Figure S1. Real-time PCR quantification of bacterial 16S gene copies in vaginal (**A**) and seminal (**B**) samples. ΔRn is plotted against PCR cycle number.

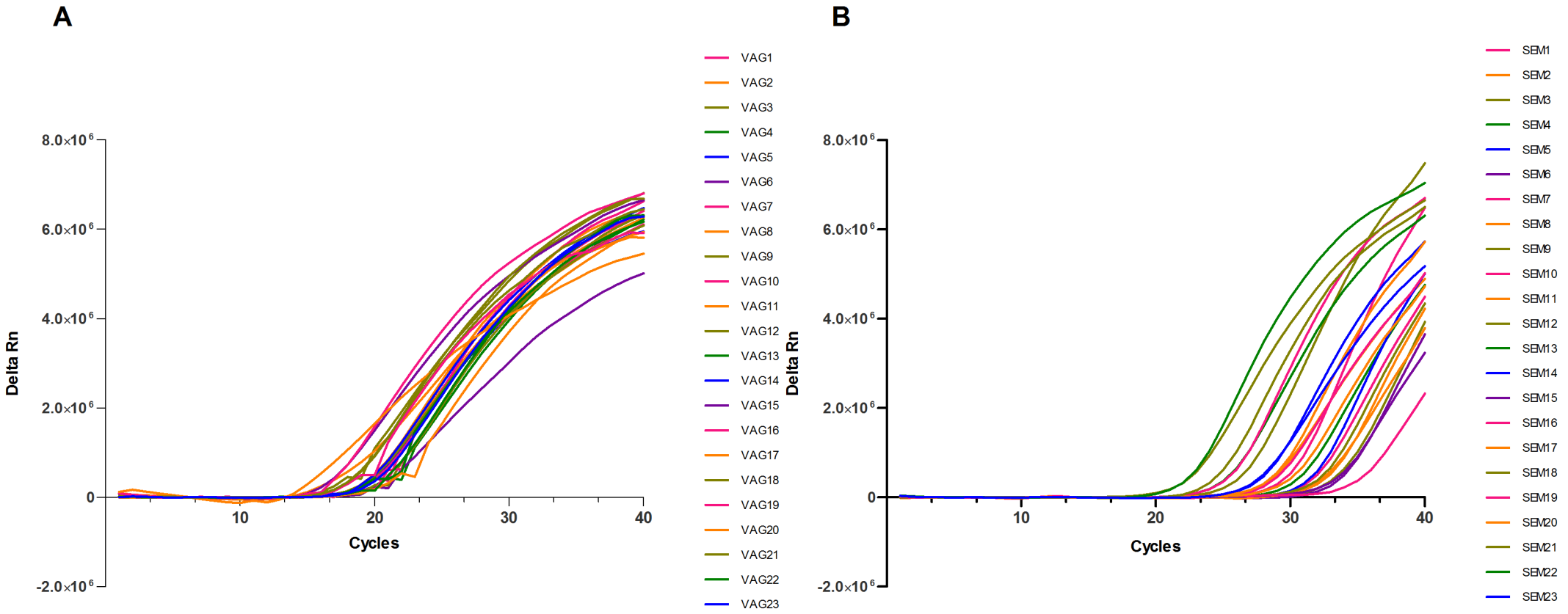

Figure S2. Vaginal microbiome composition at family level for the 23 women with idiopathic infertility.

