A Novel Non-invasive Approach to Sample Female Genital Tract Immune Cell Populations

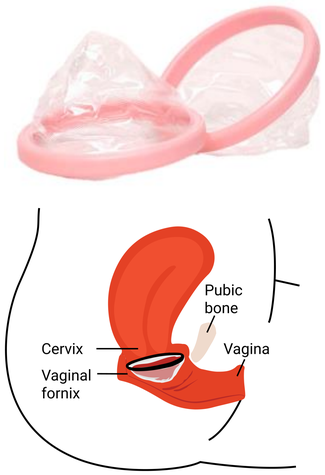
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Background

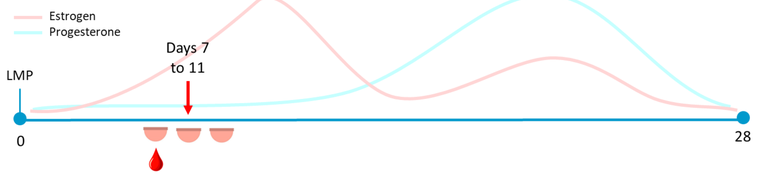
- T cells in the human female genital tract (FGT) are key mediators of susceptibility to and protection from infection, including HIV and other sexually transmitted infections.
- There is a critical need for increased understanding of the distribution and activation of T cell populations in the FGT
- Current sampling methods require a healthcare provider and are expensive, limiting the ability to study these populations longitudinally.
- Menstrual discs are a disposable menstrual product that collects cervicovaginal fluid (secretions from the cervix and fluid pooled in the vaginal fornixes)



Objective

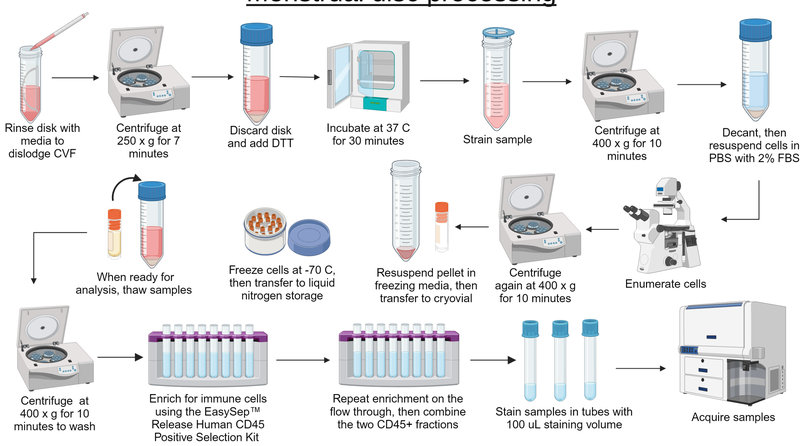
Develop a non-invasive approach to sample the FGT for immune cells

Approach



- 5 healthy, adult, reproductive-aged participants were enrolled
- Each participant donated CVF 3 days in a row, 7 to 11 days from LMP.
- Blood was also drawn and processed for PBMCs

