**Impact of a FSHR positive allosteric modulator on FSH glycosylation variant-dependent FSHR homomerisation and signal pathway activation**

The heterodimeric pituitary glycoprotein hormone, follicle-stimulating hormone (FSH) and its target G protein-coupled receptor (FSHR) are essential for reproduction. As an important drug target for IVF, the need for more effective treatment drives interest in understanding what modulates FSH/FSHR functions. In vivo, two predominant FSH glycosylation variants have been identified; partially glycosylated FSH (FSH21) has faster binding kinetics to the FSHR and is more potent at activating cAMP-dependent signal pathways, in comparison to fully glycosylated FSH (FSH24). An important mechanism of regulating GPCR function is the formation of dimers and oligomers. FSHR has been shown to self-associate, and our unpublished data suggests that the increased bioactivity of FSH21 may be mediated via dissociation of FSHR oligomers into dimers and monomers, with no effect of FSH24 observed. As FSH24 displays slower binding kinetics to FSHR, we aimed to determine the effect of Compound 2 (C2), a FSHR positive allosteric modulator on FSH glycoform receptor binding, FSHR oligomerisation and signal pathway activation. C2 increased both FSH21 and FSH24 receptor binding. In HEK293 cells transiently expressing FSHR, 30-minute pre-treatment +/- 1µM C2, enhanced the concentrations-dependent effects of FSH21 on CREB phosphorylation. Moreover, co-treatment with C2 enhanced FSH24-dependent CREB activation at low concentrations but had no effect on FSH24-dependent CREB-P at concentrations of FSH24 >1ng/ml. Super-resolution imaging via PD-PALM of FSHR homomers showed that C2 pre-treatment had no effect on the number of FSHR monomers and homomers observed. However, C2 pre-treatment caused rapid FSH24-dependent dissociation of FSHR homomers into monomers and lower order trimers. Interestingly, C2 had little effect on FSH21-dependent regulation of FSHR complexes. These data suggest that allosteric modulation of FSHR is a powerful tool for enhancing FSH-FSHR action, modulating signal strength, with potential as a novel therapeutic strategy for enhancing ovarian responses during IVF protocols.