

Decreased production of TNF- α and IL-6 inflammatory cytokines in non – pregnant idiopathic RPL women. (Immunomodulatory effect of sildenafil citrate on the cellular response of idiopathic RPL women).

Monika Kniotek¹, Michał Zych¹, Aleksander Roszczyk¹, Monika Szafarowska², Małgorzata Jerzak²

¹Department of Clinical Immunology, Transplantation Institute, Medical University of Warsaw, Nowogrodzka 59, Warsaw, 02-006, Mazovian Voivodeship, Poland

²Department of Gynecology and Oncological Gynecology, Military Institute of Health Sciences, Szaserów 128, Warsaw, 04-141, Mazovian Voivodeship, Poland

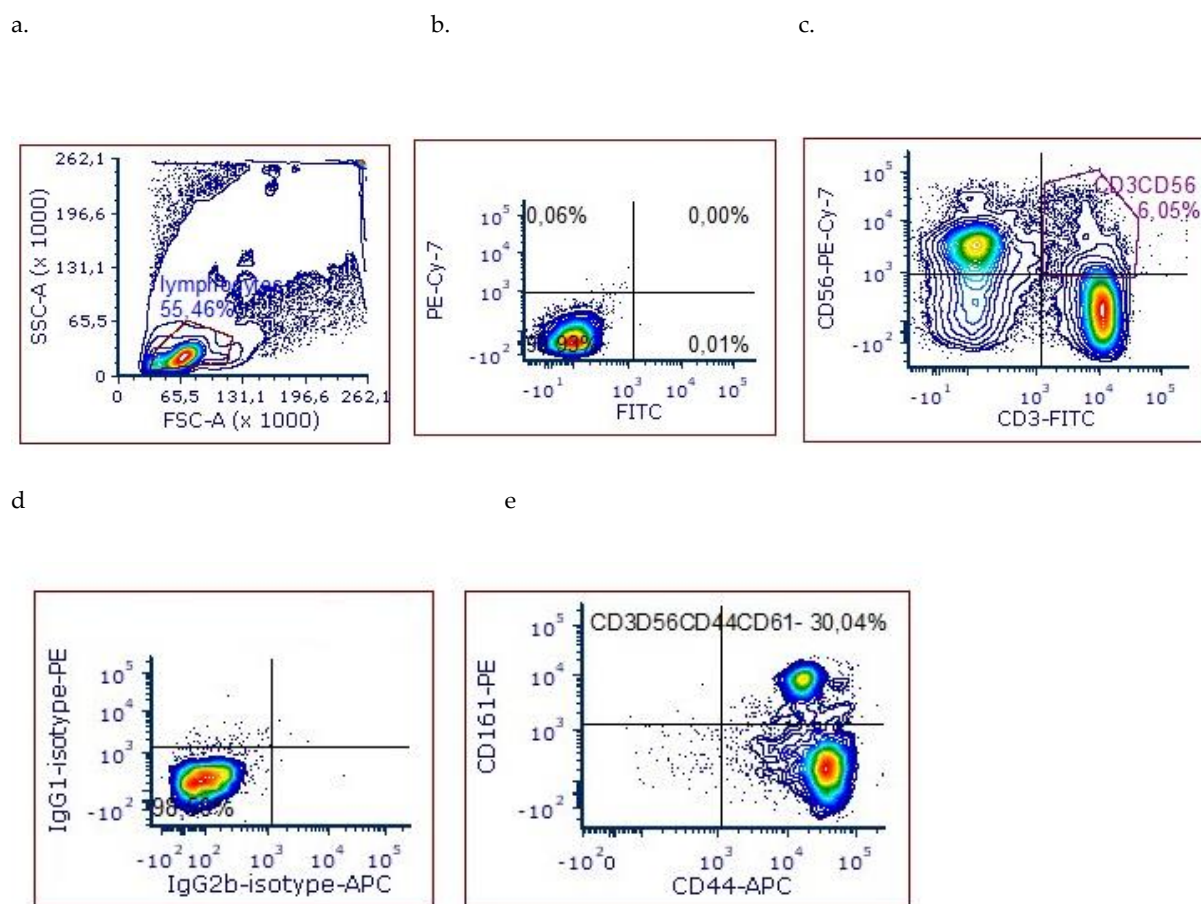


Figure S1. Gating strategy for CD3CD56CD44CD161 NKT cells. a) a region based on forward light scatter (FSC) versus side scatter (SSC) was drawn around the major lymphocyte population, b) unstained cells serve as fluorescence control for CD3CD56 positive cells, c) CD3CD56-positive cells among lymphocytes were identified as Pe-Cy-7 (fluorescence above 10³ decades on the y-axis) and CD56-FITC positive cells (fluorescence above 10³ decades on the x-axis), d) isotype controls for CD161-PE and CD44-APC antibodies were used to set the boundaries of specific fluorescence, e) among gated CD3⁺CD56⁺ cells CD161-PE (the red fluorescence above 50x10³ decade) and CD44-APC cells (fluorescence above 10³ decades for APC) were identified.