Menstrual disc processing

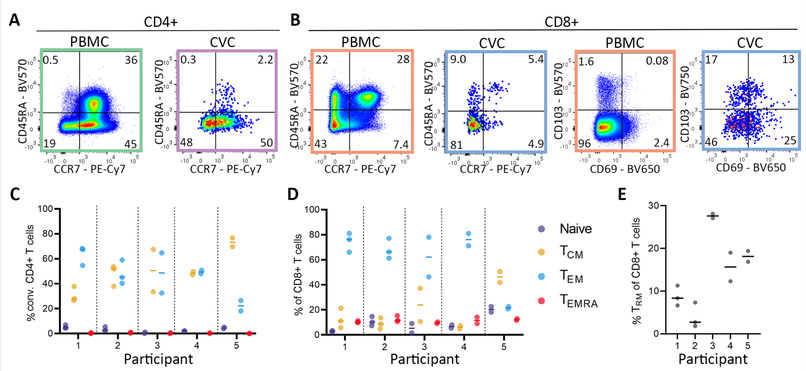


- Samples stained with a published 28-color T cell-directed flow cytometry panel

Immune Cell Recovery from CVF

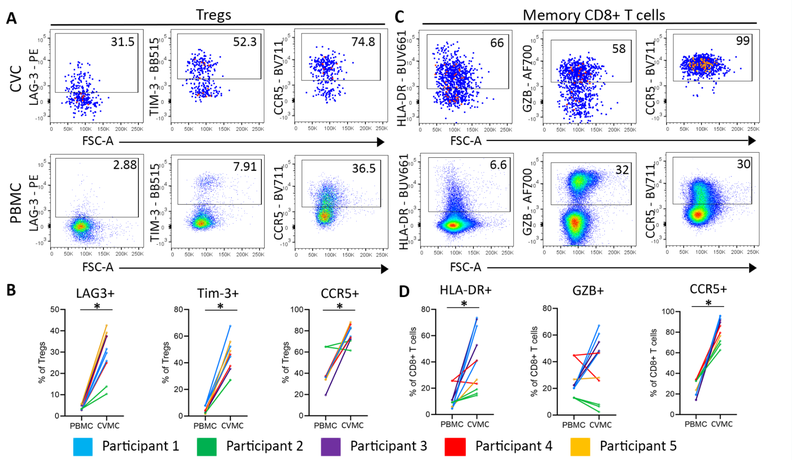
▼**Table 1.** Counts of immune cell populations across CVF samples.

Participant	1			2			3			4			5			
Sample	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	Median
CD45+	14,772	11,157	57,703	4,533	13,401	20,587	1,225	2,036	11,860	6,233	212	31,479	2,885	965	2,762	6,233
CD3+	3,174	1,182	11,554	1,052	1,966	3,512	38	392	3,897	1,808	12	7,823	521	56	384	1,182
CD4+	2,037	938	7,484	518	1,197	1,460	14	223	2,037	1,567	5	5,449	350	34	271	938
CD8+	902	157	3,404	191	331	659	21	118	1,387	163	2	968	77	4	31	163
CD19+	2,166	727	3,638	1,072	2,070	2,599	51	456	1,220	1,548	8	4,690	170	25	201	1,072
CD3/CD19-HLA-DR+	5,539	4,082	12,849	605	3,052	3,726	482	392	3,019	611	32	3,251	775	64	452	775



