Supporting Information to

Rapid Mouse FSH Quantification and Estrus Cycle Analysis Using an Automated Microfluidic Chemiluminescent ELISA System

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Ovariectomy in recipient mice

Bilateral ovariectomies were performed on adult female mice (B6CBAF1) aged 12–16 weeks to induce infertility. The females were anesthetized by isoflurane. Before the first cut was made, preemptive analgesics (Carprofen, Rimadyl, Zoetis, USA) were administered to the mice. A midline incision was made in the abdominal wall and the intraperitoneal space was exposed with an abdomen retractor. The ovaries were removed and the remaining reproductive tract was gently reinserted into the body cavity. The muscle layer and the skin of the abdominal wall were closed with 5/0 absorbable and non-absorbable sutures (AD Surgical, USA) in two separate layers. The mouse was then placed in a clean warmed cage for recovery. Post-surgery, mice received analgesia for at least 24 h or as needed.

Collection of donor ovaries

Ovaries from 6 to 8 days old B6CBAF1 mice were collected and transferred to Leibovitz L-15 media (Sigma-Aldrich, USA). The ovaries were then transferred in the maintenance media (a-MEM, Gibco, USA) in a CO_2 incubator till further use.

Subcutaneous implantation of ovaries in ovariectomized mice

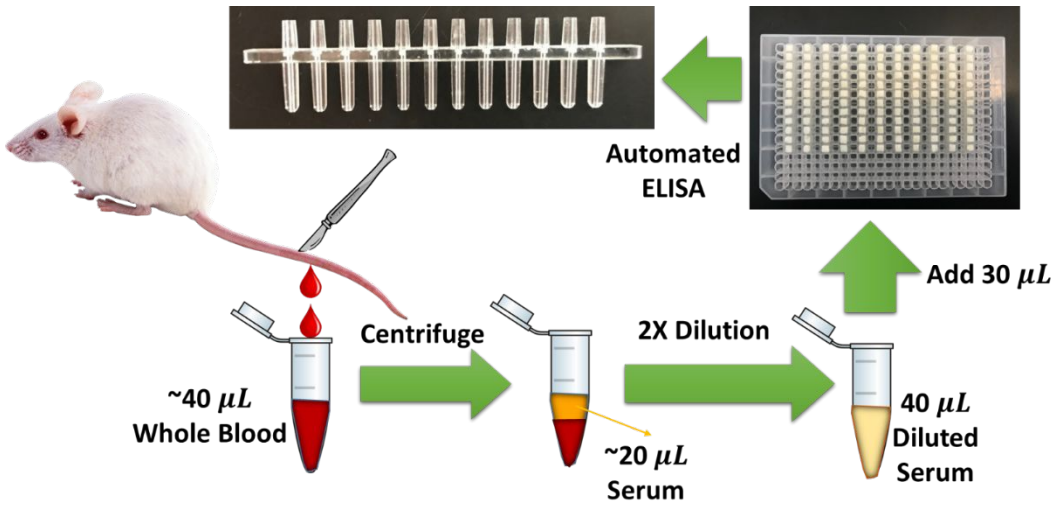
A small incision was made on the dorsal side of the anesthetized mice (B6CBAF1) and whole ovaries were implanted subcutaneously. The skin was closed using 5/0 non-absorbable sutures. After implantation, the mice were euthanized at 63 days. For controls, the ovariectomized mice were not implanted with ovaries.