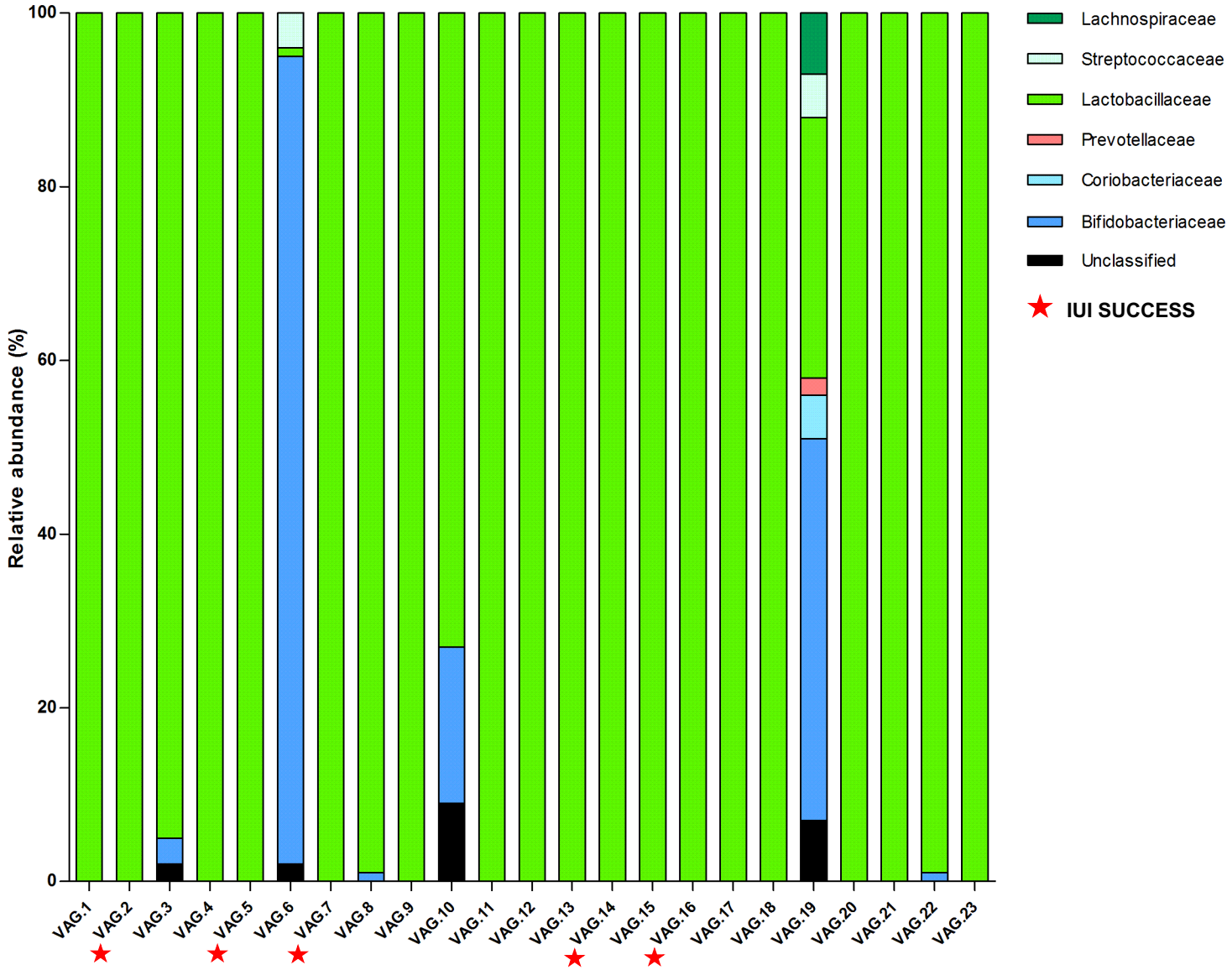

Figure S3. Shannon diversity rarefaction curves for the cohort of women (**A**) and men (**B**) with idiopathic infertility (IUI success, no IUI success) and controls.

