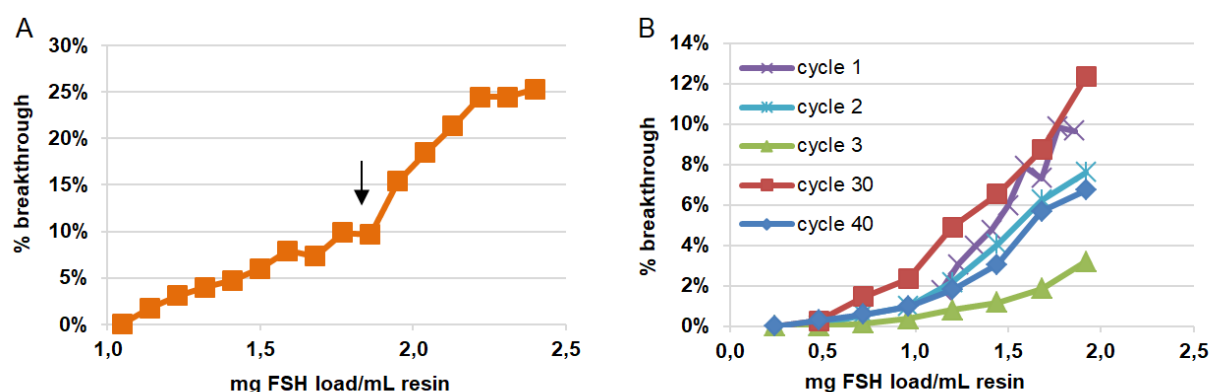
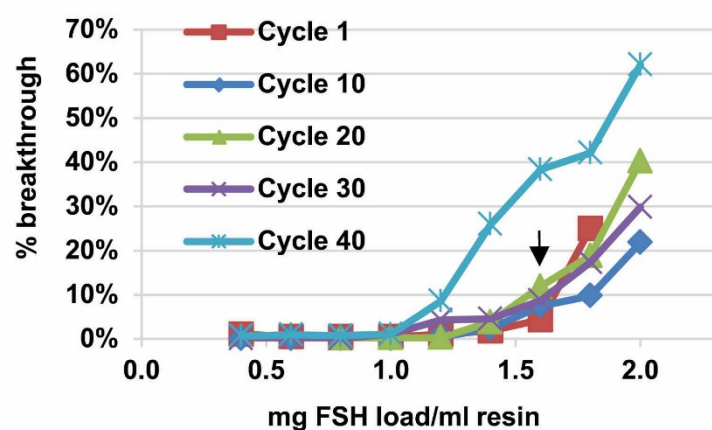


# Supplementary Materials: Purification Process of a Recombinant Human Follicle Stimulating Hormone Biosimilar (Primapur®) to Yield a Pharmaceutical Product with High Batch-to-Batch Consistency

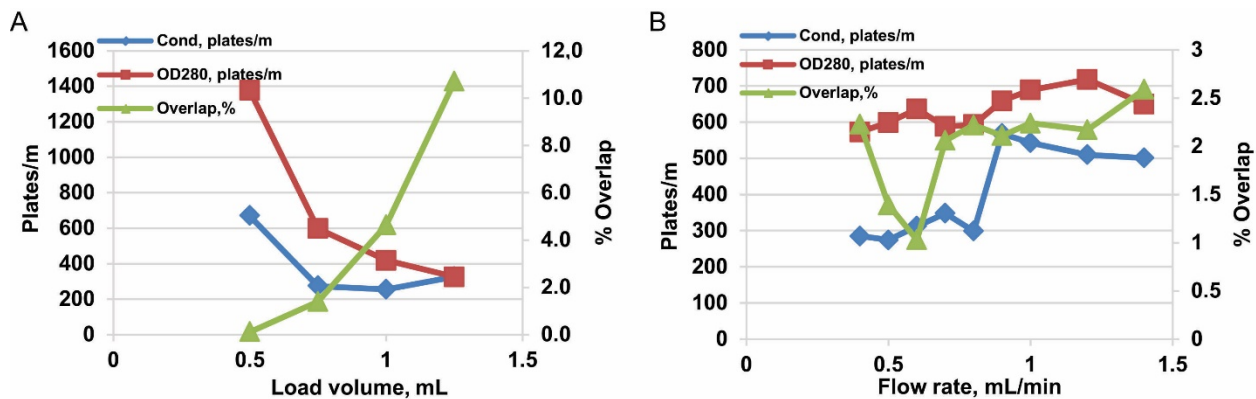
Maria Sinegubova, Ivan Vorobiev, Anatoly Klishin, Dmitry Eremin, Nadezhda Orlova, Natalya Orlova and Mikhail Polzikov