Serum hormone analysis with RIA

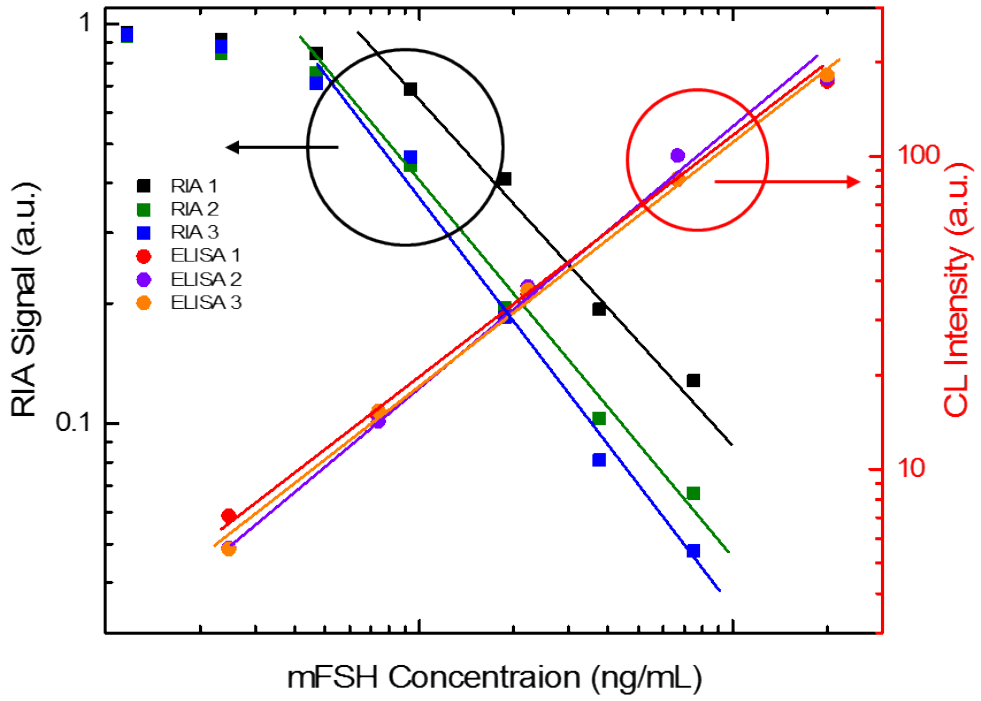
The samples were analyzed for mFSH using a radio-immunoassay (Ligand Assay and Analysis Core Facility, University of Virginia Center for Research in Reproduction). The core at the University of Virginia used mouse FSH reference preparation AFP5308D for assay standards and mouse FSH antiserum (guinea pig; AFP-1760191) diluted to a final concentration of 1:400,000 as primary antibody. The secondary antibody is from Equitech-Bio, Inc. and was diluted to a final concentration of 1:25. The radio-immunoassay has a sensitivity of 2.0 ng/mL (and <0.5% cross-reactivity with other pituitary hormones. When needed, the samples were diluted 5–10-fold.

Purification of detection antibody

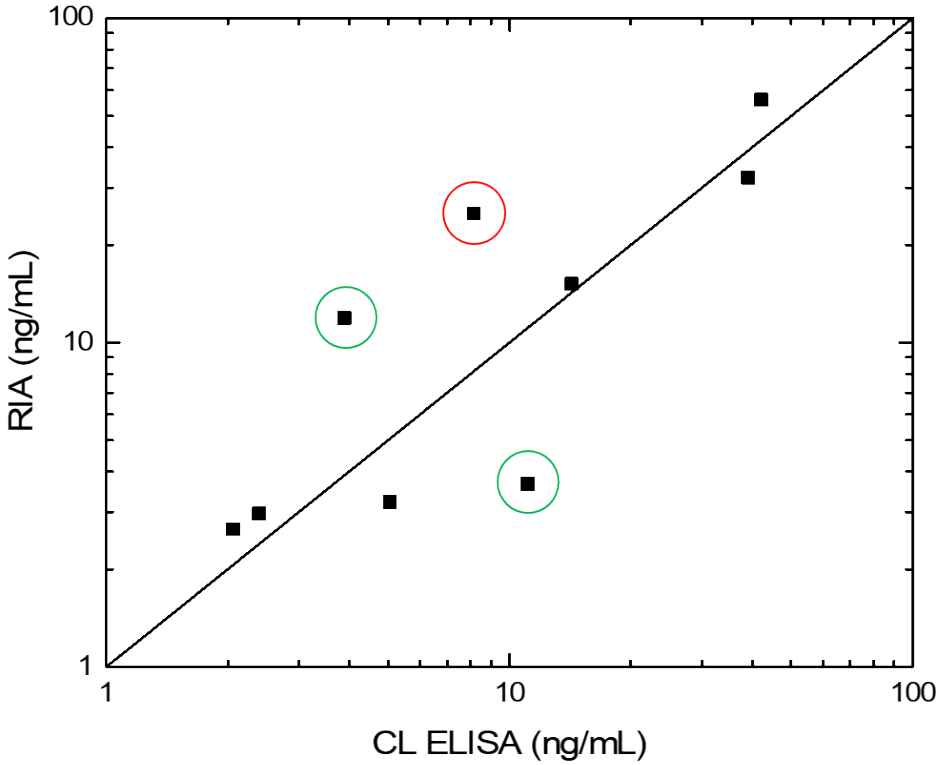
The detection antibody purification was carried out at the University of Michigan through a bead-based protein A adsorbent assay with the Abcam's antibody purification kit (ab102784). First, the guinea pig anti-mFSH antiserum (AFP-1760191) was incubated with protein A resin for 2 hours under room temperature with periodic shaking. Then the unbound portion of the antisera was washed away from the resin (through centrifugation). The rinsing procedure was repeated four times to eliminate non-specific binding. Finally, the purified IgG was eluted with antibody elution buffer. The eluted antibody was stored in a clean PCR tube for further treatment. The purified antibody usually had a yield concentration of 0.6–0.8 mg/mL, which meets the requirement for performing HRP conjugation.



