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## OBJECTIVE:

It is known that human endometrial receptivity is affected by T cell subpopulations and CD4+ (T-helpers) / CD8+ (T-killers) ratio. To ensure successful embryo implantation, an active state of maternal immune tolerance mediated by regulatory T cells in the endometrium is essential.

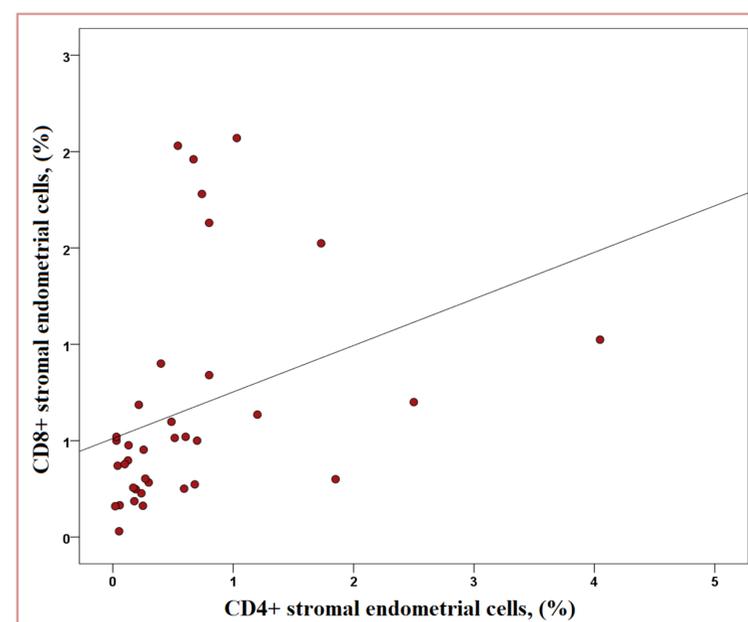
The purpose of this study was to quantify and compare endometrial CD4+ (T-helpers) and CD8+ (T-killers) cells during the mid-luteal phase in women, with repeated implantation failures (RIF) using immunohistochemistry.

## MATERIALS AND METHODS:

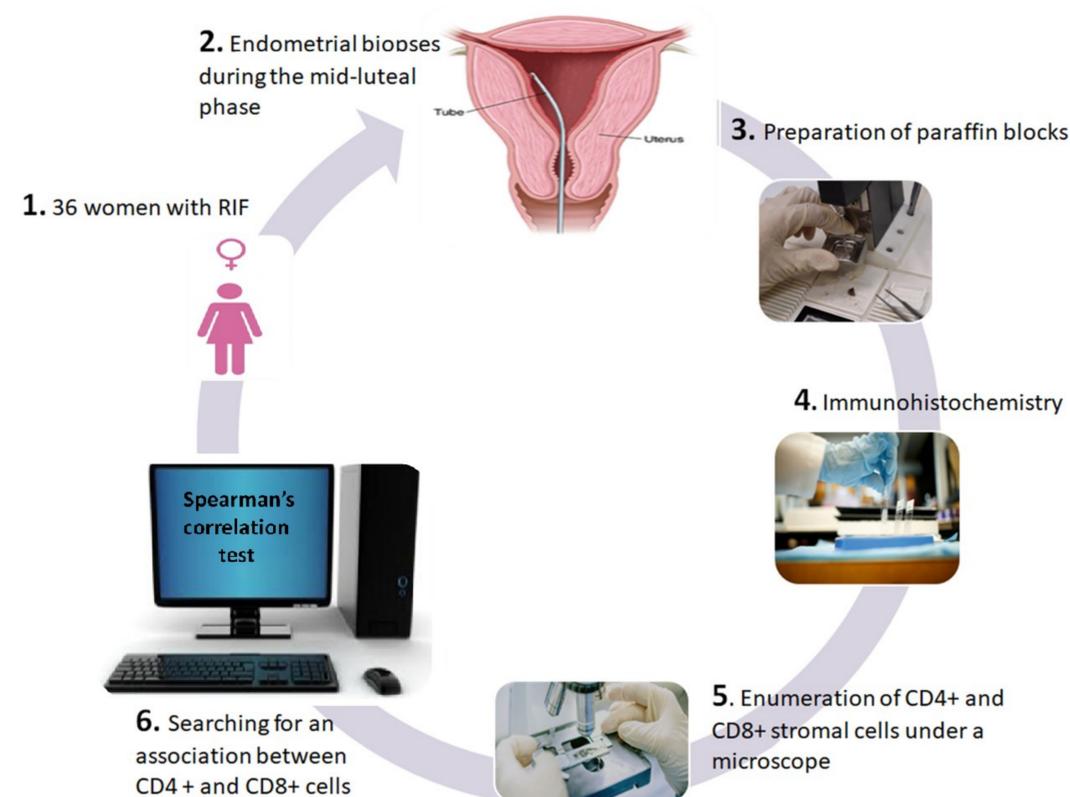
- Endometrial biopsies from 36 women with RIF during the mid-luteal phase of the cycle.
- Immunohistochemistry of CD4+ and CD8+ positive T cells with rabbit polyclonal CD4 antibody (E-AB-65682, Elabscience) and rabbit polyclonal CD8 antibody (E-AB-60717, Elabscience)
- Visualization by Novolink Max Polymer Detection System (RE7280-K, Leica).
- Results are presented as percentage of CD4+ or CD8+ positive cells of the total number of stromal cells.
- Spearman's correlation test by SPSS v.21 (IBM Corp., Armonk, NY, USA).

