction. *Curr Opin Obstet Gynecol* 2015 **27** 175-181. (doi.org/10.1097/GCO.0000000000000164).

43 Takahashi M. Oxidative stress and redox regulation on in vitro development of mammalian embryos. *J Reprod Dev* 2012 **58** 1-9.

44 Vasconsuelo A, Pronsato L, Ronda AC, Boland R & Milanesi L. Role of 17beta-estradiol and testosterone in apoptosis. *Steroids* 2011 **76** 1223-1231. (doi.org/10.1016/j.steroids.2011.08.001).

45 Treidel LA, Whitley BN, Benowitz-Fredericks ZM & Haussmann MF. Prenatal exposure to testosterone impairs oxidative damage repair efficiency in the domestic chicken (Gallus gallus). *Biol Lett* 2013 **9** 20130684.

46 Zhao H, Zhao Y, Li T, Li M, Li J, Li R, Liu P, Yu Y & Qiao J. Metabolism alteration in follicular niche: The nexus among intermediary metabolism, mitochondrial function, and classic polycystic ovary syndrome. *Free Radic Biol Med* 2015 **86** 295-307. (doi.org/10.1016/j.freeradbiomed.2015.05.013).

47 Vasconsuelo A, Milanesi L & Boland R. Actions of 17beta-estradiol and testosterone in the mitochondria and their implications in aging. *Ageing Res Rev* 2013 **12** 907-917. (doi.org/10.1016/j.arr.2013.09.001).

48 Karamohamed S & Guidotti G. Bioluminometric method for real-time detection of ATPase activity. *Biotechniques* 2001 **31** 420-425.

49 Barsky M, Yang L, Hosseinzadeh P, Dunn JC, Gibbons WE & Blesson CS 2019 Fetal Programming of Polycystic Ovary Syndrome (PCOS): In Utero Androgen Exposure Alters Murine Prenatal Ovarian Mitochondrial Structure and Function. In *REPRODUCTIVE SCIENCES*, pp 282A-282A: SAGE PUBLICATIONS INC 2455 TELLER RD, THOUSAND OAKS, CA 91320 USA.

50 Sirard MA. Factors affecting oocyte and embryo transcriptomes. *Reprod Domest Anim* 2012 **47 Suppl 4** 148-155. (doi.org/10.1111/j.1439-0531.2012.02069.x).

51 Sorensen AE, Udesen PB, Wissing ML, Englund ALM & Dalggaard LT. MicroRNAs related to androgen metabolism and polycystic ovary syndrome. *Chem Biol Interact* 2016 **259** 8-16. (doi.org/10.1016/j.cbi.2016.06.008).

52 Wood JR, Ho CK, Nelson-Degrave VL, McAllister JM & Strauss JF, 3rd. The molecular signature of polycystic ovary syndrome (PCOS) theca cells defined by gene expression profiling. *J Reprod Immunol* 2004 **63** 51-60. (doi.org/10.1016/j.jri.2004.01.010).
53 Pan JX, Zhang JY, Ke ZH, Wang FF, Barry JA, Hardiman PJ & Qu F. Androgens as double-edged swords: Induction and suppression of follicular development. *Hormones (Athens)* 2015 **14** 190-200. (doi.org/10.14310/horm.2002.1580).

54 Hayes MG, Urbanek M, Ehrmann DA, Armstrong LL, Lee JY, Sisk R, Karaderi T, Barber TM, McCarthy MI, Franks S, et al. Genome-wide association of polycystic ovary syndrome implicates alterations in gonadotropin secretion in European ancestry populations. *Nat Commun* 2015 **6** 7502. (doi.org/10.1038/ncomms8502).

55 Liu H, Zhao H & Chen ZJ. Genome-Wide Association Studies for Polycystic Ovary Syndrome. *Semin Reprod Med* 2016 **34** 224-229. (doi.org/10.1055/s-0036-1585403).

56 Zhao H, Lv Y, Li L & Chen ZJ. Genetic Studies on Polycystic Ovary Syndrome. *Best Pract Res Clin Obstet Gynaecol* 2016 (doi.org/10.1016/j.bpobgyn.2016.04.002).

