cells. To assess DC maturity, geometric means of HLADR expression on DCs were derived using CellQuest software. Blood flow impedance was determined using the resistance index (RI) according to the formula: RI=(peak systolic velocity–end diastolic velocity)/peak systolic velocity.

RESULTS: The percentage of DCs out total $CD45^+$ hematopoietic cells within the FF was strongly correlated to both day 3 FSH levels (r=0.853, $P<0.001$) and LH levels (r=0.83, $P<0.0001$). The presence of $CD45^+$ hematopoietic cells correlated with end diastolic velocity of blood flow to the follicle (r=0.475, $P=0.003$). Although the RI to blood flow did not correlate with the presence of *total* DCs within the FF (r=-0.08,$P=0.97$), it was inversely correlated significantly with the *maturity* of DCs as indicated by their expression of HLADR (r=-0.36, $P=0.043$).

CONCLUSION: The presence of *total* DCs in the follicular fluid is associated with poor ovarian reserve parameters. In accordance to our previous findings that the presence of *mature* DCs within the ovarian follicle correlates with ovarian response to gonadotropins, we now report that their presence correlates with improved follicular blood flow. The notion that *mature* DCs within the ovarian follicle are important for angiogenesis and oocyte development is intriguing and warrants further investigation.

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NON-SYNONYMOUS SINGLE NUCLEOTIDE POLYMORPHISM IN MCM8 DECREASED IN A COHORT OF PREMATURE OVARIAN FAILURE PATIENTS. E. M. Munch,[a] Q. Liu,[a] A. Balasa,[a] W. E. Gibbons,[a] J. L. Simpson,[b] E. Kovanci.[a] [a]Obstetrics & Gynecology, Baylor College of Medicine, Houston, TX; [b]Obstetrics & Gynecology, Florida International University, Miami, FL.

OBJECTIVE: Genome-wide association studies have identified genetic loci that may be involved in the onset and senescence of ovarian function. A non-synonymous single nucleotide polymorphism (SNP rs16991615), located in exon 9 of MCM8, was found to be associated with age at natural menopause in a recent GWAS analysis (He, 2009). We speculate that this mutation changes secondary protein structure sufficiently to affect the function of MCM8. Therefore, we hypothesize that this SNP may be present in more women with premature ovarian failure (POF) compared to controls.

DESIGN: Experimental laboratory study.

MATERIALS AND METHODS: We collected genomic DNA from predominantly North-American Caucasian women with premature ovarian failure (defined as amenorrhea prior to age 40 with FSH > 20IU/L) and control patients of similar background. A primer set specific for the region of MCM8 containing exon 9 was used for polymerase chain reaction to amplify the segment of interest. Restriction enzyme AciI was used to cut the 597-bp product at the recognition site; cleaved products indicated wild-type, and intact products indicated a PCR product containing the SNP. Statistical comparison was performed using the Fisher exact test.

RESULTS: The frequency of SNP rs16991615 was lower in women with POF. Although there was a trend, it did not reach statistical significance. Of 60 women with POF, only 1 (1.6%) had the SNP rs16991615 identified, compared to 6 of 56 (10.7%) control women ($P=0.055$).

CONCLUSION: SNP rs16991615 is more common in our control women than those with POF. Our finding suggests that this SNP could confer a reproductive advantage to women or, at a minimum, provides supporting evidence for the association of MCM8 with genes influencing age at natural menopause. Overall frequency of this SNP was low in both groups of women. In future studies, an increased sample size may lead to a statistically significant difference in SNP frequencies.

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MECHANISM OF BENZO[a]PYRENE-INDUCED INHIBITION OF FOLLICLE GROWTH. J. C. Sadeu W. Foster. Obstetrics and Gynecology, McMaster University, Hamilton, ON, Canada.

OBJECTIVE: To determine the mechanisms mediating the adverse effects of B[a]P on ovarian follicle growth and function.

DESIGN: Prospective laboratory study.

