Culture of human ovarian tissue in xeno-free conditions using laminin components of the human ovarian extracellular matrix

ONLINE RESOURSES

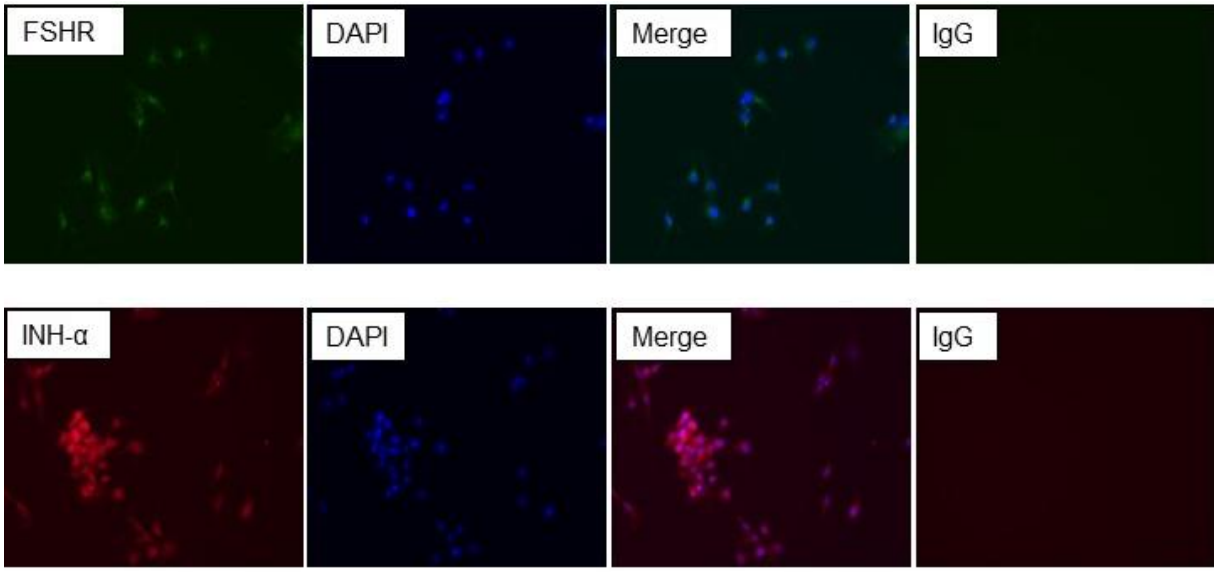
Figure 1. Validation of mural granulosa cells

Figure 2. Follicle density

Figure 3. Endocrine function of the tissue

Figure 4. Ki67 expression

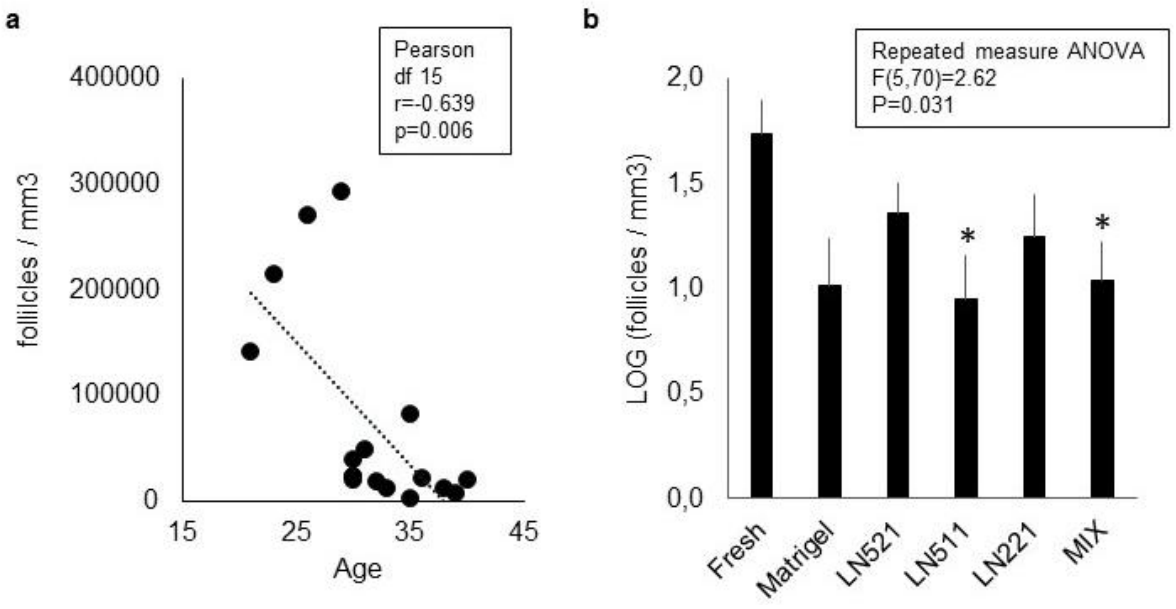
Figure 1. Validation of mural granulosa cells



