Single-cell RNA Sequencing of human endometrium with Asherman's syndrome

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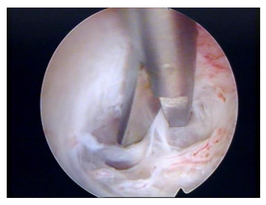
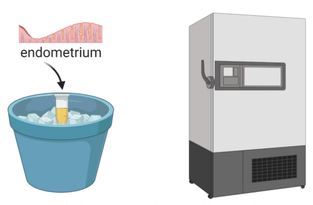
Introduction

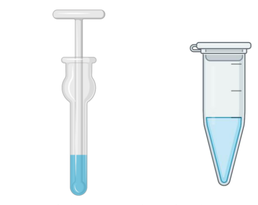
Asherman's syndrome (AS) is characterized by the presence of intrauterine adhesions and clinical symptoms including menstrual cycle disorder , cyclic pain and/or infertility or sterility. The formation of the adhesion is primarily due to intrauterine procedures, especially those connected with pregnancy. At a lower frequency, the synechia may occur after other gynaecological surgeries such as myomectomy or uterine artery embolization. The current gold standard therapy used is hysteroscopic adhesiolysis, but it can still result in reassurances of the syndrome. Here, we focused on the comparison of the endometrium of patient with and without AS. We used single-nucleus RNA Sequencing method combined with hashing antibodies and compared 20 healthy patients with 20 patients with AS.



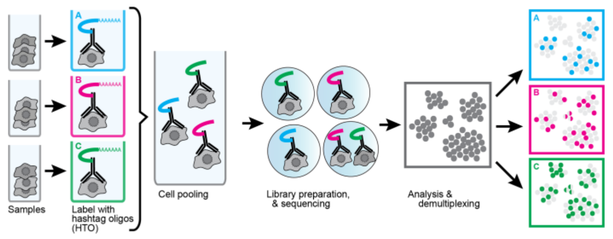
1. Endometrial samples were collected during hysteroscopic adhesiolysis



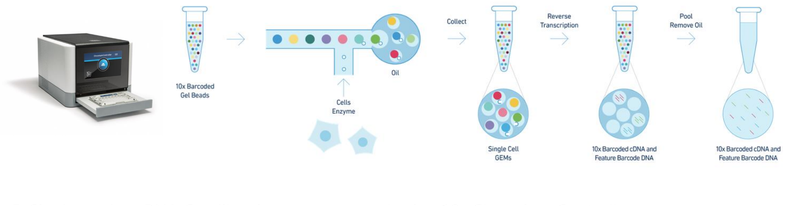
2. Collected tissue was immediately frozen on dry ice and stored at -80°C



3. Nuclei were isolated using Dounce tissue grinder in medium with sucrose and centrifuge



4. Nuclei were hashtaged using Biolegend Totalseq-A antibodies and nuclei from 10 samples were pooled together.



5. Single-nucleus RNA-Seq libraries were prepared using 10x Chromium Controller.

Results

Subject group characteristics

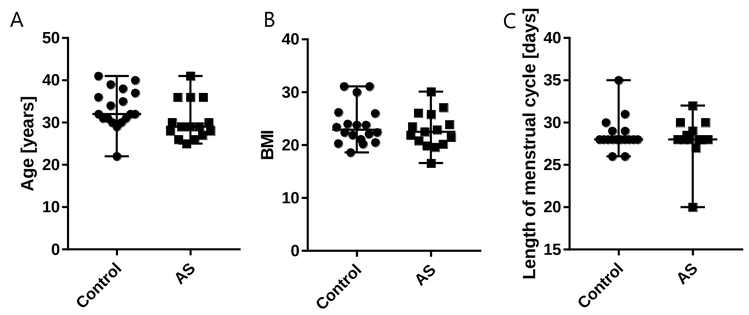


Fig. 1. Characteristics of subject groups. (**A**) Age, (**B**) BMI, (**C**) length of menstrual cycle before surgery.

