# Endometrial cycle phase -specific cortisol metabolism and local paracrine regulation of cortisol action in the endometrium

Kalle T. Rytkönen[1,2,*], Sebastian Zúñiga Norman[1,2], Arttu Junnila[1], Nea E. Nygård[1], Ida-Marija Hyvönen[1], Taija Heinosalo[1], Antti Perheentupa[1], Leena Strauss[1], Laura L. Elo[1,3], Matti Poutanen[1]

[1]Turku Bioscience Centre, Tykistökatu 6, 20520, Turku, Finland, [2]Research Centre for Integrative Physiology and Pharmacology, Institute of Biomedicine, University of Turku, Turku, Finland, [3] Institute of Biomedicine, University of Turku, Finland. *katury@utu.fi

## Background

Glucocorticoid signaling has been recognized as a regulator of endometrial function[1], and synthetic glucocorticoids are now being explored as treatment option for heavy menstrual bleeding[2] ref2. However, the menstrual cycle-specific regulation and cell type -specific mechanisms of glucocorticoid action remain poorly understood.

## Methods

Serum and endometrial tissue steroidomics was conducted with mass spectrometry and tissue cortisol levels were correlated with transcriptomics in endometrial biopsies across the menstrual cycle. Bulk RNA was extracted and analyzed with Deseq2 with standard protocols. Public single-cell transcriptomics data [3,4] were utilized to analyze cell type -specific glucocorticoid pathway with Seurat v5.0.1[5], including Harmony [v1.2], and clusters were annotated with markers from the original publications. In ongoing work, we will confirm the results with IHC in uterine biopsies.