57 Wang H, Wang X, Zhu Y, Chen F, Sun Y & Han X. Increased androgen levels in rats impair glucose-stimulated insulin secretion through disruption of pancreatic beta cell mitochondrial function. *J Steroid Biochem Mol Biol* 2015 **154** 254-266. (doi.org/10.1016/j.jsbmb.2015.09.003).

58 Selen ES, Bolandnazar Z, Tonelli M, Butz DE, Haviland JA, Porter WP & Assadi-Porter FM. NMR Metabolomics Show Evidence for Mitochondrial Oxidative Stress in a Mouse Model of Polycystic Ovary Syndrome. *J Proteome Res* 2015 **14** 3284-3291. (doi.org/10.1021/acs.jproteome.5b00307).

59 Cree-Green M, Rahat H, Newcomer BR, Bergman BC, Brown MS, Coe GV, Newnes L, Garcia-Reyes Y, Bacon S, Thurston JE, et al. Insulin Resistance, Hyperinsulinemia, and Mitochondria Dysfunction in Nonobese Girls With Polycystic Ovarian Syndrome. *J Endocr Soc* 2017 **1** 931-944. (doi.org/10.1210/js.2017-00192).

---

**Legends:**

Figure 1. Lean PCOS mice displayed similar body weight (A) and BMI (B) when compared to controls. They also showed irregular cycles when compared to controls. The mean cycle length (C) in the lean PCOS group was longer, more mice exhibited irregular cycles (D), and had more variation in the overall time spent in diestrus (E), with a wider variation in the days spent per estrus cycle (F). (n=11-15 in Controls and 18-19 in Lean PCOS, *- p<0.05 and **- p<0.001)
Figure 2. The lean PCOS mouse model displayed glucose intolerance (A) with increased glucose area under the curve (AUC) (B). Additionally, the lean PCOS mouse model also demonstrated hyperinsulinemia (C) with elevated insulin AUC (D). (n=7-8 in Controls and n=8 Lean PCOS, *- p<0.05 and **- p<0.01)

Figure 3. Pictorial representation (A) of control (upper panel) and lean PCOS (lower panel) model. Phase contrast microscopy, followed by green fluorescence, red fluorescence, and merged fluorescent images using JC-1 dye at 60x magnification. The lean PCOS mice show a lower red to green ratio compared to controls (B) indicating compromised mitochondrial function. (n=5 in Controls and n=8 in Lean PCOS, *- p<0.05)

Figure 4. Pictorial representation (A) of control (upper panel) and lean PCOS (lower panel) model. Phase contrast microscopy, followed by green fluorescence using CellRox Green dye at 60x magnification. The lean PCOS mice have a higher mean RFU value indicating presence of higher reactive oxygen species concentration when compared to controls (B). (n=5 in Controls and n=6 in Lean PCOS, *- p<0.05)

Figure 5. Pictorial representation (A) of control (upper panel) and lean PCOS (lower panel) model. Phase contrast microscopy, followed by green fluorescence using BODIPY dye at 60x magnification. The lean PCOS mice have a similar mean RFU value when compared to controls indicating no differences in lipid peroxidation between the groups (B). (n=5 in Controls and n=5 in Lean PCOS)

Figure 6. RNA transcript abundance in the control group versus lean PCOS mouse model. (n=5-8, *p<0.05, **p<0.01)

Figure 7. Representative transmission electron microscopy imaging of control (A) and lean PCOS (B) mouse oocyte mitochondria. Oocytes from lean PCOS mouse show abnormal mitochondrial structure with swollen cristae (n=4 in each group).
Supplementary Materials

Glucose Tolerance Test:
Mice (12 weeks old) in diestrus were fasted for six hours in wire bottom cages. After 6 hours, baseline blood samples for blood glucose were collected from the saphenous vein using the Accuchek Nano Glucometer with Accuchek Smart View Test Strips (Roche, Switzerland). Following baseline measurements, a dose of 1 gram/kilogram glucose (Sigma-Aldrich, MO, USA) was given intra-peritoneally. This was marked as time zero. Further blood samples were obtained at 15, 30, 60, 120 and 180 minutes. Insulin levels were measured with serum collected at each time point in the GTT using a mouse insulin ELISA kit (Mercodia, Sweden) per manufacturer’s instructions.

