Decoding the functional significance of follicle stimulating hormone glycosylation variants

Follicle stimulating hormone (FSH) and its G protein-coupled receptor (FSHR) are essential for coordinating reproductive functions. They are a primary target of most assisted reproductive technologies, thus understanding the physiology regulating their function is paramount. Two naturally occurring glycosylation variants of FSH have been identified, termed hyperglycosylated FSH (FSH24) and hypoglycosylated FSH (FSH21). FSH21 and FSH24 have different bioactivites, with FSH21 more potent at activating FSHR signalling and displaying faster binding kinetics to FSHR. Pituitary expression levels of FSH21 and FSH24 have been reported to change with age, with a decline in the more bioactive FSH21 and increase in FSH24, we hypothesise that this age-related change in FSH may underpin ovarian resistance that precedes the menopause. This study aimed to determine how the differential effects of FSH21 and FSH24 are mediated by the FSHR, and if ovarian granulosa cell responses to FSH21 and FSH24 change with age. Isolation of granulosa cells from 8-10 week old mice representing post-pubertal age and 11 month old female, representing perimenopause, showed differential signalling profiles to FSH21 and FSH24, as assessed by phosphokinase array. As we have previously shown receptor di/oligomerisation to be an important way of regulating of gonadotrophin receptors signalling, we next determined if FSH21 and FSH24 differentially modulated FSHR di/oligomerisation using the super resolution imaging. In HEK293 cells transiently expressing FSHR, acute 2-minute treatment with FSH21 resulted in a significant decrease in the number of FSHR homomers observed in comparison to basal. In contrast, treatment with FSH24 had no effect on basal FSHR homomerisation. Analysis of the individual FSHR homomeric forms revealed an enrichment in dimeric population and decrease in higher order FSHR oligomers following FSH21-treatment, suggesting the enhanced signalling properties of FSH21 may be mediated by alterations in FSHR associations. These data provide evidence of ageing-related modulation of FSHR function by FSH glycosylation, and suggest this may be mediated via modulating FSHR di/oligomerisation.