MATERIALS AND METHODS: Isolated mouse follicles (100-130 μm) were cultured for 12 days in the absence (controls) or presence of increasing B[a]P concentrations (1.5 - 45 ng/ml). Follicle growth and viability were assessed on days 4, 8 and 12 (preovulatory follicles) of culture. On days 8 and 12, morphologically healthy-looking follicles were collected and stored frozen in liquid nitrogen. Using real-time RT-PCR, AhR, Cyp1a1, Cyp1b1, aromatase, StAR, Cyp11A1, cdk4, cdk2, ccnd2, bax, and hsp90ab1 transcripts were quantified. The statistical differences were determined by one-way ANOVA followed by Tukey's post hoc test.

RESULTS: AhR mRNA expression in follicles in the control groups was not different from B[a]P-exposed follicles. On days 8 and 12 of culture, B[a]P exposure induced an increase in CYP1a1 and CYP1b1 mRNA expression, respectively, which became significant at 45 ng/ml B[a]P. B[a]P exposure had no effect on mRNA expression of StAR, Cyp11A1 and aromatase compared to controls. On day 12, preovulatory follicles exposed to 45 ng/ml B[a]P throughout growth had significantly increased cdk4 and cdk2 mRNA expression. Transcript levels for Ccnd2 were profoundly lower at day 12 compared to day 8 of culture. Preovulatory follicles exposed to 45 ng/ml B[a]P throughout growth also had significantly increased bax and hsp90ab1 mRNA expression levels compared to controls.

CONCLUSION: This study suggests that the decrease in human fecundity associated with cigarette smoking may be due to B[a]P activation of the AhR signaling, and inadequate gene expression in the steroidogenic, cell-cycle proliferation and apoptosis pathways all culminating in inhibition of follicle growth and follicular dysfunction.

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MUTATIONS IN MITOCHONDRIAL HISTIDYL tRNA SYNTHETASE (HARS2) IS NOT ASSOCIATED WITH PREMATURE OVARIAN FAILURE IN A COHORT OF 69 PATIENTS. Q. Liu,[a] S. K. Mahadevan,[a] W. E. Gibbons,[a] J. L. Simpson,[b] E. Kovanci.[a] [a]Ob&Gyn, Baylor College of Medicine, Houston, TX; [b]March of Dimes Foundation, White Plains, NY.

OBJECTIVE: Previous linkage and sequencing analysis revealed the genetic basis of Perrault syndrome (ovarian dysgenesis and sensorineural hearing loss) in a non-consanguineous family with five affected siblings. Compound heterozygosity for two highly conserved amino acids, L200V and V368L were found in the mitochondrial histidyl tRNA synthetase (HARS2) gene (Pierce, 2011). Functional studies suggested that the Perrault syndrome in this family was caused by the reduction of HARS2 activity. We hypothesized that these mutations may be overrepresented in women with sporadic premature ovarian failure (POF).

DESIGN: Genetic study with DNA sequencing.

MATERIALS AND METHODS: We collected genomic DNA from predominantly North American Caucasian women with POF defined as amenorrhea prior to age 40 with elevated FSH >20 IU/L. Primer sets specific for all 13 exons of HARS2 were designed for polymerase chain reaction (PCR). The PCR products were then sequenced using a commercial ABI3730XL Sanger sequencing platform and systematically compared to the human genome database sequence (ENSG00000112855) using Sequencher v5.0 (Gene Codes Corp, Ann Arbor, MI). Chromatograms were also manually examined to identify sequence variants.

RESULTS: In 69 women with POF, we did not detect the previously reported mutations. Moreover, no other novel or known SNPs were identified in any exon of HARS2.

CONCLUSION: All 13 exons of *HARS2* are highly conserved in our study population. Therefore, HARS2 mutations are not a common cause of sporadic POF in Caucasians.

POLYCYSTIC OVARY SYNDROME

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EFFECT OF CALCIUM AND VITAMIN D SUPPLEMENTATION ON FOLLICULAR FLUID TUMOR NECROSIS-ALPHA AND INTERLEUKIN-6 AND IVF/ICSI OUTCOMES IN INFERTILE PATIENTS WITH POLYCYSTIC OVARY SYNDROME. S.-Y. Choi,[a] C. H. Kim,[a] J.-W. Ahn,[b] S.-K. Kwon,[a] K.-H. Lee,[a] B.-M. Kang.[a] [a]Obstetrics and Gynecology, College of Medicine, University of Ulsan, Asan Medical Center, Seoul, Korea; [b]Obstetrics and Gynecology, College of Medicine, Ulsan University Hospital, Ulsan, Korea.