Into the "Sexome": Genital Microbiome Transfer and Applications

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ABSTRACT

Conventional sexual assault evidence testing that relies on preferential lysis faces challenges when low to no spermatozoa are present. Furthermore, low concentrations of male DNA and mixtures can complicate STR profile interpretation. The emerging concept of the "Sexome," or genital microbiome is gaining attention as a potential solution to these limitations.

The purpose of this research was to investigate the potential post-coital transfer between vaginal and penile microbiomes. Pre-coital and post-coital vaginal and penile swabs were collected from two donor couples. In both showered and non-showered post-coital microbiomes, *Lactobacillus* was observed to be transferred from vaginal to penile skin microbiomes. While all penile microbiomes were significantly more diverse than vaginal microbiomes, severe post-coital bacterial diversity loss was observed in penile microbiomes.

INTRODUCTION

Relying on the presence of spermatozoa for screening and identifying male perpetrators in sexual assault evidence leaves forensic DNA laboratories ineffective when there are no spermatozoa present in the evidence. With the increasing accessibility of next-generation sequencing, the analysis of genital microbiota in such cases is an appealing alternative[1]. Potential advantages for sequencing bacteria in these microbiomes include persistence of microbiota and resistance to DNA degradation[2].

This study investigates the transfer and persistence of microbiota found in penile and vaginal swabs. Pre-coital and post-coital swabs were collected from two couples to characterize transfer. Persistence was interrogated by comparing post-coital swabs collected with and without a post-coital shower. Microbiome bacterial libraries were prepared by targeting the hypervariable regions V3 and V4 of the 16S ribosomal RNA gene. The diversity between individuals of each couple were determined to identify transfer and persistence of unique bacterial genera.

ACKNOWLEDGEMENTS

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RESULTS & DISCUSSION

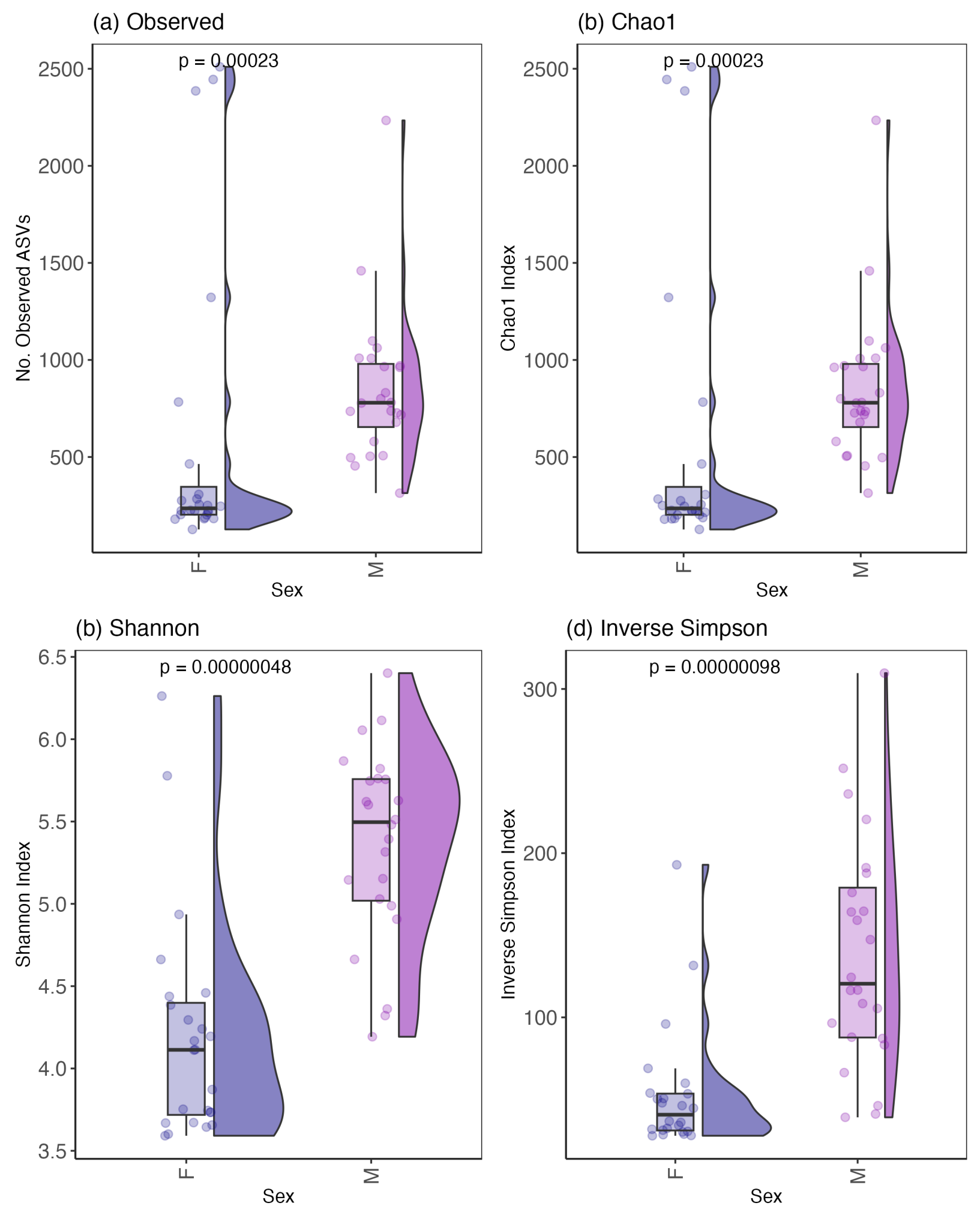
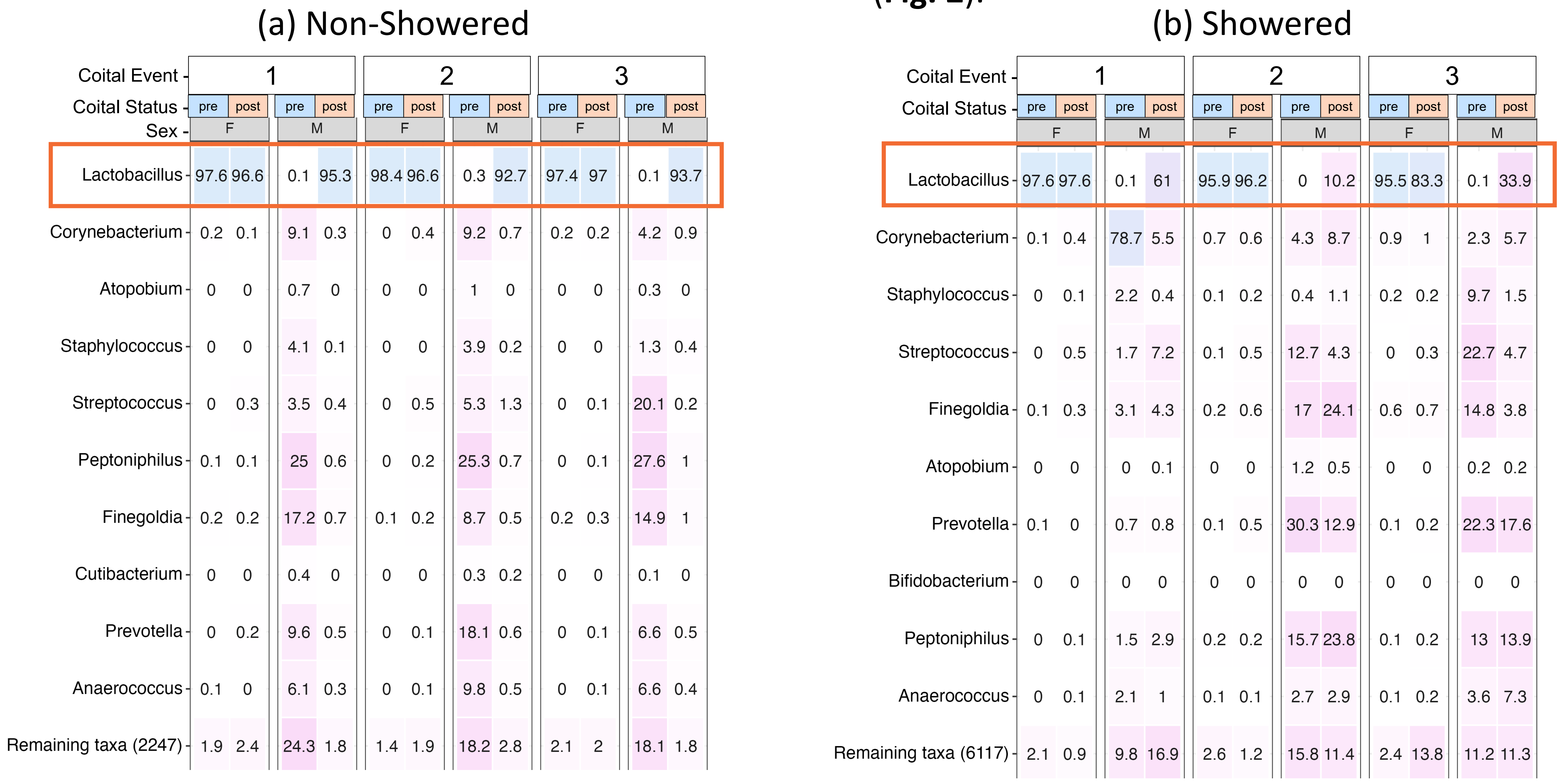