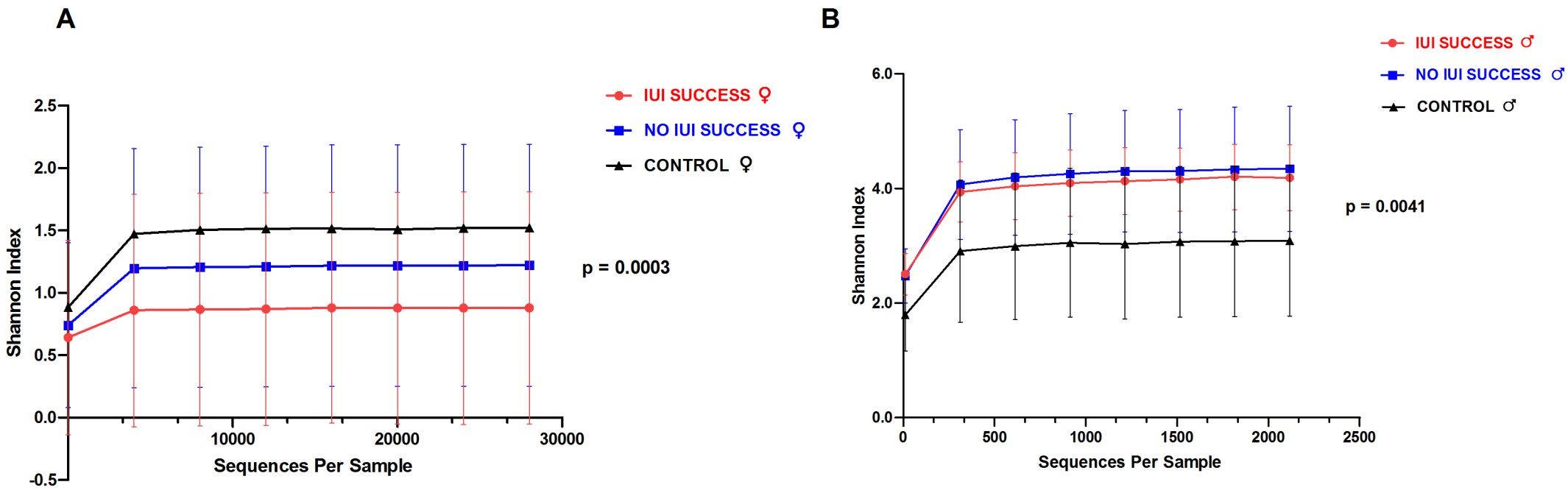

Figure S4. Seminal microbiome composition at family level for the 23 men with idiopathic infertility.