Online Resource Figure 1. Validation of mural granulosa cells

Mural granulosa cells were isolated from human ovarian follicular fluid by density gradient centrifugation and depletion of red blood cells and leukocytes. Cell identify was verified by immunostaining for granulosa cells markers follicle stimulating hormone receptor (FSHR) and inhibin-alpha (INH-a). Cell nuclei were stained with DAPI. IgGs were used as negative control. Positive staining for FSHR and INH-a was observed, confirming the identify of the cells.

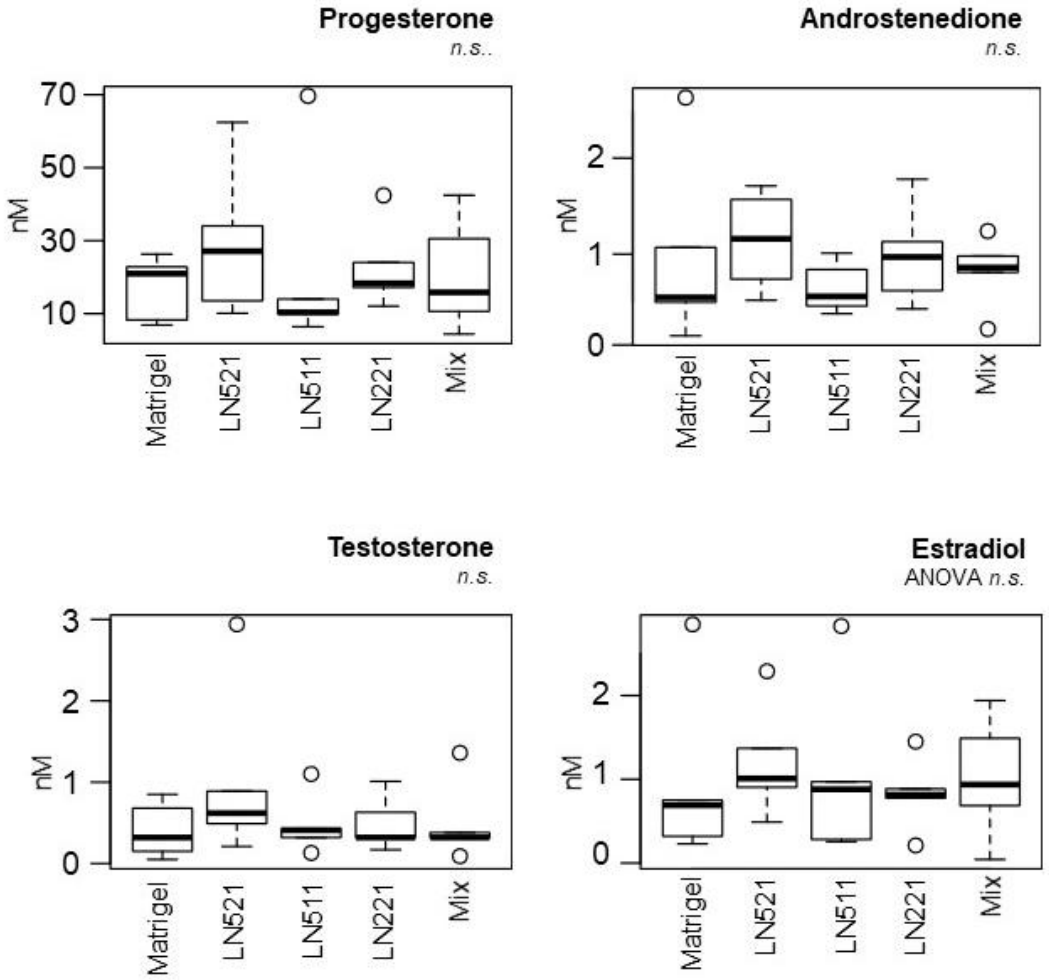
Figure 2. Follicle densities



Online Resource Figure 2. Follicle density

Ovarian cortical tissue from 17 caesarean section patients was used in the culture studies. (a) Total follicle density (sum of all detected follicles) in the samples correlated significantly with patient age as expected. Pearson correlation coefficient and associated p-value are shown. (b) Total follicle density in samples was compared before and after culture. The density was significantly lower in tissue cultured on LN511 and MIX compared to fresh control tissue. Repeated measures ANOVA followed by Bonferroni-Holm post hoc test, * $p<0.05$.