Superovulation and Oocyte Retrieval:
Superovulation was performed using pregnant mare serum gonadotropin (PMSG, Fisher Scientific, Hampton, NH) and human chorionic gonadotropin (hCG, EMD Millipore, Germany). These were both diluted in sterile water and injected intraperitoneally. First, 10 IU of PMSG were injected, followed by 10 IU of hCG approximately 47-52 hours after the PMSG. Mice were euthanized by cervical dislocation under isoflurane anesthesia approximately 18 hours following the hCG injection.

Upon euthanization, the abdominal cavity was opened, and the uterine horns were identified. These were dissected in a cephalad fashion to isolate the distal portion of the uterine horn, the ovary, and the bursa. This section of the reproductive tract was removed and placed in approximately 1000 µl KSOM EmbryoMax media (Sigma, MO, USA) that had been pre-incubated at 37°C with 5% CO₂ and 6% O₂ conditions. The material was then removed and placed on a pre-incubated petri dish in 200 µl of KSOM. The ampulla was isolated under a microscope and was removed from the remainder of the tissue using sharp dissection and placed in a separate 100 µl droplet of KSOM. Here, gentle traction was applied to the ampulla until a micro-perforation was created, allowing the cumulus oophorous complex (COC) containing the superovulated oocytes...
to be released. These were gathered with a 135 µm stripper tip (Origio, Charlottesville, VA) and moved to a clean 100 µl droplet of KSOM. After collection of COCs from both ampullas, they were placed in 0.3 mg/mL hyaluronidase (Sigma, MO, USA) diluted in KSOM for under 30 seconds to denude to cumulus cells and isolate the oocytes. The oocytes were washed 3-5 times in KSOM. All immature oocytes were removed from analysis, and metaphase II (MII) oocytes were isolated and allocated for further study.

Mitochondrial Imaging Studies

- Inner Mitochondrial Membrane (IMM) Potential

Oocytes were isolated from each mouse as described above. For IMM, 1 mL of JC-1 dye was mixed daily from stock solution stored at -30°C by diluting 2 µl of stock into 998 µl of PBS, resulting in 2µM concentration of JC-1. Oocytes were incubated in 250 µl of dye for 30 minutes at 37°C, then rinsed in serial PBS washes. The oocytes were then placed on pre-labeled positively charged microscope slides (vWR, Sugarland, TX) in approximately 1 µl of PBS and covered with Prolonged Gold Antifade mounting agent (ThermoFisher, Waltham, MA) and cover slip. Oocytes were immediately visualized using fluorescent microscopy with 20x and 60x objectives using appropriate filters. By comparing the red to green fluorescence ratio, the strength of the IMM was quantified using Image J software.

- Reactive Oxygen Species (ROS)

For ROS measurement, 1 mL of CellRox Green dye was mixed daily from stock solution stored at -30°C by diluting 2 µl of stock into 998 µl of PBS. This would result in 5 µM concentration. Oocytes were incubated in 250 µl of dye for 30 minutes at 37°C, then rinsed in serial PBS washes. The oocytes were then placed on pre-labeled positively charged microscope slides (vWR, Sugarland, TX) in approximately 1 µl of PBS and covered with Prolonged Gold Antifade mounting agent (ThermoFisher, Waltham, MA) and cover slip. Oocytes were then immediately visualized using fluorescent microscopy with 20x and 60x objectives and quantified using Image J software.
Lipid Peroxidation

For lipid peroxidation measurement, 1 mL of BODIPY dye was also mixed daily from stock solution stored at -30°C by diluting 1 µl of stock into 999 µl of PBS. After isolation, oocytes were incubated in 4% paraformaldehyde solution (Sigma, MO, USA) for 45 minutes at 37°C. Following this step, oocytes were then placed in 250 µl of BODIPY dye for 1 hour at room temperature, then washed serially in PBS. Oocytes were then placed in approximately 1 µl of PBS on a pre-labeled microscope slide and covered with Prolonged Gold Antifade mounting agent (ThermoFisher, Waltham, MA). The oocytes were immediately visualized with a fluorescent microscope with 20x and 60x objectives. Measurement of fluorescence was performed using Image J software.

