SERUM AND FOLLICULAR FLUID EXTRACELLULAR MATRIX (ECM) FINGERPRINTS REVEAL NEW FIBROTIC SIGNATURES OF OVARIAN AGING

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Background: Ovarian aging is characterized by progressive stromal fibrosis and declining follicular function. In this study, we report the first application of extracellular matrix (ECM) fingerprinting to ovarian aging. This immunoassay platform enables quantification of neo-epitopes of collagen formation and degradation peptides in both serum and follicular fluid (FF). By matching systemic and ovarian ECM profiles, this approach provides a novel, minimally invasive modality to assess dynamic ovarian fibrotic remodeling and establish an ovarian fibrotic index to define reproductive aging.

Objective: To investigate age-related ECM formation and degradation biomarker profiles in the serum and FF of women undergoing fertility preservation or IVF.

Materials and Methods: Paired serum and FF samples were collected from reproductively young (≤33 years) and old (≥39 years) individuals undergoing planned oocyte cryopreservation or IVF for male factor. The older group also included IVF patients with diminished ovarian reserve or unexplained infertility, where female age was the likely etiology. Serum was collected in the early follicular phase at the baseline visit, and FF was aspirated from three dominant follicles per ovary at oocyte retrieval. Samples from each ovary were analyzed individually and as pooled FF. ECM biomarkers were measured using competitive enzyme-linked immunosorbent assays (ELISAs) that quantify collagen formation (PRO-C3, PRO-C6, PRO-C8) and degradation (C3M, C6M) neo-epitopes as indices of matrix remodeling. ECM fingerprints were analyzed by age group and intra-individually for both serum and FF. Ratios of degradation/formation for relevant peptide fragment pairs (C3M/PRO-C3 and PRO-C6/C6M) were examined by age. Magnitude and direction of age-related shifts in pooled FF and serum was analyzed.

Results: Ten individuals were enrolled per group. Mean age (± SD) was 29.8 ± 1.9 (young) and 40.9 ± 2.1 years (old). Anti-Mullerian hormone (AMH) levels were higher in the young cohort (2.8 ± 1.6 vs. 1.6 ± 0.9 ng/mL, p= 0.05) as expected. In the combined FF from each ovary, PRO-C8 was significantly higher in the old compared to young participants (0.64±0.5 vs 0.34 ± 0.2 ng/mL, p=0.01), while serum PRO-C8 trended higher, but did not significantly differ by age (3.4±1.5 vs 3.0 ± 2.3 ng/mL, p=NS). No age-related differences were observed for PRO-C3, PRO-C6, C3M, C6M, C3M/PRO-C3, or C6M/PRO-C6 in serum or combined FF from each ovary. A $\log_2$(old/young) fold-change analysis revealed that PRO-C8 (collagen VIII) had the most pronounced age-related increase (1.8-fold in pooled FF), whereas collagen degradation markers, C3M (collagen III) and C6M (collagen VI), trended lower with age. These reciprocal shifts indicate reduced ECM turnover and enhanced collagen VIII deposition in older patients, consistent with age-associated ovarian fibrosis.

Conclusions: This study delivers the first paired circulating and ovarian ECM fingerprinting profile of reproductive aging, defining a molecular framework for quantifying ovarian fibrosis. FF PRO-C8 emerges as a promising biomarker of increasing ovarian age and warrants further study in a larger cohort. Establishing an ovarian fibrotic index through ECM turnover analysis combined with other clinical technologies, such as shear wave elastography, may enable early, minimally invasive detection of ovarian fibrosis and guide evaluation of emerging anti-fibrotic therapies aimed at extending reproductive longevity.

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