

in these women circulating and ovarian levels of *H19* are increased as compared to controls.

IMPACT STATEMENT: Research into novel genetic targets which can be harnessed for the diagnosis and treatment of PCOS is critical. While further study is needed, our findings that loss of *H19* may disrupt androgen production via *Cyp17*, and that elevated circulating and local *H19* are associated with hyperandrogenic PCOS, may implicate *H19* as a novel diagnostic and therapeutic target for this life-altering condition.

References

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O-198 12:00 PM Wednesday, October 20, 2021

GRANULOSA CELLS OF WOMEN WITH PCOS WITH OR WITHOUT INSULIN RESISTANCE DISPLAY SIGNS OF METABOLIC DISTRESS.

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OBJECTIVE: PCOS is diagnosed based on the presence of oligo/anovulation, hyperandrogenemia/hyperandrogenism, and polycystic ovaries. In addition, many women with PCOS have hyperinsulinemia and insulin resistance (IR), which are associated with significant cellular metabolic abnormalities. In this study, we investigated whether metabolic dysfunction in women with PCOS induces granulosa cell stress and causes activation of endoplasmic reticulum and mitochondrial unfolded protein response (UPR^{er} and UPR^{mt}).

MATERIALS AND METHODS: Women with PCOS diagnosed based on the Rotterdam criteria were included in the study and divided into two groups: PCOS with insulin resistance (PCOS-IR) when HOMA index was ≥ 2 (n=20) and PCOS with no insulin resistance (PCOS-nIR) when HOMA index was < 2 (n= 20). In addition, a healthy control group (CONT) included women undergoing IVF as oocyte donors (n=20). Granulosa cells (GCs) were collected on the day of oocyte retrieval and total RNA was isolated. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed to determine expression of UPR^{er} genes *BIP*, *ATF4*, *ATF6*, *IRE1*, *CHOP*, *XBPI* and UPR^{mt} genes *HSP60*, *HSP10*, *CLPP*, *HSP40*. ANOVA, student's t-test, and Chi-Square analysis were used for statistical analyses.

RESULTS: Women with PCOS-IR and PCOS-nIR were older than the CONT group (30.65 \pm 5.25 and 29.94 \pm 6.2 vs 25.12 \pm 2.26 years, respectively). Body mass index (BMI) of women in the PCOS-IR group was higher (27.64 \pm 6.92) compared to both the PCOS-nIR (24.58 \pm 3.64; p = 0.032) and CONT (23.76 \pm 1.86; p = 0.012). In the GCs of women with PCOS (both -IR and -nIR), UPR^{er} and UPR^{mt} genes were up-regulated compared to the CONT group. Among the genes that regulate UPR^{er}, chaperone proteins *HSP10* and *HSP40*, involved in the folding of proteins transported into the mitochondria were upregulated in the PCOS-IR (p < 0.01 and p < 0.001) and PCOS-nIR (p < 0.05 and p < 0.05) groups compared to CONT, while no difference in the expression of *CLPP* and *HSP60* was found. UPR^{er}-related transcription factors *ATF4*, *IRE1*, and *XBPI*, activated by stress in the endoplasmic reticulum, were significantly increased in GCs of PCOS-IR compared with CONT (p < 0.05 for all), while *IRE1* was increased in nIR-PCOS (p < 0.05). The chaperone *BIP*, involved in the protein folding in the endoplasmic reticulum, was specifically increased in PCOS-IR compared to CONT and PCOS-nIR (p < 0.05). No difference was found in *ATF6* gene expression. The transcription factor C/EBP homologous protein

(CHOP), involved in apoptosis initiation in response to organelle stress, was overexpressed in both PCOS-IR and PCOS-nIR (p < 0.05) compared to CONT.

CONCLUSIONS: Granulosa cells of women with PCOS with or without insulin resistance display signs of metabolic distress and up-regulation of UPR^{er} and UPR^{mt} genes.

IMPACT STATEMENT: Endoplasmic reticulum and mitochondrial stress responses are activated in the granulosa cells of women with PCOS. Our study provides novel mechanistic insights for pathological changes that take place in the follicular environment of women with PCOS.

ART LAB 2

O-199 10:45 AM Wednesday, October 20, 2021

COULD THE EMA ARTIFICIAL NEURAL NETWORK GRADE BLASTOCYSTS AS AN EMBRYOLOGIST?

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OBJECTIVE: To validate the accuracy of the EMA automated embryo grading system compared to senior embryologists.

MATERIALS AND METHODS: Time-lapse sequences of 10,000 individual blastocysts were graded by 20 senior embryologists according to ASE-BIR criteria (based on the morphology of the inner cell mass and trophectoderm, which defined 3 categories of viable blastocyst from A to C), and subsequently used to train artificial neural network (ANN) model. This ANN model was used to develop the EMA automated multi-class blastocyst grading method. The ANN EMA model was trained to distinguish between grades of blastocysts; A, B and C. After training on 8,000 sequences, the model was tested on 2,000 sequences, to determine accuracy, sensitivity and specificity values. The area under the receiver operating characteristic (ROC) curves (AUC) was used to calculate performance evaluations.

RESULTS: The accuracy, sensitivity and specificity values for the automated EMA ANN blastocyst grading model (A, B, C) compared to the ASE-BIR grading by the embryologists were 0.92, 0.94 and 0.95, respectively. The EMA ANN model had an extremely high accuracy for distinguishing between the three grades of blastocysts, with a corresponding AUCs of 0.99 for A versus B; 0.97 for B versus C; and 0.98 for A versus C.

CONCLUSIONS: The EMA ANN model showed remarkable consistency with embryologist grading. Moreover, the model was highly accurate in differentiating between different grades of blastocysts. This study demonstrates that the EMA ANN model can serve as an efficient and objective tool for blastocyst evaluation.

IMPACT STATEMENT: The blastocyst evaluation process could be automated with neural network-based models improving laboratory workflow.

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O-200 11:00 AM Wednesday, October 20, 2021

THE INFLUENCE OF BLASTOCYST MORPHOLOGICAL CONFIGURATION ON THE OCCURRENCE OF MONOZYGOTIC TWINNING IN SINGLE EMBRYO TRANSFER CYCLES.

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OBJECTIVE: To compare embryo morphology of fresh or frozen-thawed blastocysts that resulted in monozygotic twinning (MZT) after single embryo transfer (SET).

MATERIALS AND METHODS: All patients undergoing fresh or frozen-thawed blastocyst stage SET at a large infertility practice were included. Monozygotic twin pregnancies (cases) were identified by ultrasound and compared to transfer cycles that did not result in MZT (controls). A monozygotic twin pregnancy was identified when a SET resulted in either two or more gestational sacs and/or two or more yolk sacs. The incidence of