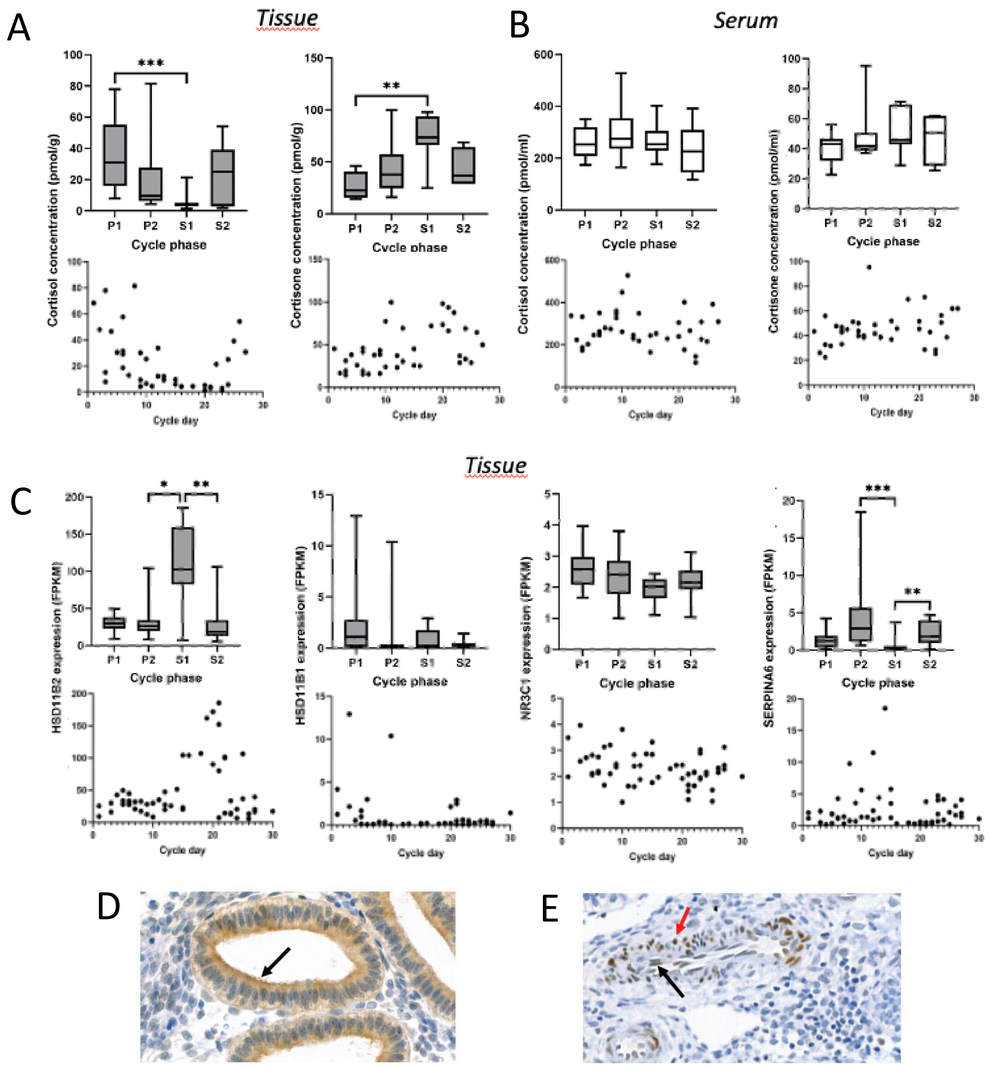


**Figure 1 Endometrial tissue (A) and serum (B) cortisol and cortisone consentrations across the menstrual cycle. P1=menstrual/early proliferative, P2=late proliferative S1=early secretory/implantation window, S2=late secretory, matched n=11, 13, 6, 6. (C) Transcription of key key cortisol metabolizing enzymes (HSD11B2, HSD11B1), glucocorticoid receptor (GR,NR3C1) and corticosteroid binding globulin (CBG, SERPINA6. (D) IHC for HSD11B2 (14192-1-AP, Proteintech), arrow indicates epithelia. (E) IHC for GR (D6H2L, Cell Sign.), black and red arrows indicate predicted endothelia and perivascular cells. ANOVA: *** p<0.001, ** p<0.01, * p<0.05.**

## Results

Cortisol levels were low in the early secretory phase (implantation window) and increased during other phases, a pattern absent in serum, highlighting tissue-specific metabolism. Transcription patterns (Fig. 1) and correlation analyses suggested that HSD11B2 predominantly governs endometrial cortisol levels (HSD11B2 r = –0.43 vs. HSD11B1 r = 0.11). We also identified elevated expression of corticosteroid-binding globulin (CBG (SERPINA6) r = 0.35) in the late proliferative phase, indicating another regulatory layer. Transcriptomic results (Fig. 2) indicate that endometrial cortisol metabolism takes place in endometrial epithelia and stroma. Glucocorticoid receptor is expressed in endothelia, perivascular cells, immune cells and to a lesser degree in stroma. Intersection of known GR targets in other tissues predicts targets activated by GR such as FOSL2, DUSP, THBD, ARID5B, and also targets repressed by GR such as PTGDS (Fig. 2E,F).