## RESULTS:

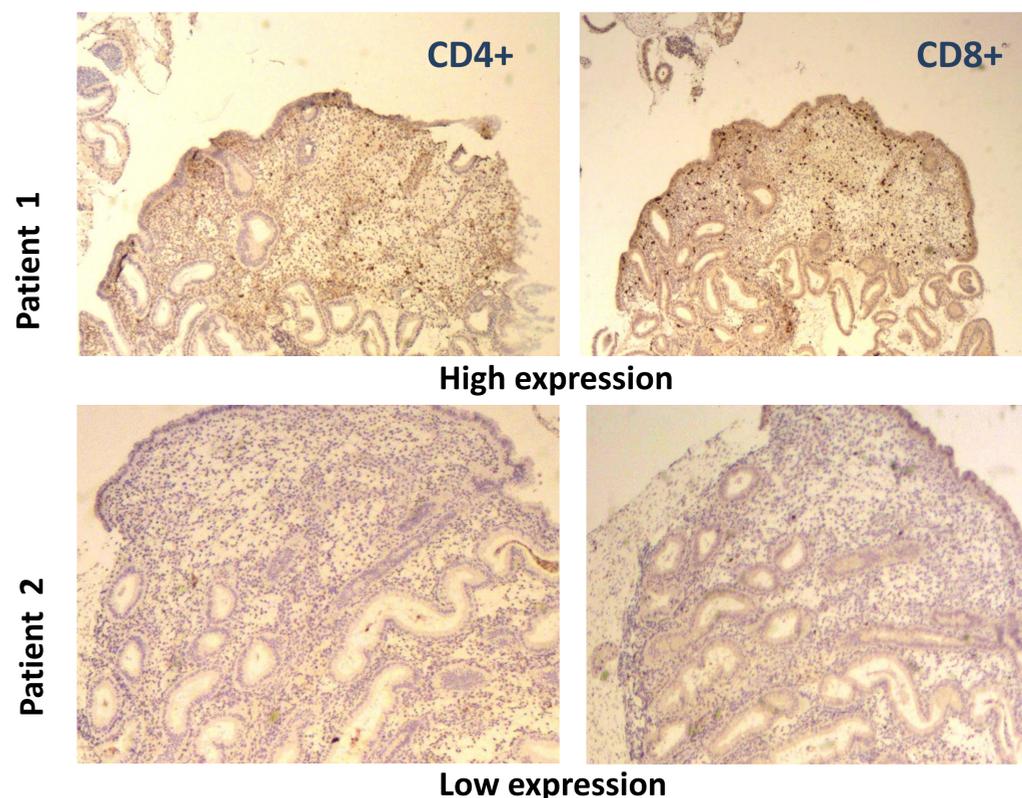
CD4+ and CD8+ cells were found in all endometrial samples. The percentage CD4+ cells and CD8+ cells in the endometrial stroma varied between 0.01% and 4.05%, and between 0.16 and 1.52%, respectively. The mean CD4+ percentage and CD8+ percentage were  $0.58\% \pm 0.84\%$  and  $0.4526\% \pm 0.30\%$ , respectively. Spearman correlation analysis showed significant positive correlation between the percentage of CD4+ and CD8+ cells in the endometrial samples of the studied RIF patients ( $r=0.52$ ;  $p=0.01$ ).



**Fig. 2** Scatter plot between the percentage of CD4+ stromal endometrial cells and CD8+ stromal endometrial cells during the mid-luteal phase of the cycle.



**Fig. 1** Schematic presentation of the experimental design



**Fig. 3** Representative immunohistochemical staining and view of the endometrium with CD4 and CD8-positively stained cells in the endometrial stroma of two patients (100x magnification).

## CONCLUSIONS:

Our study showed that CD4+ cells (T-helpers) are more abundantly distributed than CD8+ (T-killers) in the endometrial stroma of RIF patients during the mid-luteal phase. The observed association between CD4+ and CD8+ could provide a valuable data on the endometrial receptivity.