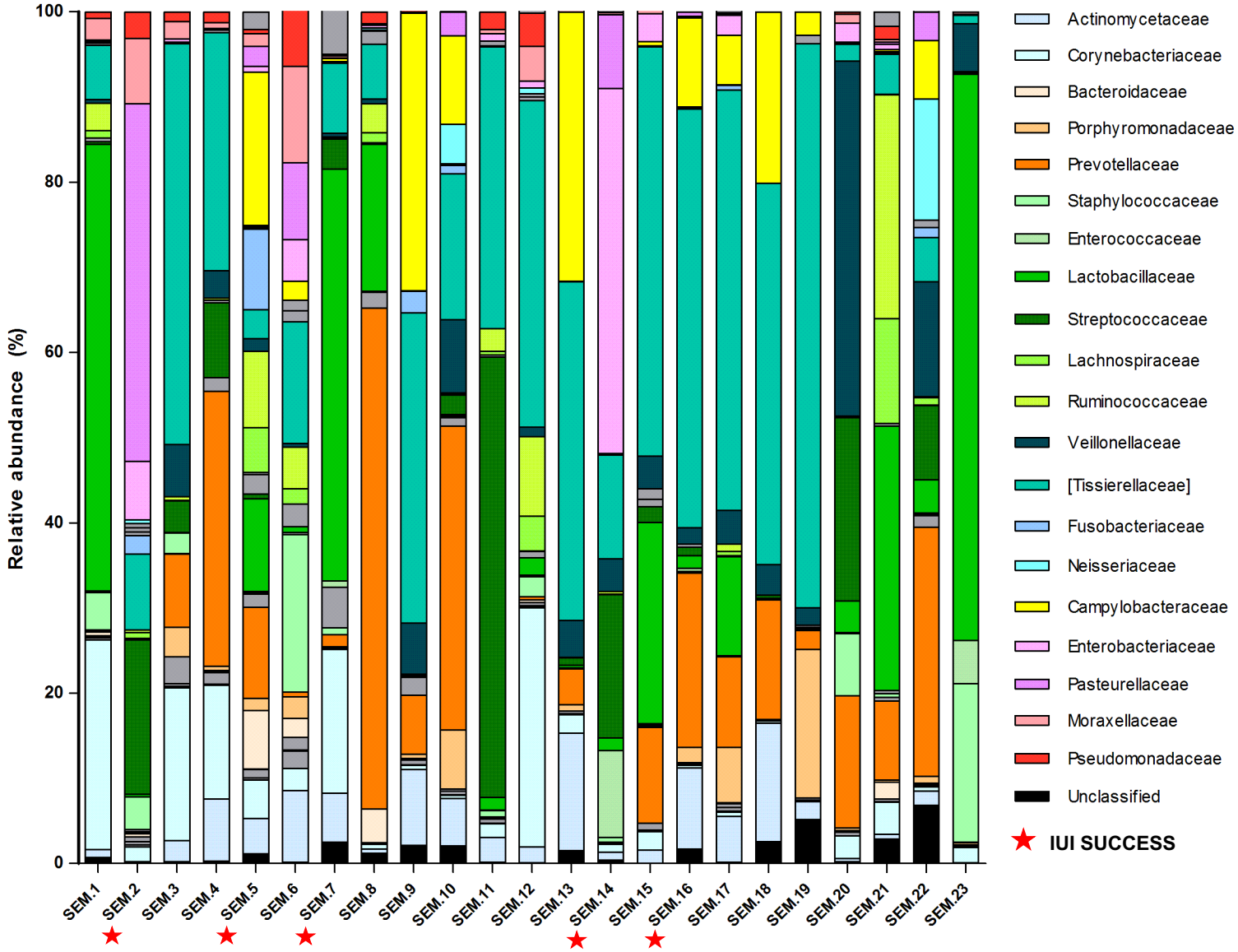

Figure S5. Heatmap of the relative abundance of *Lactobacillus* species found in the seminal microbiome of 23 men with idiopathic infertility.

Color key is indicated in the upper left corner. The red star indicates IUI success.