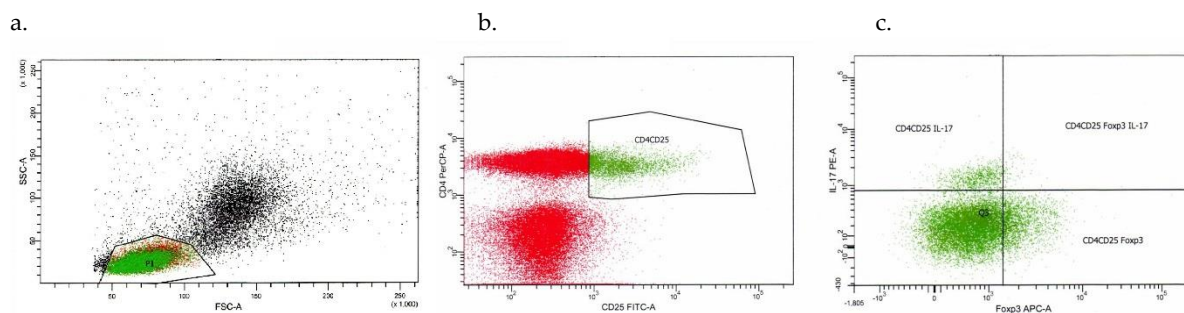
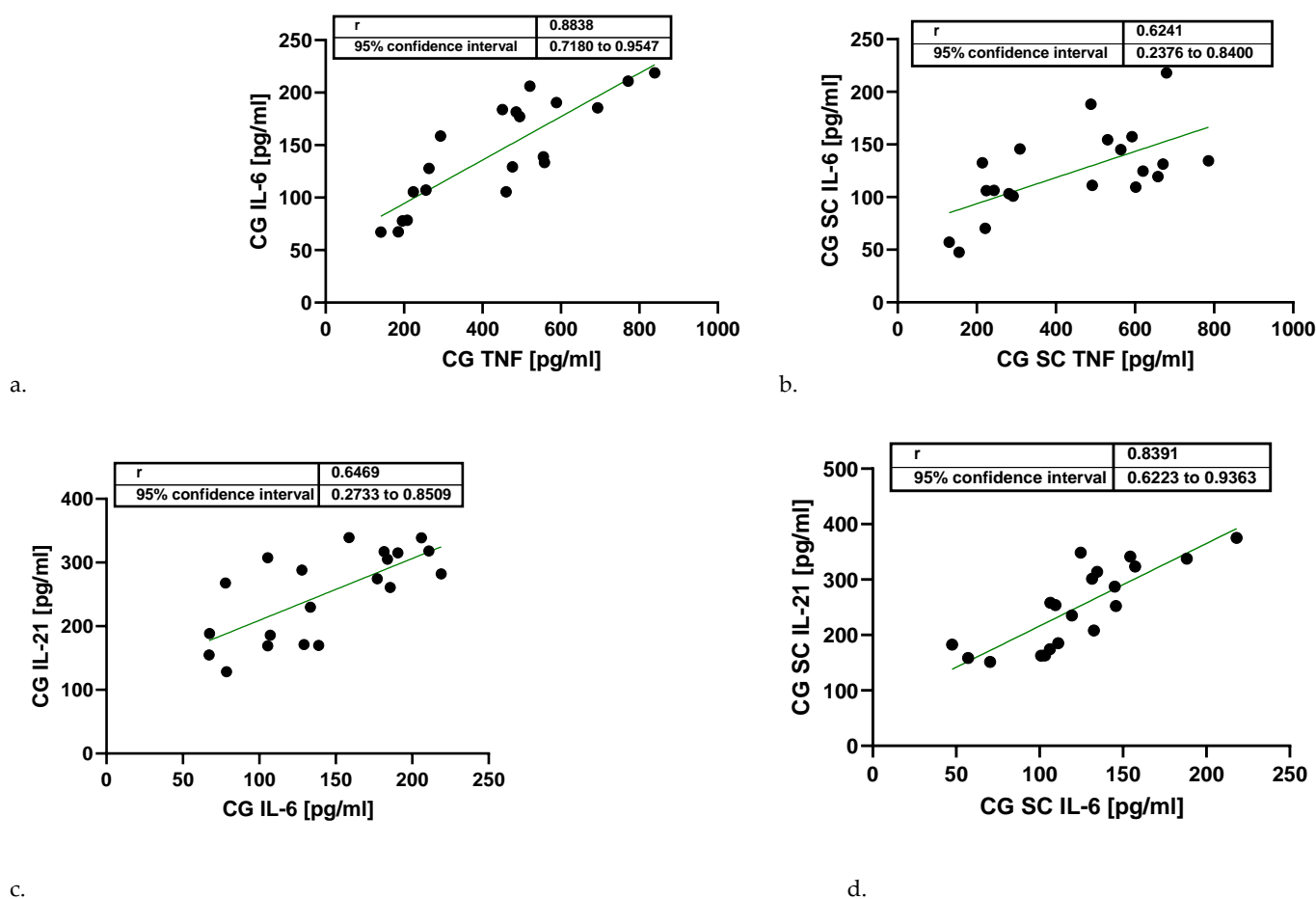
