Endometrial Aging Evidence at Transcriptomic Level

**Author Block:** Almudena Devesa-Peiro†,1,2 Patricia Sebastian-Leon*,1 Antonio Pellicer*,2,3 Patricia Diaz-Gimeno*,1 1IVI-RMA IVI Foundation / Biomedical Research Institute La Fe, Valencia, Spain; 2University of Valencia, Valencia, Spain; 3IVI-RMA Rome, Rome, Italy.

**Abstract:**

**Introduction:** It is widely known aging begins in all human organs and tissues as a natural process after the age of 35. Female fertility also decreases suddenly after this age, and especially after 40 years old. Although there is no doubt that ovarian aging affects oocyte quality and ovarian reserve, the concept of endometrial aging does not exist in infertility under the dogma that age does not affect endometrial receptivity. The aim of this study was to investigate the effect of age on endometrium under the hypothesis that endometrial aging is affecting gene expression and fertility.

**Methods:** Endometrial biopsies from norm-ovulatory women (n=27) with regular cycles and free of endometriosis were classified according to age into two groups (<35y.o.: n=8; ≥35y.o.: n=19). Raw gene expression data from these patients were downloaded from the public repository Gene Expression Omnibus and pre-processed and normalized by the quantile method. In this sample cohort, variables as biopsy menstrual cycle phase, ethnicity or the presence of other uterine pathologies were considered in the analysis using linear models to investigate the real effect of age on the endometrium (limma R-package). Differential expression analysis and the functional meaning of these differences was analysed by gene set enrichment analysis (limma and mdgsa R-package) consulting Gene Ontology (GO) functional database.

**Results:** Principal component analysis revealed a clear effect of age on endometrial gene expression behaviour, dividing the samples into two transcriptomic groups at the age of 35 as threshold. A total of 6858 genes were found as affected in the endometrium of women older than 35 years old (False Discovery Rate (FDR)<0.05). The functional meaning of these changes showed 28 altered GO terms (FDR<0.05), 89% of them upregulated and mainly involved in ciliary motility. The remaining 3 terms found downregulated were associated to endocrine system and insulin-like growth factor.

**Conclusion:** Our study suggests for the first time that aging affects endometrial factor. As it has been reported in previous studies, the parameter number of cilia decreases in the endometrium during the window of implantation. Nevertheless, our results showed that ciliary processes were upregulated at gene expression level in women older than 35 years old, indicating a potential effect of endometrial aging in infertility.

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Please describe the translational relevance below: Our results have translational value because our findings related to age-associated ciliary alterations could be used as a biomarker for endometrial factor evaluation in infertility.

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Society for Reproductive Investigation
555 East Wells Street, Suite 1100
Milwaukee, WI 53202-3823
(414) 918-9888

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