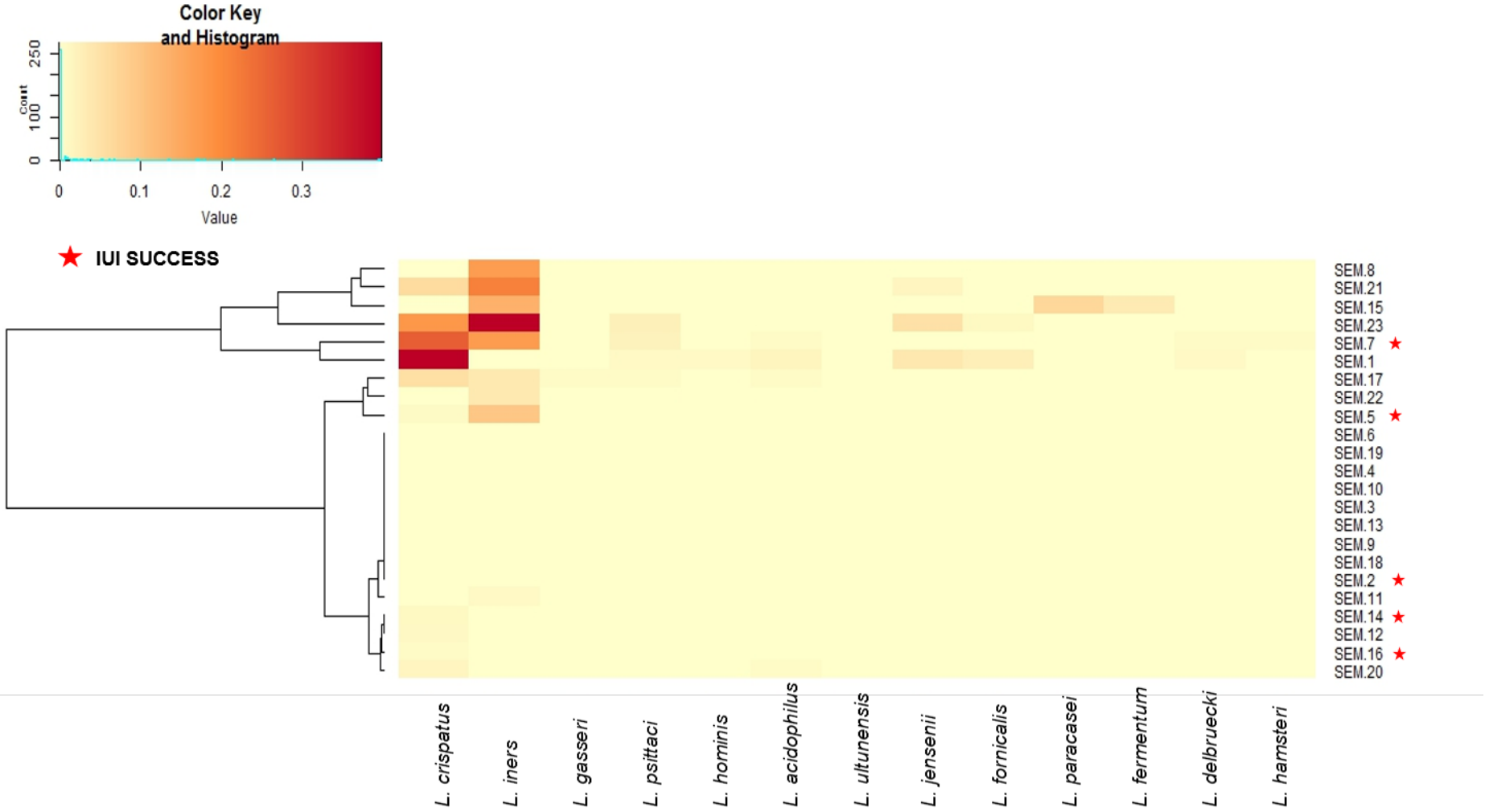

Table S1. Real-time PCR quantification of bacterial 16S gene copies in vaginal and seminal samples. Standard curve values are reported as well.

Sample ID	Ct	Ct (mean)	16S Copies	ng/uL total DNA
VAG1	18,17	18,465	5,08E+11	5,2
	18,76			
VAG2	18,77	18,755	4,09E+11	7,5
	18,74			
VAG3	16,61	16,655	1,97E+12	1,5
	16,7			
VAG4	19,55	19,54	2,27E+11	17
	19,53			
VAG5	18,49	18,52	4,87E+11	17,5
	18,55			
VAG6	19,82	19,72	1,98E+11	7
	19,62			
VAG7	18,81	18,84	3,83E+11	8,5
	18,87			
VAG8	18,31	18,38	5,41E+11	36,5
	18,45			
VAG9	19,38	19,41	2,50E+11	34
	19,44			
VAG10	18,58	18,58	4,66E+11	73
	18,58			
VAG11	14,8	14,8	7,90E+12	1,1
	14,8			
VAG12	18,44	18,475	5,04E+11	14,5
	18,51			
VAG13	18,91	18,72	4,19E+11	12
	18,53			
VAG14	18,36	18,385	5,39E+11	25
	18,41			
VAG15	15,7	15,88	3,52E+12	6
	16,06			
VAG16	15,9	15,965	3,30E+12	16
	16,03			
VAG17	14,56	14,56	9,46E+12	1,2
	14,56			
VAG18	17,22	17,185	1,32E+12	6
	17,15			
VAG19	17,36	17,38	1,14E+12	18,5
	17,4			
VAG20	20,24	20,12	1,47E+11	29
	20			
VAG21	17,24	17,205	1,30E+12	8
	17,17			
VAG22	19,44	19,395	2,53E+11	37
	19,35			
VAG23	19,05	19,05	3,28E+11	28
	19,11			

Sample ID	Ct	Ct (mean)	16S Copies	ng/uL total DNA
SEM1	27,08	27,385	5,44E+08	8,4
	27,69			
SEM2	30,56	30,535	5,01E+07	6,8
	30,51			
SEM3	31,44	32,105	1,53E+07	1
	32,77			
SEM4	29,32	29,395	1,19E+08	4,7
	29,47			
SEM5	30,42	30,325	5,87E+07	0,8
	30,23			
SEM6	32,31	32,62	1,03E+07	7
	32,93			
SEM7	27,66	27,725	4,21E+08	1
	27,79			
SEM8	32,31	32,27	1,35E+07	7
	32,23			
SEM9	25,07	24,95	3,44E+09	5,2
	24,83			
SEM10	28,37	28,37	2,58E+08	32
	28,37			
SEM11	31,12	31,28	2,85E+07	15
	31,44			
SEM12	31,24	30,85	3,95E+07	0,8
	30,46			
SEM13	24,06	24,075	6,67E+09	4,3
	24,09			
SEM14	26,68	26,57	1,01E+09	21
	26,46			
SEM15	32,11	32,005	1,65E+07	6,5
	31,9			
SEM16	24,15	24,115	6,47E+09	23
	24,08			
SEM17	27,29	27,29	5,85E+08	0,5
	27,29			
SEM18	23,37	23,285	1,21E+10	8,4
	23,2			
SEM19	34,01	33,72	4,49E+06	7,8
	33,43			
SEM20	28,94	28,66	2,07E+08	5,7
	28,38			
SEM21	21,25	21,175	6,00E+10	1
	21,1			
SEM22	21,32	21,22	5,80E+10	33
	21,12			
SEM23	26,45	26,38	1,10E+09	11,1
	26,31			

