Using decellularized human uterine samples to assess the influence of the extracellular matrix on the age related decay on uterine function

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INTRODUCTION

The fertility decline and increased incidence of pregnancy complications associated with advanced maternal age are mainly caused by disturbance in uterine function and low quality/quantity of oocytes. Oxidative stress plays a major role in age-related processes contributing to abnormal uterine transformation and fertility decrease in mice1. Also, we reported an age-related uterine accumulation of carbonylated albumin that affects trophoblasts’ function and can modify the extracellular matrix (ECM)2. Hence, it is hypothesized that alterations in uterine ECM due to ageing may affect its function and contribute to fertility decline.

AIMS

To optimize the decellularization procedure to be applied on human uterine samples

To evaluate age-related changes on proteome and protein modifications in uterine ECM obtained after tissue decellularization

RESULTS

1. Histological analysis

| Histological Stainings | H & E | Picosirius Red | Masson’s Trichrome |

Decellularized uterine samples stained with H&E (B) and MT (D) showed a significant decrease of stained nuclei, compared to the non-decellularized tissue (A), while either MT (D) or PSR (C) staining evidenced abundant collagen fibers (red in PSR and cyan in MT) in the processed samples.

(Representative images captured with 400x magnification)

2. Proteomic analysis

SDS-PAGE

Coomassie blue staining shows different protein profiles in uterine samples submitted to decellularization (Y1, Y2, A1, A2), when compared to the non-decellularized sample (ND).

Y-young; A-aged

Western blotting

Decellularized samples (Y1, Y2, A1, A2) are enriched in ECM proteins (Fibronectin, COL1A1) while having very small amounts of cytosolic (SOD1), endoplasmic reticulum (BIP), mitochondrial (SOD2) and nuclear (Histone H3) proteins.

Carbonylated proteins are more frequent in decellularized samples (Y1, Y2, A1, A2) than in non-decellularized tissue, suggesting that ECM proteins are preferentially oxidized in human uterus.

Y-young; A-aged; ND- non-decellularized

Mass Spectrometry

Intensity Aged vs Young

Protein intensity variations from Aged vs Young decellularized samples. MS analysis identified 345 proteins in the decellularized samples, from which 94 were annotated as "extracellular region part" by gene ontology cell component (GOCC) (red dots).

Panther GOCC enrichment

Panther GOCC enrichment analysis considering protein abundance has shown proteins annotated with "extracellular matrix" as the most significantly enriched in these samples.

CONCLUSION

Both histological staining and proteomic analysis show a successful elimination of most intracellular components and an enrichment of ECM proteins in the decellularized uterine samples.

Preliminary Aged vs Young MS data analysis suggest the existence of a differential expression of some ECM proteins.

REFERENCES

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2 Mendes S., et al. FRBM 2020,152:313-322