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SUPPLEMENTARY DATA

Supplementary methods

FSH glycoform analysis

Automated western blotting was accomplished using a Protein Simple (San Jose, CA, USA) Wes to characterize the FSH glycoforms. We analyzed 100 ng samples in duplicate. The primary antibody was anti-FSHβ monoclonal antibody 7-13.B5 diluted 1:5000. The secondary antibody was anti-mouse IgG-HRP complex provided by the manufacturer. The sample, primary antibody, secondary antibody, wash reagents and detection reagents were prepared according to the manufacturer's recommendation and the automated capillary electrophoretic and western blot procedures executed by the Protein Simple Compass software. Sample densities were evaluated with the Compass software. Images are presented as conventional western blots. Band densities were used to determine the relative abundance of FSHβ variants present in each recombinant hFSH preparation. Pituitary hFSHβ bands were used to mark 24 kDa- and 21 kDa-hFSHβ mobilities only.

Recombinant hFSH half-life and glycan clearance analysis

FSH was dissolved in 0.15 M sterile saline to a final concentration of 50 μg/ml and 200 μl samples (10 μg) injected intraperitoneally (i.p.). Blood samples were collected from the retroorbital plexus using a Pasteur pipet calibrated to collect 20 μl. Once preliminary experiments established, FSH was undetectable at time 0, up to six samples were collected beginning 10 min and continuing at 20, 40, 60, 120, 240 min. At 300 min, the mouse was euthanized, and a final blood sample taken. Blood samples were allowed to clot and centrifuged to obtain serum. Ten μl samples of serum were diluted to 1 mL with PBS and FSH determined by ELISA using two, 50 μl serum dilutions. The ELISA reagents were obtained from IBL and followed a modified protocol. The animal handling procedures and related experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the Wichita State University.

RNA sequencing data analysis

NGS short reads from RNA-seq experiments were downloaded from the HiSeq2500 server in FASTQ format. The original FASTQ reads were trimmed by the fqtrim tool (https://ccb.jhu.edu/software/fqtrim) to remove adapters, terminal unknown bases (Ns) and low quality 3′ regions (Phred score < 30). The quality of trimmed sequence reads was assessed using the quality control tool FastQC (Andrews, 2014). The trimmed reads passing FastQC were aligned to the mouse reference genome (GRCm38) by STAR aligner (Dobin *et al.*, 2013), and the RSEM tool (Li and Dewey, 2011) was used to annotate and quantify the mapped reads from STAR aligner into both isoform and gene levels. In RSEM quantification, the raw read counts (abundance) of genes/isoforms were normalized for gene/isoform length and sequencing depth to transcripts per million (TPM) values. T-tests were performed using R/Bioconductor packages to identify differentially expressed genes (DEG) followed by the Benjamini-Hochberg (BH) procedure (Benjamini and Hochberg, 1995) for multiple-testing adjustment of the raw *P*-values. The DEGs were determined by the criteria of the adjusted $P \leq 0.01$ and $\log_2$ fold change (log2FC) of 2. Principal component analysis (PCA) was performed using the built-in R function prcomp ().

Pathway analysis was performed using QIAGEN (Germantown, MD, USA) Ingenuity Pathway Analysis (IPA) to identify significantly activated and inhibited pathways according to the calculated z-scores ($|z\text{-score}| > 2$) based on overlap of observed and predicted expression profiles of DEGs (FSH21 vs Control, FSH24 vs Control, FSH21 vs FSH24) in the pathway.

STRING v11 (Szklarczyk *et al.*, 2019) was used to identify interesting associations between DEGs identified in our study. The nodes were colored by the expression value obtained from the RNAseq analysis. Functional enrichment (GO, KEGG, InterPro, *etc.*) for the network was retrieved using the default *P*-value 0.05 in the STRING Enrichment App, a Cytoscape plugin. The table shows the top enriched GO terms along with their corresponding FDR values obtained using the functional enrichment analysis for a whole STRING network using the StringApp (Doncheva *et al.*, 2018).

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