Pseudo-bulk differential expression analysis in stromal fibroblasts

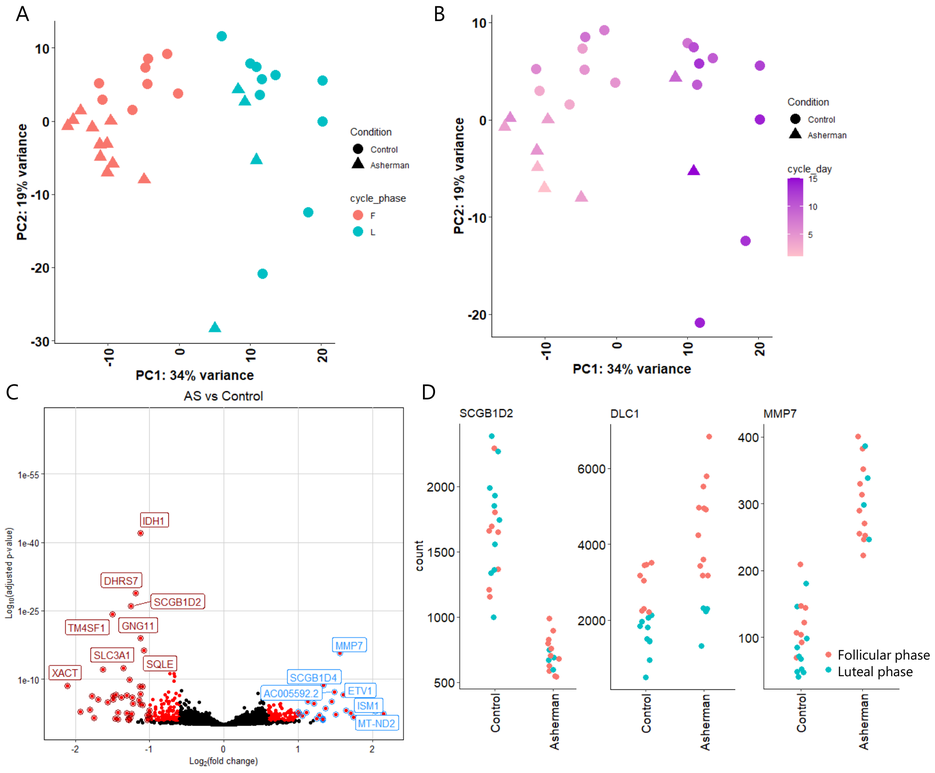


Fig. 6. PCA and DE analysis of population of stromal fibroblasts. (**A**, **B**) PCA plot of pseudobulk population of stromal cells. (**A**) Clear separation of samples based on the phase of menstrual cycle and (**B**) based on the day of the cycle. (**C**) Differential expression analysis identified overexpressed genes connected with scar formation (*MMP7*), cell adhesion (*DLC1*) in AS patient or downregulated genes responsible for steroid hormone binding (*SCGB1D2*). (**D**) Visualisation of expression of these genes.

Quality of isolated nuclei and hashing efficiency

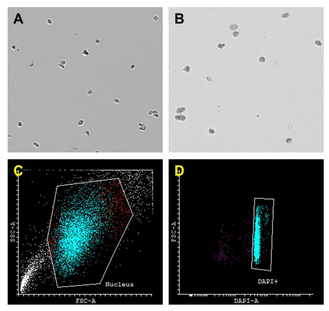


Fig. 2. Quality control of single-nucleus suspension obtain from human endometrium. (**A**) nucleus without staining (**B**) nucleus stained using 0.4 % Trypan Blue solution (**C**, **D**) cytometry analysis of suspension of nucleus stained using DAPI. Light-blue dots represents high quality singlet's nuclei

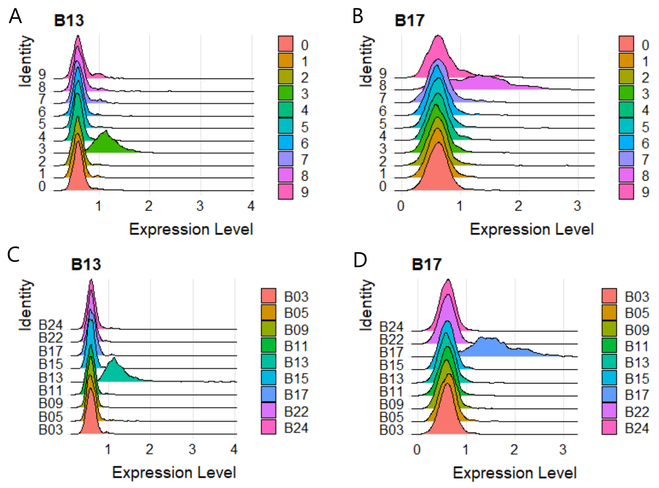


Fig. 3. Identification of demultiplexed samples using freemuxlet (1) and souporcell (2) with based on the hashing antibodies.