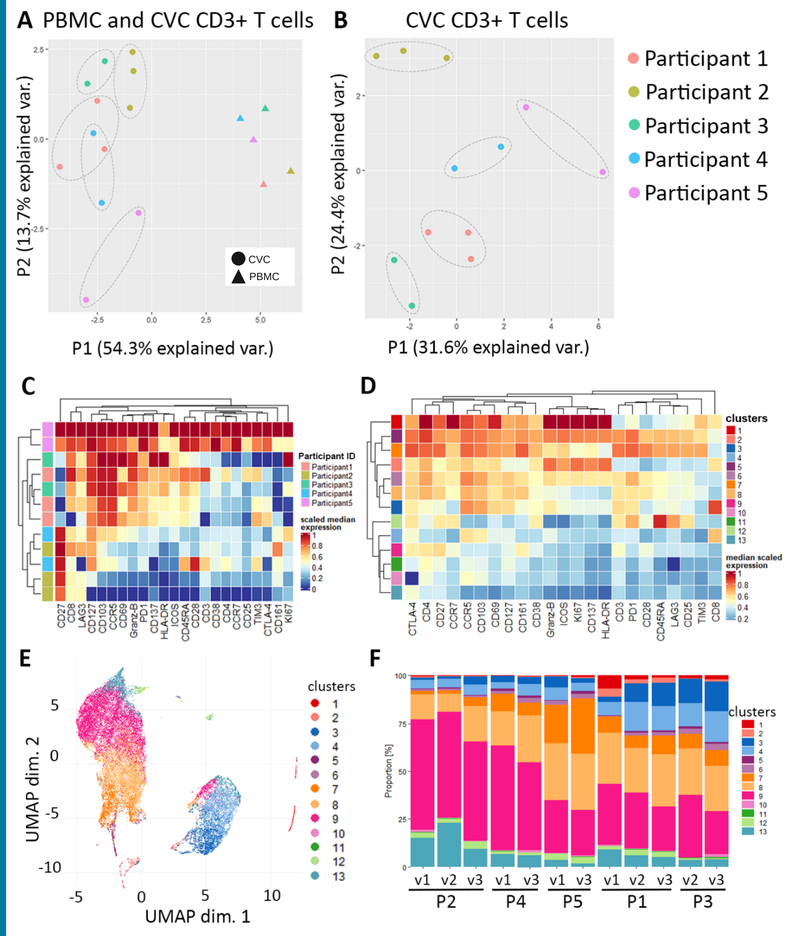
▲**Figure 1**. Overview of CVC T cell populations. Data from five individuals sampled across three days. **(A)** After gating out Tregs (CD25^{high}, CD127^{low}), conventional CD4+ T cells were further gated by CCR7 and CD45RA to define T_{EM}, T_{CM}, T_{EMRA}, and T_N, populations for PBMC (left) and CVC (right) samples. **(B)** CD8+ T cells were also gated by CCR7 and CD45RA to determine memory subtypes, as well as by CD69 and CD103 to determine tissue residency (CD69+ CD103+). **(C)** Frequency of conventional CVC CD4+ memory subtypes across samples. **(D)** Frequency of CVC CD8+ memory subtypes across samples. **(E)** Frequency of CVC CD8+ T_{RM} across samples.

Activation and Suppression of CVC T cells



▲Figure 2. Characterization of key CVC T cell populations. Data from five individuals sampled across three (CVC) and one (PBMC) day. Results for samples with greater than 50 cells in the parent population for each analysis are presented. The mean CVC frequency of each population of interest across sampled days was compared to the PBMC frequency using a Wilcoxon signed rank test. Star indicates p value less than or equal to 0.05. **(A)** Representative flow plots of activation and suppression marker expression from paired CVC and PBMC CD4+ Treg populations. **(B)** Summary plots of frequencies of activation and suppression markers between paired CVC and PBMC CD4+ Treg populations. **(C)** Representative flow plots of activation and suppression marker expression from paired CVC and PBMC memory CD8+ T cells. **(D)** Summary plots of frequencies of activation and suppression markers between paired CVC and PBMC memory CD8+ T cells.

Reproducibility of T cell Population Structure



▲Figure 3. CVC T cell populations are reproducible within individuals but vary across individuals. Data from five individuals sampled across three (CVC) and one (PBMC) day. Results for samples with greater than 100 cells in the parent population for each analysis are presented. **(A)** PCA utilizing high parameter flow data to characterize paired CVC and PBMC CD3+ memory T cell populations. **(B)** PCA characterizing CVC CD3+ memory T cell populations. **(C)** Unsupervised hierarchical heat map characterizing the median expression of CVC T cell markers across individuals and samples **(D)** Heat map characterizing the median expression of T cell markers in each identified cluster. **(E)** Dimensional reduction of all markers relevant to T cells was performed and is represented as a UMAP including all CD3+ T cells across CVC samples. **(F)** Proportions of cell clusters composing each sample, arranged by participant and visit.

Conclusions

- We have established a low cost, self-applied method that will facilitate longitudinal studies and reduce the need for participants to interface with a clinical setting
- This method is reproducible and allows for cryopreservation with sufficient cell recovery

Future Questions

- How does the phenotype and function of cells isolated from CVF compared to tissue?
- What is the role of the luminal B cell population?
- How do longitudinal factors such as the vaginal microbiome, menstrual cycle, and menopause modulate T cell milieu?
- Can we assess mucosal vaccine responses with this method?