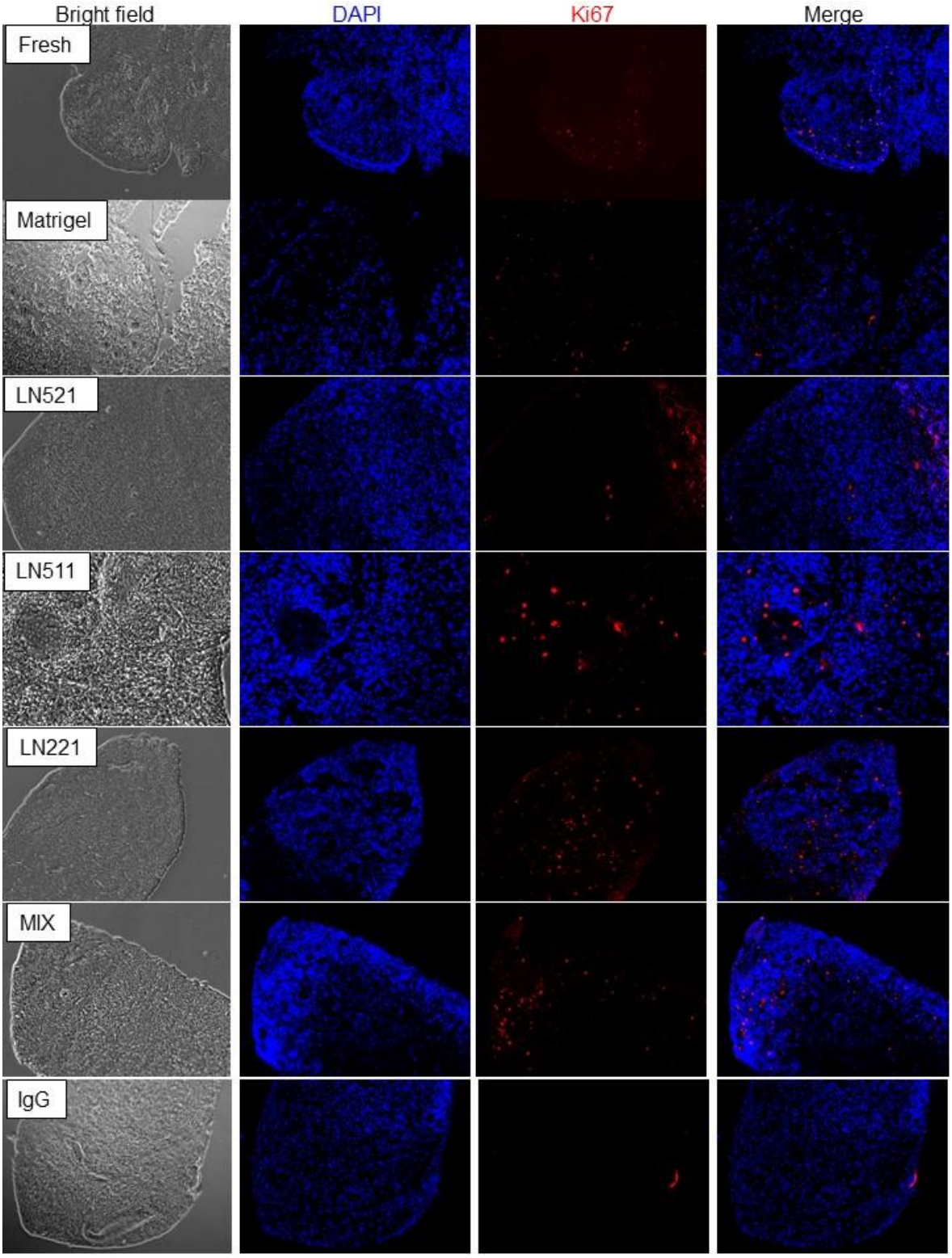
Figure 3. Endocrine function of the tissue in culture



Online Resource Figure 3. Endocrine function of the tissue

Steroid hormones were measured in five independent media samples per group at the end of the culture. Progesterone, androstenedione, testosterone and estradiol were deteced in all cultures. There were no significant differences between the groups. Repeated measures ANOVA.

Figure 4. Ki67 expression



Online Resource Figure 4. Ki67 expression

Proliferation marker Ki67 expression in tissues. Samples from three patients per group were stained and inspected for the presense of positive cells. Scattered nuclear staining was observed throughout the samples in all patients, suggesting the presense of viable cells after the culture. Nuclei were stained with DAPI and IgG were used as negative control.