(**A**, **B**) Example of two identification of demultiplexed samples in one pool. Samples demultiplexed using freemuxlet without known SNP. Hashing antibodies were used for identification of patient. (**C**, **D**) Final samples after merging 3 demultiplexing strategies.

Identification of major cell types in human endometrium

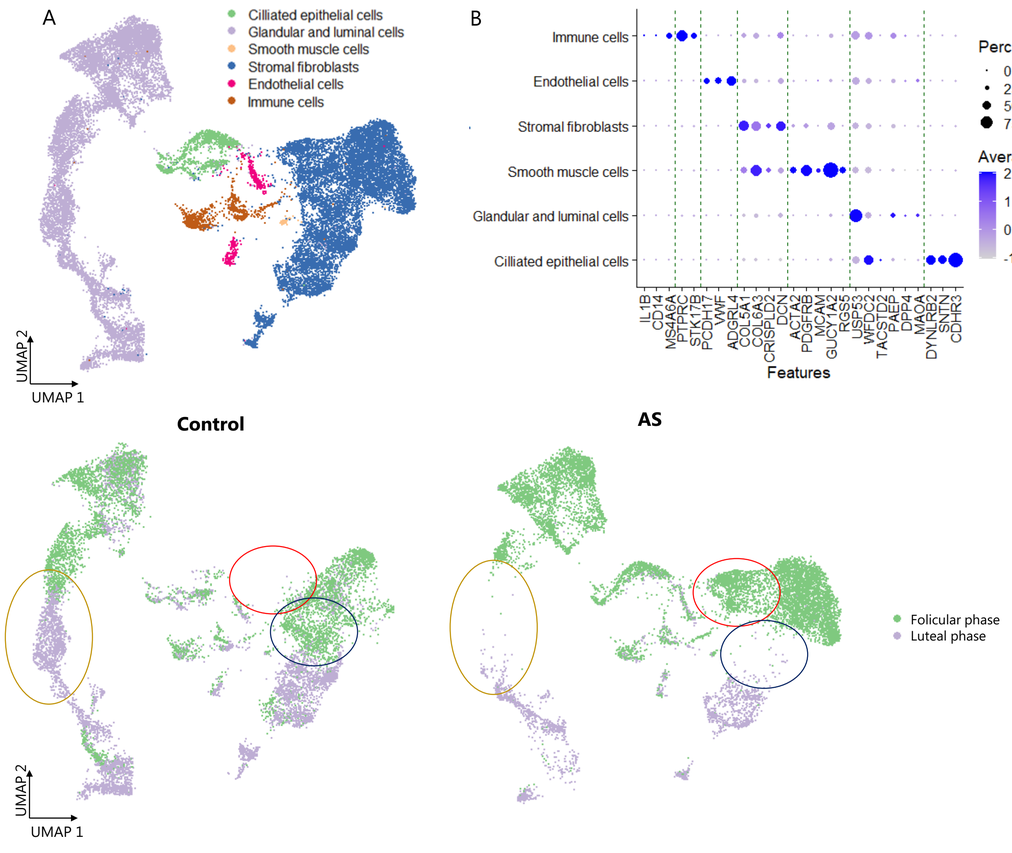


Fig. 4. Annotation of clusters of main cell types. Ten major populations were identified. (**A**) UMAP plot with annotated cell types. (**B**) Dot plot is showing expression level of known markers of main cell types of human endometrium.

Fig. 5. Differences in clustering between cell population from healthy control and AS patient. The main additional population were identified in AS patient only in stromal fibroblasts cells at follicular phase of menstrual cycle. On the other hand AS patient are missing of some subcluster of glandular and luminal cells at luteal phase of menstrual cycle.