Sample ID	Ct	Ct (mean)	16S Copies
STANDARD (1)	32,9	32,9	6,81E+06
	32,98		
STANDARD (2)	31,03	30,91	6,81E+07
	30,79		
STANDARD (3)	27,62	27,53	6,81E+08
	27,44		
STANDARD (4)	23,87	24,015	6,81E+09
	24,16		
STANDARD (5)	21,2	21,2	6,81E+10
	21,2		
STANDARD (6)	17,86	17,95	6,81E+11
	18,04		

Table S2. Characteristics of male controls.

Male controls (n = 16)	**Mean**	**SD**
Age (y)	41,44	8,68
Semen Volume (mL)	2,48	1,67
Semen pH	7,93	0,28
Sperm concentration (10^6/mL)	82,08	34,94
Motility grade a (%)	35,92	16,63
Motility grade b (%)	18,23	9,61
Motility grade c (%)	8,13	9,68
Motility grade d (%)	38,93	12,73
Morphology (% normal)	4,53	2,07
Vitality (% alive)	80,00	8,10

Table S3. Relative abundance of species found in the vaginal microbiota of 23 women with idiopathic infertility.

Sample ID	*L. crispatus*	*L. iners*	*L. gasseri*	*L. psittaci*	*L. hominis*	*L. acidophilus*	*L. ultunensis*	*L. jensenii*	Total *Lactobacillus* spp.	*Bifidobacterium breve*	*Gardnerella vaginalis*	*Bifidobacterium minimum*	Total Bifidobacteriaceae
VAG.19	8%	2%	4%	0%	2%	1%	12%	0%	29,7%	0%	39%	3%	42%
VAG.6	0%	0%	1%	0%	0%	0%	0%	0%	1,4%	93%	0%	0%	93%
VAG.22	13%	47%	0%	12%	0%	15%	0%	11%	98,8%	0%	0%	0%	0%
VAG.4	31%	2%	0%	1%	0%	29%	0%	34%	97,3%	0%	0%	0%	0%
VAG.10	8%	4%	55%	0%	6%	0%	0%	1%	73,1%	0%	17%	0%	17%
VAG.3	13%	6%	68%	0%	8%	0%	0%	0%	95,3%	3%	0%	0%	3%
VAG.13	8%	81%	7%	1%	3%	0%	0%	0%	99,3%	0%	0%	0%	0%
VAG.9	17%	77%	6%	0%	0%	0%	0%	0%	99,9%	0%	0%	0%	0%
VAG.21	22%	76%	0%	1%	0%	0%	1%	0%	99,9%	0%	0%	0%	0%
VAG.11	19%	80%	0%	0%	0%	1%	0%	0%	100,0%	0%	0%	0%	0%
VAG.8	2%	92%	3%	0%	3%	0%	0%	0%	98,8%	0%	0%	0%	0%
VAG.18	66%	24%	5%	3%	1%	1%	0%	0%	99,9%	0%	0%	0%	0%
VAG.15	62%	28%	5%	1%	1%	1%	0%	2%	100,0%	0%	0%	0%	0%
VAG.20	50%	35%	6%	0%	2%	4%	0%	3%	99,8%	0%	0%	0%	0%
VAG.17	42%	53%	2%	3%	0%	0%	0%	0%	99,6%	0%	0%	0%	0%
VAG.12	79%	4%	4%	0%	3%	7%	0%	3%	100,0%	0%	0%	0%	0%
VAG.1	82%	5%	3%	3%	5%	2%	0%	0%	99,6%	0%	0%	0%	0%
VAG.23	80%	12%	0%	3%	0%	4%	0%	1%	99,5%	0%	0%	0%	0%
VAG.14	91%	4%	0%	2%	1%	2%	0%	0%	99,9%	0%	0%	0%	0%
VAG.5	91%	5%	0%	0%	0%	3%	0%	1%	100,0%	0%	0%	0%	0%
VAG.7	91%	5%	1%	1%	1%	0%	0%	1%	99,7%	0%	0%	0%	0%
VAG.2	90%	7%	1%	1%	0%	0%	0%	1%	100,0%	0%	0%	0%	0%
VAG.16	87%	7%	1%	3%	1%	1%	0%	0%	99,8%	0%	0%	0%	0%

Table S4. Relative abundance values of *Lactobacillus* species, shown as mean (M) and standard error of the mean (SEM) for the two groups of women. P-values were calculated by permutation analysis and subsequently corrected for multiple comparisons with false discovery rate (FDR).

