1	Title: Immune parameters of HIV susceptibility in the female genital tract before and after
2	penile-vaginal sex
3	
4	Avid Mohammadi ¹ , Sareh Bagherichimeh ^{1*} , Yoojin Choi ² , Azadeh Fazel ¹ , Elizabeth
5	Tevlin ^{3†} , Sanja Huibner ¹ , Sara V Good ⁴ , Wangari Tharao ³ , Rupert Kaul ¹
6	
7	Affiliations:
8	¹ Department of Medicine, University of Toronto, Toronto, Ontario, Canada.
9	² Department of Immunology, University of Toronto, Toronto, Ontario, Canada.
10	³ Women's Health in Women's Hands Community Health Center, Toronto, Ontario, Canada.
11	⁴ Department of Biology, University of Winnipeg, Winnipeg, Manitoba, Canada
12	* Present address: Department of Pathology and Laboratory Medicine at Schulich Medicine and
13	Dentistry, University of Western, London, Ontario, Canada.
14	Present address: Street Health Community Nursing, Toronto, Ontario, Canada.
15	Materials and Correspondence: Dr. Rupert Kaul and/or Mrs. Avid Mohammadi. Department
16	of Medicine, University of Toronto, Medical Sciences Building #6356, Toronto, Ontario,
17	Canada, M5S 1A8. Tel: (1-416) 978-8607; Fax: (1-416) 978-8765. <u>Rupert.kaul@utoronto.ca</u> ;
18	Avid.mohammadi@mail.utoronto.ca
19	Conflict of interests: The authors declare no competing interests.
20	

21 SUPLEMENTARY TABLE 1. Participant characteristic in women who had condomless

22 sex based on circumcision status of the male partner

24	Characteristic	Uncircumcised (n=16)	Circumcised (n=13)
25	Age (median; range)	22 (18-33)	21 (19-33)
26	Ethnicity		
27	Asian (East or South Asian)	7 (43.8)	3 (23.1)
20	White	5 (31.3)	5 (38.5)
20	African-Caribbean and Black	1 (6.3)	1 (7.7)
29	Latin American	1 (6.3)	0
30	Middle Eastern	0	1 (7.7)
31	Mixed	2 (12.5)	3 (23.1)
32	Contraceptive method		
33	Oral contraceptive pill	5 (31.3)	5 (38.5)
34	Condom	3 (18.8)	1 (7.7)
	Hormonal IUD	2 (12.5)	2 (15.4)
	Copper IUD	1 (6.3)	2 (15.4)
	NuvaRing	1 (6.3)	1 (7.7)
	Condom and hormonal	0	1 (7.7)
	None	4 (25)	1 (7.7)
	Vaginal douching	2 (12.5)	2 (15.4)
	Bacterial vaginosis	2 (12.5)	3 (23.1)
	Relationship length (months)	11 (1-72)	24 (2-96)
	Time since last sex (days)	3 (2-23)	4 (3-10)
	STI history	2 (12.5)	1 (7.7)

35 SUPLEMENTARY TABLE 2. Baseline cytokine levels in semen based on circumcision

- 36 status

	Sei		
Cytokines	Uncircumcised	Circumcised	p-value
	(Median, range)	(Median, range)	
IP-10	40,643.17	35,328.39	0.934
	(1,499.81-77,457.33)	(14,591.45,328,349.53)	
MIG	23,426.74	23,820.07	0.561
	(471.79-38,100.08)	(4,233.68-333,954.49)	
MIP-1β	630.23	720.32	0.678
	(124.64-10,281.91)	(134.80-8,346.62)	
IL-8	1,045.19	1,182.54	0.901
	(159.87-18,608.36)	(265.75-12,110)	
MIP-3a	1,159.77	919.04	0.901
	(20.54-5,4791.11)	(394.81-19,212.50)	
IL-1α	188.92	176.11	0.901
	(79.46-2,695.76)	(72.10-64,079)	
IL-6	8.11	8.42	0.506
	(1.20-126.27)	(0.88-144.80)	
IL-17	11.79	11.25	0.835
	(2.43-258)	(4.20-644.94)	
IFNa2a	39.63	39.04	0.771
	(6.93-65.61)	(17.99-705.95)	
sE-cadherin	290,649.64	266,298.45	0.739
	(35,808.05-786,586.53)	(129,735.20-3,053,534.76)	
MMP9	3,392.52	11,916.09	0.280
	(461.92-1,246,997.34)	(164.55-723,728.70)	