ATP Concentration:

For measurement of ATP concentration, 5-15 oocytes from a single mouse were pooled and placed in 45 µl lysis buffer (Promega, Madison, WI, USA), vortexed for 1 minute, then centrifuged at 12,000g for 10 minutes at 4°C. The supernatant was removed and stored in a 200 µl Eppendorf tube at -80°C. Upon collection of all ATP samples, samples were analyzed using an ATP kit utilizing a luciferase assay (ThermoFisher, Waltham, MA) per kit instructions. Standards used for this ELISA ranged from 0, 1, 2.5, 5, 10, 25, 50, and 100 µM, all samples were run in duplicate.

Gene Analysis:

For the collection of DNA and cDNA, three to five oocytes were collected from each mouse and underwent whole genome amplification. For DNA, the Repli-G Single Cell Kit (Qiagen, Germany) was used. The DNA libraries were stored at -80°C until further use. For the creation of cDNA libraries, the WTA Single Cell Kit (Qiagen, USA) was utilized. The cDNA library was stored at -80°C until subsequent use.

- Mitochondrial DNA Copy Number
Mitochondrial DNA copy number was assessed using the DNA library created as described above. Real time quantitative PCR (rt-PCR) was performed with 1:500 diluted samples. Reaction mix was prepared fresh daily, and 8 µl was added to each well. Following this, 2 µl of diluted DNA was added. Incubation steps of the protocol were preprogrammed on a thermal cycler. All samples were run in duplicate. For comparison of mitochondrial DNA, a somatic gene (tubulin) was compared with a mitochondrial gene (mtCo1) as previously described [58].

- RNA Transcript Abundance

RNA transcript abundance was also measured using real time qPCR, again with diluted samples of cDNA of 1:500. Reaction mix was prepared daily fresh, 8 µl of this mix was added to each well, followed by 2 µl of cDNA sample. Incubation steps of the protocol were preprogrammed on a thermal cycler. Samples were run in duplicate, and GAPDH was used as a housekeeping gene.

*Transmission Electron Microscopy:*

Electron microscopy imaging was obtained using the contralateral ovary from each mouse described above. The ovaries were placed in a drop of cold primary fix containing 2.5% glutaraldehyde in 0.1M cacodylate buffer to a pH of 7.4 at 4°C. The ovary was then washed in buffer for fifteen minutes 3 times and placed in 1% OsO4 in 0.1M cacodylate for 45 minutes at 4°C. Following this, the specimens were washed for fifteen minutes 3 times in distilled water, then 50% ethanol twice, 70% ethanol twice, and finally 100% ethanol three times for 20 minutes each. The specimens were then incubated with 2 parts 100% ethanol to one part resin for one hour, then one part 100% ethanol to one part resin overnight, then by one part 100% ethanol to three parts resin for one hour, and finally by pure resin with a catalyst for four hours. The ovaries were then embedded in fresh resin and cured at 62°C for two days. These blocks were used to prepare slides for light microscopy to identify oocytes in the ovarian stroma. Once identified, the tissue block was cut with an ultramicrotome at 50 nanometers and the thin sections were placed on a copper grid and stained with heavy metals to be viewed via free access.
under the electron beam of a transmission electron microscope. Images of individual oocytes from each specimen were captured at 2500x, 6000x, 15000x, and 25000x.

