Effect of sialylation and complexity of FSH oligosaccharides on inhibin production by rat granulosa cells.

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Introduction: Production of inhibin A (InhA) and B (InhB) by granulosa cells is differentially regulated by FSH, activin A, TGF-ß, oestradiol and oocyte-derived factor(s). FSH is released from the pituitary gland as a mixture of isoforms that act on the target cells inducing different biological responses *in vitro* and *in vivo*. The present study has examined the question of whether FSH isoforms with specific degree of sialylation and complexity of their oligosaccharides may differentially stimulate *in-vitro* inhibin A and B production by rat granulosa cells (GC). **Methodology:** Recombinant human FSH (rhFSH) was used to isolate FSH charge analogues in a pH range of 2.6-7.5 using preparative isoelectrofocusing. Two preparations were obtained combining pH 3 to 4 (more acidic, AC) and 5 to 7 (less acidic, BA) fractions. rhFSH was applied to a Concanavalin-A column to isolate three FSH glycosylation variants on the basis of glycan complexity: unbound and weakly bound FSH isoforms (UB and WB) bearing complex type and firmly bound (FB) FSH isoforms bearing hybrid type oligosaccharides. GC were obtained from immature, DES treated Sprague-Dawley rats. Cultures were stimulated with either rhFSH or its glycoforms in a dose range of 0.5-16 ng/ml, for 72h. Levels of inhibin A and B were determined using specific ELISAs.
Results and Discussion: Basal inhA and B production by granulosa cells was: 933±138 and 134±44 pg/ml, respectively (mean±SEM). rhFSH stimulated granulosa cell inhA and B production, with a more pronounced effect on inhA in a dose dependent manner. AC FSH charge analogues (i.e. 4ng/ml dose) favoured inhB production when compared to inhA: 8.9±1.5 vs 2.0±0.3, $p< 0.05$; conversely, BA FSH charge analogues (i.e. 4ng/ml dose) favoured inhA production: 7.1±1.2 vs 1.5±0.2, $p<0.05$ (results are expressed as mean±SEM, fold increase over basal).

FSH isoforms bearing complex oligosaccharides, UB and WB (i.e. 2ng/ml dose) showed a more potent stimulatory effect on inhB compared to inhA production (6.9±0.6 vs 2.2±0.5, and 8.2±0.6 vs 3.5±0.3, $p<0.01$ fold stimulation, respectively). FB FSH isoforms showed no effect on inhA and induced a slight decrease on inhB production.
Conclusion: These results suggest that the degree of sialylation as well as the complexity of carbohydrate chains present in FSH molecules may be considered as additional factors that differentially regulate dimeric inhibin production by granulosa cells. (Supported by CONICET and FONCYT, Argentina)