41 SUPLEMENTARY TABLE 3. Baseline cytokine levels in cervico-vaginal secretion based

42 on circumcision status of the male partner

	Cervico-vaginal secretion		
Cytokines	Uncircumcised	Circumcised	p-value
	(Median, range)	(Median, range)	
IP-10	2,214.90	1,458.19	0.599
	(108.91-66,407.83)	(9.90-8,181.43)	
MIG	124.60	153.57	0.792
	(49.40-5,393.74)	(15.82-940.45)	
MIP-1β	307.07	518.92	0.303
	(139-3,979)	(139-4,784.98)	
IL-8	28,435.45	24,851.75	0.630
	(364.73-37,935.93)	(2,082.25-42,978.93)	
MIP-3a	766.29	793.03	0.965
	(119.82-19,736)	(73.43-17,573.81)	
IL-1α	23,507.52	43,041.81	0.079
	(3,334.60-545,981.71)	(5,468.27-233,021.32)	
IL-6	47.78	116.68	0.335
	(3.71-2,524.54)	(5.05-2,302.65)	
IL-17	25.89	44.04	0.403
	(12-717.60)	(12-411.25)	
IFNα2a	16.4	24.54	0.826
	(5.16-124.31)	(2.80-42.11)	
sE-cadherin	66,766.81	111,666.7	0.456
	(1,980.38-2,169,304.19)	(2,136.33-1,904,214.72)	
MMP9	21,473.07	576,234.27	0.288
	(124.49-3,560,343.5)	(763.33-2,712,754.25)	

47 SUPPLEMENTARY FIGURE 1. The levels of cytokines in cervico-vaginal secretions and



49



50

51

52 SUPPLEMENTARY FIGURE 1. The levels of cytokines in cervico-vaginal secretions and

53 semen. The level of (a) soluble E-cadherin (pg/ml), (b) IP-10 (pg/ml), (c) MIG (pg/ml), in

54 cervico-vaginal secretion (baseline, 1 hour and 72 hours) after condomless (N=29) and condom-

55 protected sex (N=8), and semen (baseline). The box plots represent the median, with interquartile

56 and minimum to maximum ranges. The red box plots represent cervico-vaginal secretions, and

57 the blue box plots represent semen.

SUPPLEMENTARY FIGURE 2. Baseline levels of cytokines in cervico-vaginal secretion of

BV+ and BV- women.



63 SUPPLEMENTARY FIGURE 2. Baseline levels of cytokines in cervicovaginal secretions of

BV+ and BV- women. The level of cervico-vaginal cytokine concentrations is compared

between BV+ (N=5) and BV- women (N=24) in condomless group. Statistical comparisons were

66 performed using Mann-Witney U test. BV stands for bacterial vaginosis.

70 SUPPLEMENTARY FIGURE 3. Gating strategy and representative plots for endocervical



72



73

74

SUPPLEMENTARY FIGURE 3. Gating strategy and representative plots for endocervical activated, CCR5+ and memory CD4+ T cells subsets.

- 77 Cells were gated on lymphocytes, singlets, live, CD3+ cells, CD4+ cells, HLA-DR+CD4+ T
- cells (activated CD4+ T cells), CCR5+CD4+ T cells and CD4+ memory subsets (Central
- 79 memory: CCR7-CD45RA+; effector memory: CCR7-CD45RA-).

80

SUPPLEMENTARY FIGURE 4. Gating strategy and representative plots for endocervical



81 Dendritic cells population.

82

89 SUPPLEMENTARY FIGURE 4. Gating strategy and representative plots for endocervical

Dendritic cells population. Cells were gated on monocytes, CD45+ cells, singlets, CD3/CD19

91 negative, CD66b negative cells, based on CD14 and HLA-DR expressions two subsets of

92 dendritic cells (DCs): monocyte-derived DCs (CD11c+HLA-Dr highCD14+), CD14- DCs

93 (CD11c+ HLA-DR highCD14-).

94

95