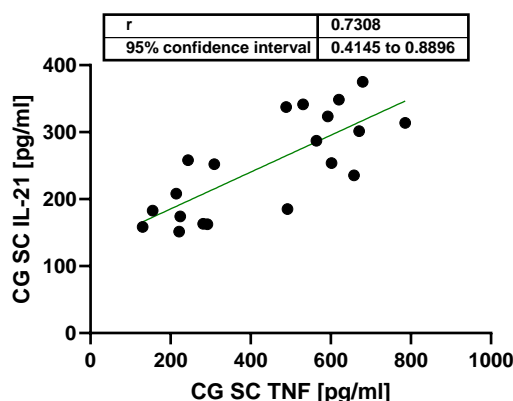
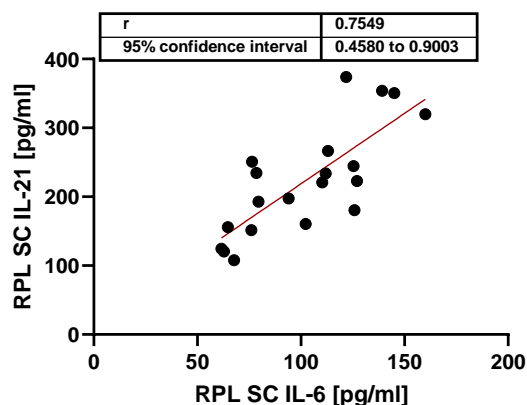