**Primers Used:**

| Gene   | Gene ID  | Sequence ID   | Forward 5'-3'          | Reverse 5'-3'          |
|--------|----------|---------------|------------------------|------------------------|
| Gdf9   | 14566    | NM_008110.2   | GGCAGTCTCTTCTAGTCTCC   | GGGGAGATCTTTCAGACCT    |
| Bmp15  | 12155    | NM_009757.5   | ACAGTGTACCTAGCCTTCC    | ACAGGACTGGAAGTACATCCC  |
| Ikbkb  | 16150    | NM_010546.2   | TTCCAGTGGAGAAATGTTGG   | GCCATGCGGTTTCTACCTTAT |
| Itga1  | 109700   | NM_008397.4   | GACTCTCAACTGACGCTCA    | GTGCTGTCTCCAAACTCGGA   |
| Itga4  | 16401    | NM_008402.3   | ACTGCCGGAGATACACAGAG   | CAGACCGGAAAGTGAACAT    |
| Itgax  | 16411    | NM_021334.2   | GGCCATGACCGGAGATACAC   | CAGACGGGAAAGTGAACAT    |
| Pdgfra | 18595    | NM_011058.3   | GCCATGCCACCAGTGAAGTCTAT| CGGCAACAGGTTCTACCTAG   |
| Pgr    | 18667    | NM_008829.2   | GCCATGCCACCAGTGAAGTCTAT| CGGCAACAGGTTCTACCTAG   |
| Ppp2ca | 19052    | NM_010288.3   | TGATTTCCCTGACGACAGCC   | ATCTCCAGTCTACGAGCC     |
| Gja1   | 14609    | NM_010546.2   | TTCCAGCTGAGGAAATGTTGG  | GCCATGCGGTTTCTACCTTAT |
| Egfr   | 13649    | NM_007912.4   | CAACATCCTGGAGGGGAAC    | TGTATCACTGAGGCAACTGCT  |
| Tuba1b | 22143    | NM_011654.2   | TGTTGTGGTCTTCTGGAAG    | GCCATGCGGTTTCTACCTTAT |
| Tuba1c | 22146    | NM_009484.5   | TGTTGTGGTCTTCTGGAAG    | GCCATGCGGTTTCTACCTTAT |
| Lhcg   | 18687    | NM_013582.2   | TTGCTGAGCAGATTCTTGGTC  | AGCTTGGGAAGCTTACTTG    |
| Oxtr   | 18430    | NM_00110814.7 | TCTTCCATGCTGTCTTGAGACGAG | TACTCTGAGGAGCTTACTTG  |
| Pgrmc1 | 53328    | NM_016783.4   | TTGCCACCTCAGGACTTGGACGAG | AGATATGCTCCAGGACATGCT  |
| Plz1   | 114875   | NM_054066.4   | GCCGAAGTCCAGGATTAGTA   | GCTTGGGAACTCAGAAGACCT  |
| Sfrp4  | 20379    | NM_016687.3   | GCCCAAAAAGTCTAAGACCT   | AGTGGTGCTTACGGCACGAG   |
| Zmiz1  | 328365   | NM_01310666.1 | TGCAAGGAAATCTACGACCAC  | AGAGGCCCCACTGAGTGA     |
| Amhr2  | 110542   | NM_01356571.5 | CCTGATGAGGCAAGACTGTCTCA| ACGCAACAGAAATGGAACAG   |
| Kat2b  | 18519    | NM_009484.5   | CTACAAATGCGCCAGCATC    | ACGCAACAGAAATGGAACAG   |
| Xrcc1  | 22594    | NM_009532.4   | AACTTTGAGGAGGCCCTGAT   | GTGCTGAGGGAAGTACTTG    |
| Ppp2r1a| 51792    | NM_016891.3   | GCTGGGCCAAGAGATCTTCTCA| ACGCAACAGAAATGGAACAG   |
| Nbl    | 18291    | NM_0130869.3  | GCCCATGACAGGAGTCTGG    | ACGCAACAGAAATGGAACAG   |
| Ifg1f1 | 16000    | NM_001111274.1| TACTTCAACAACGACCAGAC   | ATAGAGCCGCTGCTTTGTTT   |
| Ifg1r1 | 16001    | NM_010513.2   | TACAGACAGAACGCAACAG    | TGTCTTACGGCAGGACAG     |
| Ecd2   | 13605    | NM_01177626.1 | GCAACATCCTCAGGACTGTA   | GTGCTGAGGGAAGTACTTG    |
| Eml1   | 68519    | NM_001043335.1| TCAAGTGCCGGGACTGCTA    | GGGACTAATGACTCGGAC     |
| Diaph1 | 54004    | NM_172493.2   | AATCCGACATAACGGACCCG   | ATGCTCCATCTCAGCGGCG    |
| Atrx   | 22589    | NM_009532.4   | TGTACAACTGTCTTCCGAGCA  | GCTTGGGACATGAGGACTGCT  |
| Nbn    | 27354    | NM_013562.3   | GCCAGATCTCTGAGAAGTGA   | TGTCTTACGAGAGCAGCAG    |
| Cep70  | 68121    | NM_023873.3   | ATCTTAGGATCTGAGCCTGAG  | CTTTGCTCAAGAGCAGCGCT   |
| Fgfr1-1| 14182    | NM_001079909.2| TGCAGAACAACATCCTGGAG   | TGGGAGGAGATCTACCATCT   |
| Bmp8a  | 12163    | NM_007558.3   | CCAATGCCATCTTGAGCTC    | ACCGCACTGATGAGTGGT    |
| Fnr2   | 18805    | NM_010892.3   | GCTAACAAGGAGGACGCTAG   | GCCACACACAAAGGCTTCC   |
| Nek4   | 23955    | NM_01308328.1 | CACTTCCGGAGGAAAGGAG    | ACTTCTCCCTGCTGGAACTC   |