Pseudo-bulk differential expression analysis in glandular and luminal cells

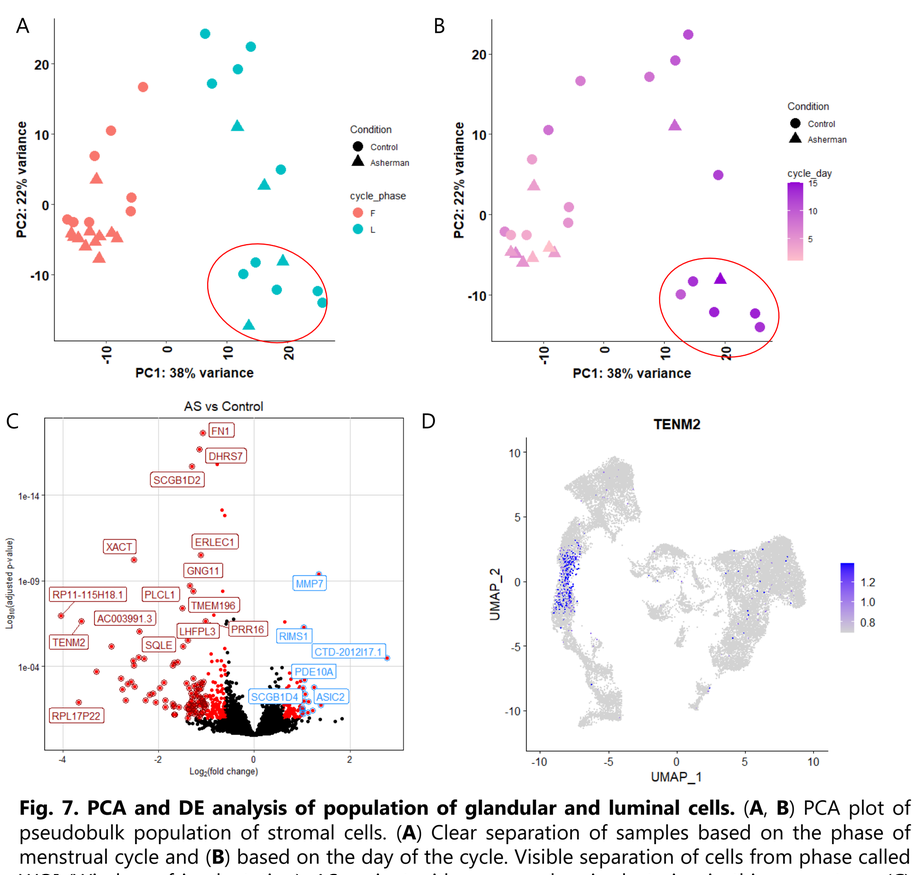


Fig. 7. PCA and DE analysis of population of glandular and luminal cells. (**A**, **B**) PCA plot of pseudobulk population of stromal cells. (**A**) Clear separation of samples based on the phase of menstrual cycle and (**B**) based on the day of the cycle. Visible separation of cells from phase called WOI (Window of implantation). AS patient with ammenorhea is clustering in this group too. (**C**) Differential expression analysis between AS patient and healthy control. (**D**) Visualisation of expression of *TENM2* gene, which regulate cell-cell adhesion.

Conclusion

We have prepared a single-cell libraries from scared human endometrial tissue using cost effective protocol and get about 1000 cells per sample.
The main differences between control and AS samples are observed in stromal fibroblasts.
The processes connected with menstrual cycle are preserved in the endometrium and AS has probably no effect on receptivity of endometrium.

Future plans

- bulk RNA-Seq validation using samples of patient with stronger problems and not included in the single-cell RNA-Seq analysis
- integrative analysis (cell-cell communication, bulk RNA-seq deconvolution, meta-analysis) and network analysis
- identification of targets for potential therapeutic intervention and their manipulation

Ethic statment

All patients agreed with participation in clinical study and signed the written informed consent prior to surgery and sample collection. The study was approved by the hospital Ethical Committee (1443/19 S-IV).

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References

1 - Zhang et al., popscle: A suite of population scale analysis tools for single-cell genomics data (freemuxlet). Software package. https://github.com/statgen/popscle. 2020
2 - Heaton et al., Souporcell: robust clustering of single-cell RNA-seq data by genotype without reference genotypes. Nature Methods, 2020.