Abstract # 1768

Age-dependent Dysregulation of Hyaluronan and Collagen Matrices Alters Ovarian Biomechanical Properties. Farners Amargant, Northwestern University, USA

Female reproductive aging is associated with infertility due to decreased egg quality and quantity. We recently identified that there is an increase in collagen (fibrosis) in the ovarian stroma with advanced reproductive age, likely affecting gamete quality. Hyaluronan (HA) is an extracellular matrix glycosaminoglycan that maintains tissue homeostasis, and its loss can change tissue micromechanical properties, leading to a mechanically stiff microenvironment. Therefore, we hypothesized that reproductive aging is associated with a loss of ovarian HA, which promotes stromal stiffness and fibrosis. HA levels are dictated by the relative activities of enzymes that regulate its synthesis (hyaluronan synthases) and degradation (hyaluronidases). To investigate whether there were age-associated changes in these enzymes, we performed real time PCR to examine the expression of hyaluronan synthases (Has1, Has2, Has3) and hyaluronidases (Hyal1, Hyal2, Tmem2, Kiaa1199) in enriched ovarian stromal tissue from reproductively young (6-12 weeks) and old (14-17 months) CB6F1 mice. Of these genes, Has3 and Hyal1 were the only ones whose expression changed with age, with Has3 expression decreasing (1.45-fold change) and Hyal1 expression increasing (1.38-fold change). We further validated their stromal expression and localization using RNA in situ hybridization. These gene expression changes would predict a net loss of ovarian stromal HA with age. To investigate this, we assessed the HA content in the ovary using a hyaluronic acid binding protein (HABP) assay. HA was detected in follicles, corpora lutea (CL), and the ovarian stroma, and the total ovarian HA content was significantly reduced in reproductively old mice compared to young controls ($p=0.008$). This reduction in HA occurred specifically in the stroma, as HA loss in other ovarian sub-compartments was not significant between age cohorts (follicles $p=0.056$; CL $p=0.55$). To examine how advanced reproductive age affects ovarian micromechanical properties, we performed nanoindentation analysis to measure ovarian stiffness. It took more force to indent ovaries from reproductively old mice (3.57±2.4kPa) compared to young mice (1.69±2.4kPa; $p<0.001$). We then examined whether the increase of ovarian stiffness with age was dependent on the increase in collagen and the decrease in HA content by quantifying the micromechanical properties of collagenase-treated and Has3-/- mice ovaries, respectively. Reducing collagen content in reproductively old mouse ovaries restored the micromechanical properties to those of ovaries from young mice (reproductively young 1.98±0.42kPa; reproductively old

4.36±1.24kPa; reproductively old collagenase 2.28±0.61kPa). On the other hand, Has3-/- ovaries were stiffer than ovaries from age-matched wild-type (WT) mice (WT 2.51±0.66kPa; Has3-/- 6.67±2.00kPa; p=0.0079). These results demonstrate that both increased collagen and HA loss in the ovarian stroma contribute to the increase in ovarian stiffness observed with age. These findings are significant because the use of pharmacological approaches to prevent collagen and HA changes in the stroma with age may enhance reproductive longevity. This work was supported by the National Institute of Child Health and Human Development (R01HD093726).