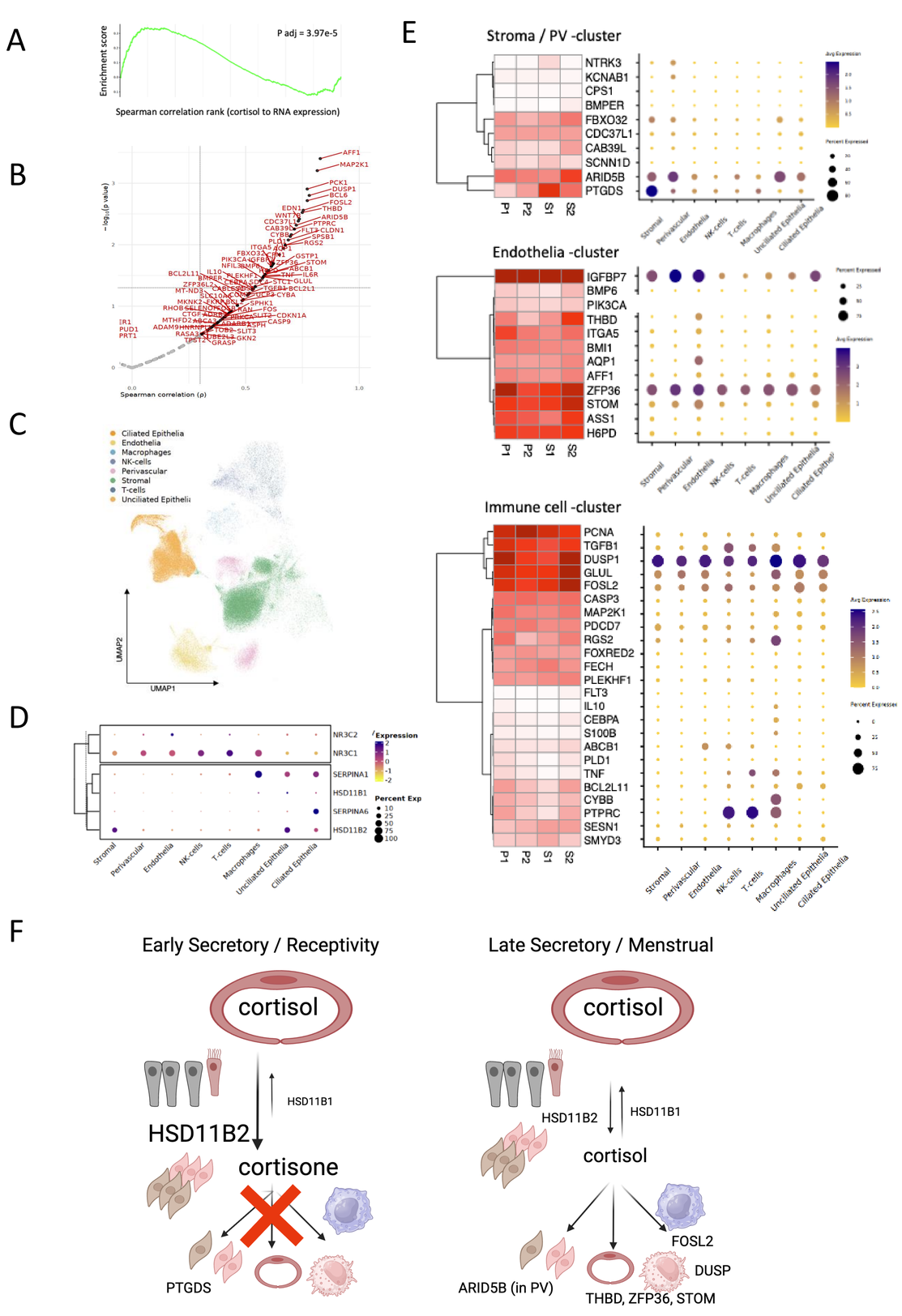


**Figure 2 (A) Known cortisol targets ([6],GOBP corticosteroid pathway) are enriched in the top ranked cortisol to RNA-seq Spearman correlation of the matched samples. (B) Top positively correlating genes during secretory phase. (C) Uterine scRNA-seq data visualized with UMAP and cell type annotations. (D) cell type -specific transcription of the key cortisol pathway genes. (E) Aligned cycle-phase (RNA-seq) and cell type -specific expression (scRNA-seq) of top cortisol correlating that are also cortisol targets ([6],GOBP corticosteroid pathway). Main cell-type clusters of the targets are shown separately based on their scRNA-seq clustering (not shown). (F) Schematic model of cortisol metabolism and action. The cycle phases are shown with simplified binary division based on high and low cortisol levels.**

## Discussion

Our results indicate that endometrial cortisol levels are tightly regulated in the endometrial tissue by HSD11B2 that converts cortisol to cortisone. The integrative analysis suggests paracrine regulation where ligand levels are modulated in stroma and epithelia, whereas glucocorticoid actions mostly take place in the cells of the vascular niche and immune cells. In ongoing studies we validate selected putative targets in these cell types, and aim to conduct ChIP-seq for GR.

## References

**1** McDonald, S.E. *et al Mol Cell Endocrinol.* 248:72-8. (2006) **2** Warner, P. *et al EBioMedicine.* 69:103434 (2021) **3** Garcia-Alonso, L. *et al. Nat. Genet.* 53:1698–1711 (2021) **4** Wang, W. *et al. Nat. Med. 26:1644-1653* (2020) **5** Hao, Y. *et al. Nat. Biotechnol.* 42:293-304 (2024) **6** Choi, D. *et al. Biomolecules,* 13:978 (2023)