| Pom1   | 18536    | NM_023662.3   | TGGGAGGAGATCTACCATCT   | GCCACACACAAAGGCTTCC   |
| Spast  | 50850    | NM_016926.2   | GCTGGTCCATCTGAGCAGT    | CTGCGAGACATGATGAGATC   |
| Tacc1  | 320165   | NM_199233.3   | CAGAGGAGGAGCTGCTCTCC   | TCTTCCAGCTTGGAGATC     |
| Opa1   | 7414     | NM_133752.3   | ACAAAGACTGAGTCTGAGGAG  | CTGCGAGACATGATGAGATC   |
| Dnm1l  | 74006    | NM_152816.3   | GATGTGCCAGTTCCAGTGC    | CTTTCCAGCTTGAGATC     |
| Mfn1   | 67414    | NM_024200.4   | TCTGAGTCTCAGGACTGCTCC  | CTGCGAGACATGATGAGATC   |
| Mfn2   | 170731   | NM_001285921.1| GCCAGGTTTGGGAATCAGC    | TGTCCAGACAATTCTCCTG    |
| Gene   | Accession | Transcript ID       | Forward Primer                     | Reverse Primer                     |
|--------|-----------|---------------------|------------------------------------|------------------------------------|
| Gapdh  | 14433     | NM_008084.3         | AAGCTCATTTCCTGGTATGACAA            | TGGGATAGGGCCTCTCTGTC               |
| Ptgs1  | 19224     | NM_008969.4         | GCCCCTATGTTTCTTCTTGGT             | GGAACAAACTCCTCCTCCCTCCCA           |
| Ptgs2  | 19225     | NM_011198.4         | TGGGACATGGGATGAGCTT               | GGGGATACACCTCCCTCCCA               |
| Zp1    | 22786     | NM_009580.2         | ACTTGAGCCCTCAAGTTCCA              | GCCCCAGATCAGACCCACAAA              |
| Zp2    | 22787     | NM_011775.7         | GACATCAGGAGCCAAACG               | TGGGGTCACACCTTTGGTGTG             |
| Zp3    | 22788     | NM_011776.1         | CCAGAGTGGTGGTTCCAGTA              | CGGGGTCTGATGACTGGGA               |
| mt-ND1 | 17716     | NC_005089.1         | TCCGAGCATCTTTATCCACGC             | GTATGGTGATGTCCCGCTG               |
| mt-ND2 | 17717     | NC_005089.1         | ATCCCTCTGGCCATCTGACT              | ATCAGAACTGGGAATGGGCG              |
| mt-ND3 | 17718     | NC_005089.1         | ACAAGCTCTGGACGTCTCTAC             | TGCTCATGGTAGGAGTGAAGTAAGA          |
| mt-ND4 | 17719     | NC_005089.1         | TAATGCGACATGGCCCTCAC              | CATTGGAATGCCTCCGGGCA              |
| mt-ND4L| 17720     | NC_005089.1         | CCAATACAATCCCTACCACACC           | CGTAATCCTGCTCTTGGTTG               |
| mt-ND5 | 17721     | NC_005089.1         | TAGCCCTGGCCAGAGCAAAG              | GTATTTCGGTGAGGCCGAGG              |
| mt-ND6 | 17722     | NC_005089.1         | CCGCAAAACAAAGTACCACCCA           | CTGATGTTGAGGGGAGTTG               |
| MT-Cytb| 17711     | NC_005089.1         | TGCATAATGGCATCTTGGCT              | AGGCTTGGTGTGTGAGGT                |
| mt-Co1  | 17708    | NC_005089.1        | TGGCTATCTAGGCTGTGTC               | CCGGGTAGACCACAAACTGT              |
| mt-Co2  | 17709    | NC_005089.1        | TGGCTATCTAGGCTGTGTC               | CCGGGTAGACCACAAACTGT              |
| mt-Co3  | 17710    | NC_005089.1        | CAAATGGCCACACACTCTAT              | GTCAGGACCTCCTAGTCA                |
| mt-ATP6 | 17705    | NC_005089.1        | CAAATGGCCACACACTCTAT              | GTCAGGACCTCCTAGTCA                |
| mt-ATP8 | 17706    | NC_005089.1        | CAAATGGCCACACACTCTAT              | GTCAGGACCTCCTAGTCA                |
| mt-Rnr1 | 17724    | NC_005089.1        | TCGGGCTAAAACGTCGTCAC              | TGGGTTATGTTGATGAGGC               |
| mt-Rnr2 | 17725    | NC_005089.1        | TCGGGCTAAAACGTCGTCAC              | TGGGTTATGTTGATGAGGC               |

