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Impact of Aging on Gene Expression in Human Oocytes: A Comparative Analysis of Young and Older Patients- Preliminary Results.

Raoul Orvieto, Omri Nayshool, Louisa Cohen, Yuval Yung, Adva Aizer, Efrat Glick Saar, Dan Dominissini.

Chaim Sheba Medical Center (Tel Hashomer), Ramat Gan, Israel and Faculty of Medical and Health Science, Tel Aviv University, Tel Aviv, Israel.

Background and objective: Aging affects gene expression in pathways essential for energy metabolism, DNA repair, cell cycle regulation, and antioxidant defenses, directly affecting oocyte quality and viability. Single-cell RNA deep sequencing studies of aged versus young human MII oocytes revealed many differentially expressed genes. In addition, single human oocyte transcriptome analysis at both germinal vesicle (GV) and MII stages revealed distinct stage-dependent pathways impacted by aging, with a decrease in mitochondrial-related transcripts from GV to MII oocytes, and a much greater reduction in MII oocytes with advanced age. Our aim was to investigate the age-related differences in gene expression of germinal vesicle (GV) oocytes between young and advanced age patients.
Material and methods: Immature GV oocytes were donated by 6 patients, divided into two age groups: The "Young" group (ages 16-29) had three participants (mean age: 23.3 ± 6.6 years), and the "Elderly" group (ages 38-40) included three participants (mean age: 39 ± 1 year). After retrieval, oocytes were denuded and donated GV oocytes were cryopreserved at -196^{0C} until analysis. For library preparation, we used the NEBNext® Single Cell/Low Input RNA Library Prep Kit for Illumina, section 1 (cat no. E6420S, New England Biolabs (NEB), USA), strictly adhering to the manufacturer's instructions. All samples were processed in a single batch to control for technical variation. The NEBNext® RNA Library Prep Kit allows conversion of mRNA to barcoded cDNA for Illumina sequencing. RNA sequencing data underwent rigorous quality control and processing through a multi-step pipeline. Gene expression quantification was performed using feature Counts from the Subread package (v1.5.3), and comprehensive quality control reports were generated using MultiQC (v1.25.1).
Results: Of top 10 significantly differently expressed genes 7 (LINC02087, POMZP3, LINC02749, MYL4, AGPAT2, GCA, and LIMK1) were downregulated and 3 (CLEC3A, ARPP21, and CITED2) showed significant upregulation in young versus old oocytes. These genes underscore the impact of aging on critical oocyte pathways, including chromosomal stability, epigenetic regulation, mitochondrial function, immune response, structural integrity, and calcium signaling Moreover, among these genes, LINC02087 was the most downregulated (log2FC = -7.66), while CITED2 showed the strongest upregulation (log2FC = 3.43) in young versus old oocytes.
Conclusions: Understanding the effects of aging on the oocyte transcriptome could identify biomarkers that characterize good MII oocyte quality. The different genes expressions in aged oocytes highlight their potential contributions to oocyte quality and development. Moreover, by elucidating age-related changes across diverse cellular functions, this study opens avenues for therapeutic interventions aimed at extending reproductive longevity and optimizing outcomes in assisted reproductive technologies.

Age-Related Gene Expression in Human Oocytes: TPM-Normalized Values of Top 10 Differentially Expressed Genes.

Samples , status and gene TPM counts

new_sample_name	group	LINC02087	CLEC3A	POMZP3	LINC02749	MYL4	AGPAT2	GCA	ARPP21	LIMK1	CITED2
Oocyte_Age_39	Old_Oocyte	19.2	9.5	150.9	189.3	32.4	23.8	30.0	0.6	6.0	1.0
Oocyte_Age_38	Old_Oocyte	14.6	4.2	196.3	223.1	36.0	22.1	27.9	1.0	9.7	0.7
Oocyte_Age_40	Old_Oocyte	9.0	6.8	124.4	178.1	26.2	39.4	40.9	0.8	9.7	0.4
Oocyte_Age_25	Young_Oocyte	0.0	35.7	33.7	51.3	8.1	10.1	15.1	2.8	2.2	9.4
Oocyte_Age_29	Young_Oocyte	0.2	58.6	25.8	78.0	6.6	5.6	10.1	13.2	2.8	9.5
Oocyte_Age_16	Young_Oocyte	0.0	46.0	55.6	41.7	1.5	4.6	10.5	1.9	2.7	1.4

Volcano plot of differential gene expression between young and old oocytes