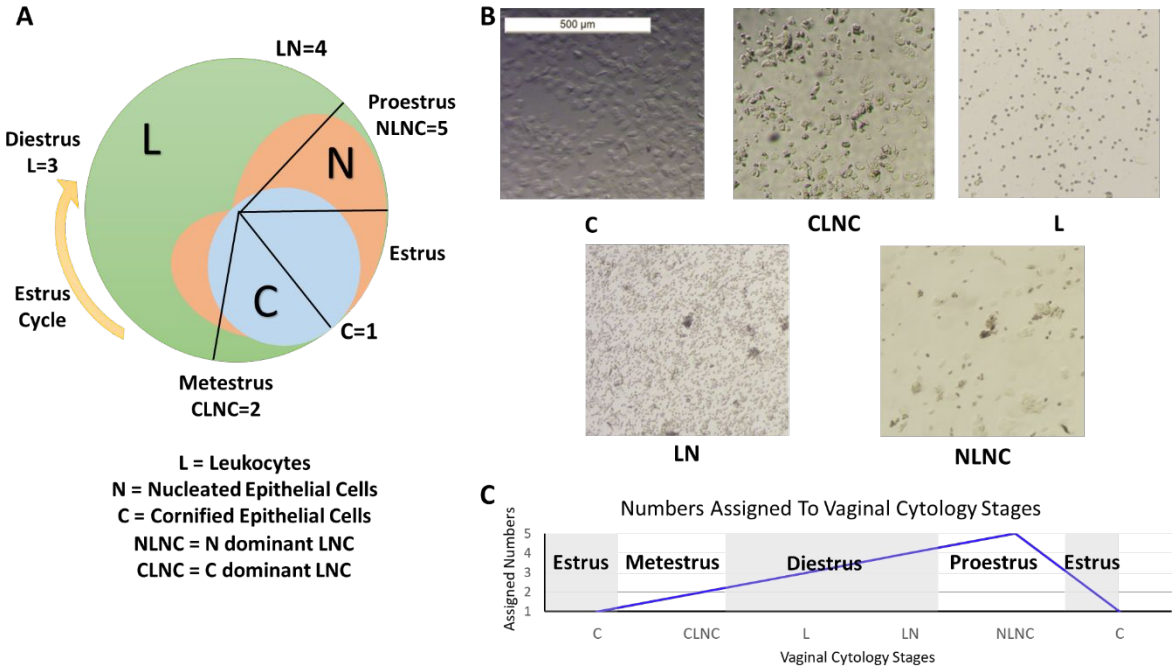
SI Fig. 1. Illustration for the procedure of the entire mFSH assay. Briefly, ~40 μL of blood was collected from mouse tail vein for each measurement. ~20 μL of serum could be collected after centrifugation and subsequently diluted to 40 μL with 50% FBS in 1% BSA. 30 μL of the diluted sample was added to a well in the reagent reservoir plate. Finally, the mFSH was analyzed using the automated ELISA instrument. Note that during the review of the manuscript, a newer version of the instrument was developed, in which only 15 μL is needed. Consequently, the above 1:1 dilution can be avoided.



SI Fig. 2. The typical dynamic ranges for mFSH RIA varies between 1.6-35 ng/mL and 3-75 ng/mL with logistic regression. When plotting the calibration points in the log-log scale, they become mostly linear within 5-75 ng/mL (10-75 ng/mL in some cases) with a slope of approximately -0.84. Both of them are much smaller than the dynamic range of the microfluidic chemiluminescent ELISA technique, which is 1-250 ng/mL with a linear regression model in the log-log scale (a slope around 0.77 with ~5 % inter-assay variance).



SI Fig. 3. Comparison between the readings measured with our ELISA system and the traditional RIA done at the University of Virginia. The results generated with these two methods generally have the same trends and most of them appears to be comparable. However, three of the data points do not correlate very well. The outliers are labeled with circles. The RIA result for one of the outliers (indicated by the red circle) is believed to have low reliability, because 25 ng/mL does not appear to be a correct serum mFSH concentration for a healthy adult mouse, which normally has an mFSH level lower than 20 ng/mL.



SI Fig. 4. The rule for assigning values to different stages in an estrus cycle. (A) The structure of a typical mice estrus cycle (from vaginal cytology) and the corresponding assigned values for each stages. (B) Exemplary vaginal cytology images for each stage. (C) A graphical illustration of value assigning strategy and the correlation between vaginal cytology readout and the stages in an estrus cycle.