***Lactobacillus* spp.**	**IUI SUCCESS ♀**		**NO IUI SUCCESS ♀**		**p-value**	**p-value adj.**
	M	**SEM**	**M**	**SEM**		
L. crispatus	0,9000	0,0036	0,3311	0,0612	**< 0,0001**	**0,0002**
L. iners	0,0560	0,0028	0,3430	0,0696	0,0760	0,3042
L. gasseri	0,0060	0,0011	0,0938	0,0399	0,2245	0,4538
L. psittaci	0,0140	0,0024	0,0100	0,0025	0,5115	0,7522
L. hominis	0,0060	0,0011	0,0199	0,0051	0,2269	0,4538
L. acidophilus	0,0120	0,0027	0,0282	0,0144	0,6581	0,7522
L. ultunensis	0,0000	0,0000	0,0074	0,0060	0,8221	0,8221
L. jenseni	0,0060	0,0011	0,0260	0,0167	0,5711	0,7522

Table S5. Relative abundance values of *Lactobacillus* species, shown as mean (M) and standard error of the mean (SEM) for the two groups of men. P-values were calculated by permutation analysis and subsequently corrected for multiple comparisons with false discovery rate (FDR).

***Lactobacillus* spp.**	**NO IUI SUCCESS ♂**		**IUI SUCCESS ♂**		**p-value**	**p-value adj.**
	M	**SEM**	**M**	**SEM**		
L. crispatus	0,0400	0,0207	0,0584	0,0241	0,7777	0,9332
L. iners	0,0555	0,0224	0,0537	0,0163	0,9884	1,0000
L. gasseri	0,0004	0,0004	0	0	0,2174	0,4307
L. psittaci	0,0024	0,0013	0,0043	0,0020	0,7408	0,9332
L. hominis	0,0005	0,0005	0	0	0,2174	0,4307
L. acidophilus	0,0019	0,0010	0,0014	0,0007	0,7696	0,9332
L. jensenii	0,0059	0,0031	0	0	0,2513	0,4307
L. fornicalis	0,0023	0,0015	0	0	0,2174	0,4307
L. paracasei	0,0037	0,0033	0	0	0,2174	0,4307
L. fermentum	0,0019	0,0017	0	0	0,2174	0,4307
L. delbruecki	0,0005	0,0005	0,0014	0,0007	1,0000	1,0000
L. hamsteri	0	0	0,0014	0,0007	0,2174	0,4307

Table S6. Relative abundance of *Lactobacillus* species found in the seminal microbiota of 23 men with idiopathic infertility.

Sample ID	*L. crispatus*	*L. iners*	*L. jensenii*	*L. psittaci*	*L. fornicalis*	*L. coleohominis*	*L. paracasei*	*L. fermentum*	*L. delbruecki*	*L. acidophilus*	*L. hamsteri*	*L. gasseri*	**Total *Lactobacillus* spp.**
SEM.1	40%	0%	4%	1%	3%	1%	0%	0%	1%	2%	0%	0%	51%
SEM.2	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
SEM.3	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
SEM.4	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
SEM.5	1%	10%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	10%
SEM.6	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
SEM.7	27%	17%	0%	2%	0%	0%	0%	0%	1%	1%	1%	0%	48%
SEM.8	0%	17%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	17%
SEM.9	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
SEM.10	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
SEM.11	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%
SEM.12	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	2%
SEM.13	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
SEM.14	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%
SEM.15	0%	13%	0%	0%	0%	0%	7%	3%	0%	0%	0%	0%	24%
SEM.16	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%
SEM.17	5%	4%	0%	1%	0%	0%	0%	0%	0%	1%	0%	1%	12%
SEM.18	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
SEM.19	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
SEM.20	2%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	4%
SEM.21	6%	21%	2%	0%	1%	0%	0%	0%	0%	0%	0%	0%	30%
SEM.22	0%	4%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	4%
SEM.23	18%	39%	5%	3%	1%	0%	0%	0%	0%	0%	0%	0%	66%