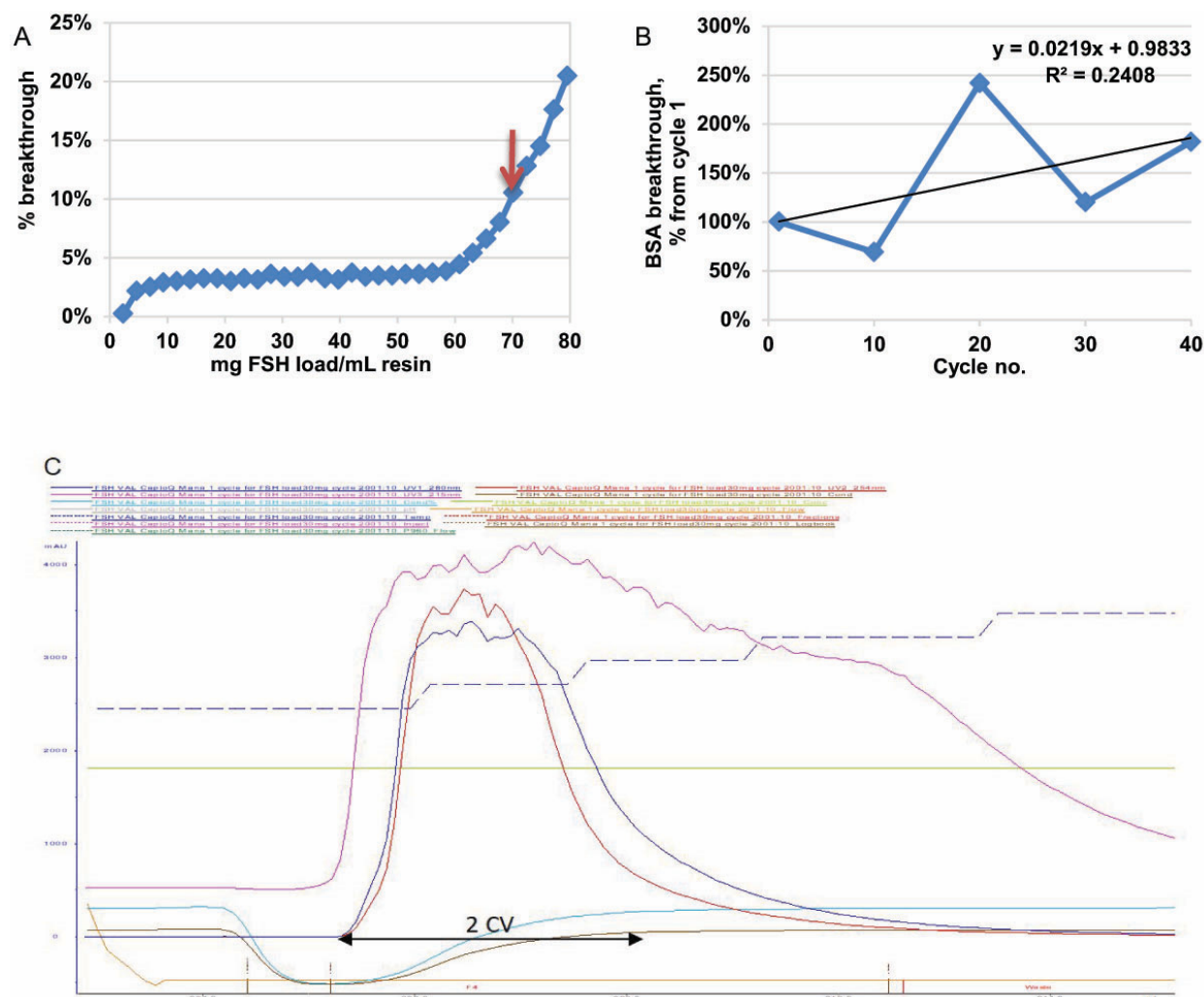
**Figure S1.** Follicle stimulating hormone (FSH) breakthrough curves for dynamic binding capacity (DBC) determination of Capto MMC resin. FSH concentration measured by enzyme-linked immunosorbent assay (ELISA). (A) Control cycle 1. Load 2.5 mg FSH/mL. Residence time 2 min, Tricorn 5/200 column, bed volume 4mL. The arrow indicates the DBC at 10% breakthrough ( $DBC_{10\%} = 1.9$  mg FSH/mL resin). (B) Control cycles 1, 2, 3, 30, and 40. Load 2.0 mg FSH/mL. Residence time 1 min, Tricorn 5/50 column, bed volume 1 mL.



**Figure S2.** FSH breakthrough curve for DBC determination of CaptureSelect FSH Affinity Matrix. FSH concentration measured by ELISA. Control cycles 1, 10, 20, 30, and 40. Load 2.0 mg FSH/mL. Residence time 5 min. Tricorn 5/50 column, bed volume 1mL. The arrow indicates the DBC at 10% breakthrough ( $DBC_{10\%} = 1.6$  mg FSH/mL resin) as determined for cycles 1–30.



**Figure S3.** Load volume optimization for Sephadex G25 Medium resin. Tricorn 5/200 column, bed volume 4mL. Applied 0.5 mg/mL bovine serum albumin (BSA) in 2M MgCl<sub>2</sub>. Detection in conductivity and optical density (OD) at 280 nm channels. **(A)** Peak overlapping and resolution for elution peaks under various load volumes (0.5-1.25 mL). **(B)** Peak overlapping and resolution for elution peaks under various flow rates (0.4–1.4 mL/min), load volume 0.75 mL.



**Figure S4.** Optimization of ion-exchange chromatography. Tricorn 5/50 column, bed volume 1 mL, CaptoQ resin. **(A)** FSH breakthrough curve for dynamic binding capacity (DBC) determination of Capto Q resin. Load 80 mg FSH/mL resin. FSH concentration in flowthrough measured by absorption at 280 nm. The arrow indicates the DBC at 10% breakthrough (DBC<sub>10%</sub> = 70 mg FSH/mL resin). **(B)** Estimation of the DBC change: BSA breakthrough in control cycles in absorbance channel at 280 nm. Load 320 mg BSA/mL resin. **(C)** Estimation of FSH elution peak volume. Loaded 30 mg FSH/mL. The elution peak volume is 2 mL, or 2 column volumes (CV, arrowed). The criterion for stopping the collection of the eluate was 5% of the peak height in absorbance channel.