Figure S2. Gating strategy for CD4⁺CD25⁺Fopx3⁺ and CD4⁺CD25⁺IL-17⁺ lymphocytes enumeration, a) lymphocytes were identified using FSC/SSC dot plot, b) CD4CD25-positive cells among lymphocytes were identified as PerCP (above 10³ decade on y axis) and CD25-FITC positive cells (fluorescence above 10³ decade on x axis) (green gate), c) then among gated CD4⁺CD25⁺ cells IL-17-PE (the red fluorescence above 10³ decade) and Fopx3-APC cells (fluorescence above 1x10³ decade for APC) were identified.





e.



f.

Figure S3. Correlations between the concentration of pro-inflammatory cytokines in culture supernatants before and after supplementation with 400 ng/mL of SC, spearman's rank correlation coefficients. green or red line - "R_s" Spearman correlation coefficient, a - e - control group, f - RPL patients, CG-control group, RPL – recurrent pregnancy loss patients, SC – 400ng/ml of sildenafil citrate.

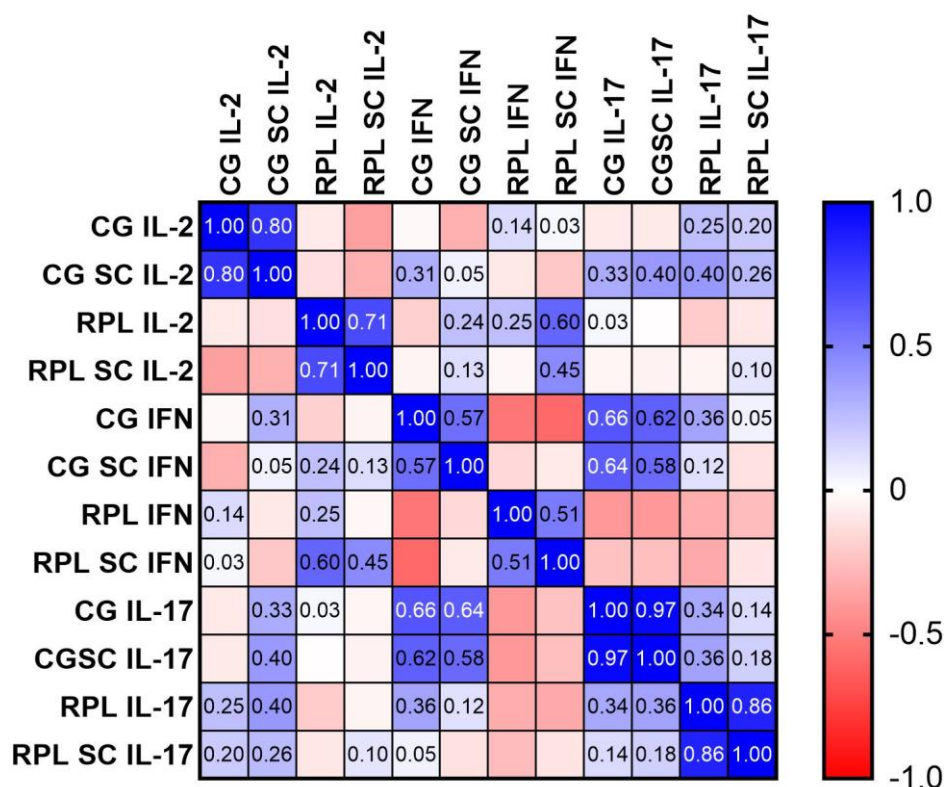


Figure S4. Pearson's "r" factor correlations between inflammatory cytokines where the concentration of the cytokine was similar in CG and RPL group before and after supplementation with SC. The blue colors stand for a positive correlation – $r > 0$, red colors for a negative correlation – $r < 0$, (CG – control group, RPL – idiopathic recurrent pregnancy loss patients, SC – sildenafil citrate).

Table S1. The concentration (pg/mL) of selected cytokines in the culture supernatants of the PBMC cells of fertile and idiopathic RPL women cultured with and without 400 ng/mL sildenafil citrate. Data presented as mean and \pm SD in case

of the normal distribution of data, or median and IQR1-IQR3 in case of the non-normal distribution of data (CG – control group, n=20; RPL – study group, n=21; SC – sildenafil citrate).

Concentration of cytokines (pg/ml)	Fertile women PBMC (CG)	Fertile women - PBMC (CG) + 400 ng/ml SC	p value CG vs. CG SC	RPL PBMC	RPL PBMC + 400ng/ml SC	p value RPL vs. RPL SC	p value CG vs. RPL
TNF- α	468.3 (247.6-556.0)	490.2 (238.5 - 606.5)	ns	231.0 (210.9-308.3)	254.0 (211.4-307.1)	0.026	0.006
IL-6	136.6 \pm 58.7	132.8 \pm 48.0	0.002	97.35 (71.5-128.3)	106.3 (78.0 - 125.5)	ns	0.007
IL-12p40	8.1 \pm 1.59	7.4 \pm 1.61	0.010	8.11 \pm 2.55	7.2 \pm 2.5	ns	ns
IL-21	271.1 (182.1-309.3)	253.1 (180.6-316.3)	ns	246.2 \pm 92.62	223.2 \pm 79.21	0.078	ns
INF- γ	198.9 (166.5-260.1)	184.7 (157.2-244.3)	ns	176.1 (154.3-211.4)	181.6 (159.8-199.08)	ns	ns
IL-2	102.8 \pm 29.39	104.8 \pm 27.45	ns	111.6 \pm 25.34	110.2 (95.0-114.2)	ns	ns
IL-17	222.6 \pm 72.1	221,0 \pm 79,4	ns	196,6 \pm 67,80	200.9 (187.4-255.4)	ns	ns
IL-4	395 \pm 104.10	385 \pm 105.51	0.078	368.8 \pm 105.35	360.7 \pm 110.91	ns	ns
IL-10	1316 (1250.0- 1444.5)	1283.5 (743.8-1684.1)	ns	1297.1 \pm 361.8	1526.0 \pm 491.7	0.019	ns
TGF- β	355.0 \pm 156.6	424.1 \pm 120.8	0.04	472.7 \pm 130.5	467.3 \pm 121.8	ns	0.02