Copyright © 2019 the authors

Downloaded from Bioscientifica.com at 02/28/2020 02:30:54PM via free access
Figure 1. Lean PCOS mice displayed similar body weight (A) and BMI (B) when compared to controls. They also showed irregular cycles when compared to controls. The mean cycle length (C) in the lean PCOS group was longer, more mice exhibited irregular cycles (D), and had more variation in the overall time spent in diestrus (E), with a wider variation in the days spent per estrus cycle (F). (n=11-15 in Controls and 18-19 in Lean PCOS, *- p<0.05 and ***- p<0.001)
Figure 2. The lean PCOS mouse model displayed glucose intolerance (A) with increased glucose AUC (B). Additionally, the lean PCOS mouse model also demonstrated hyperinsulinemia (C) with elevated insulin AUC (D). (n=7-8 in Controls and n=8 Lean PCOS, * - p<0.05 and ** - p<0.01)
Figure 3. Pictorial representation (A) of control (upper panel) and lean PCOS (lower panel) model. Phase contrast microscopy, followed by green fluorescence, red fluorescence, and merged fluorescent images using JC-1 dye at 60x magnification. The lean PCOS mice show a lower red to green ratio compared to controls (B) indicating compromised mitochondrial function. (n=5 in Controls and n=8 in Lean PCOS, *- p<0.05)
Figure 4. Pictorial representation (A) of control (upper panel) and lean PCOS (lower panel) model. Phase contrast microscopy, followed by green fluorescence using CellROX Green dye at 60x magnification. The lean PCOS mice have a higher mean RFU value indicating presence of higher reactive oxygen species concentration when compared to controls (B). (n=5 in Controls and n=6 in Lean PCOS, *- p<0.05)
Figure 5. Pictorial representation (A) of control (upper panel) and lean PCOS (lower panel) model. Phase contrast microscopy, followed by green fluorescence using BODIPY dye at 60x magnification. The lean PCOS mice have a similar mean RFU value when compared to controls indicating no differences in lipid peroxidation between the groups (B). (n=5 in Controls and n=5 in Lean PCOS)
Figure 6. RNA transcript abundance in the control group versus lean PCOS mouse model. (n=5-8, *p<0.05, **p<0.01)

100x46mm (300 x 300 DPI)
Figure 7. Representative transmission electron microscopy imaging of control (A) and lean PCOS (B) mouse oocyte mitochondria. Oocytes from lean PCOS mouse show abnormal mitochondrial structure with swollen cristae (n=4 in each group).