Figure 1. Significant alpha diversity observed between vaginal and penile microbiomes. Penile microbiomes are significantly more diverse than vaginal microbiomes in (a) observed, (b) Chao1, (c) Shannon, and (d) Inverse Simpson diversity measures.

- Penile microbiomes are significantly more diverse than vaginal microbiomes which are dominated by *Lactobacillus* (**Fig. 1**).
- Significant transfer of ***Lactobacillus*** from the female vaginal to penile environments was detected in all coital events of Couple 1 (**Fig. 2**), on average:
  - **0.2% → 93.9% (non-showered)**
  - **0.1% → 35.0% (showered)**
- Notable post-coital bacterial diversity loss was observed in non-showered penile microbiomes (**Fig. 2**).



(a) Non-Showered

Coital Event	1				2				3			
Coital Status	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post
Sex	F		M		F		M		F		M	
Lactobacillus	97.6	96.6	0.1	95.3	98.4	96.6	0.3	92.7	97.4	97	0.1	93.7
Corynebacterium	0.2	0.1	9.1	0.3	0	0.4	9.2	0.7	0.2	0.2	4.2	0.9
Atopobium	0	0	0.7	0	0	0	1	0	0	0	0.3	0
Staphylococcus	0	0	4.1	0.1	0	0	3.9	0.2	0	0	1.3	0.4
Streptococcus	0	0.3	3.5	0.4	0	0.5	5.3	1.3	0	0.1	20.1	0.2
Peptoniphilus	0.1	0.1	25	0.6	0	0.2	25.3	0.7	0	0.1	27.6	1
Finegoldia	0.2	0.2	17.2	0.7	0.1	0.2	8.7	0.5	0.2	0.3	14.9	1
Cutibacterium	0	0	0.4	0	0	0	0.3	0.2	0	0	0.1	0
Prevotella	0	0.2	9.6	0.5	0	0.1	18.1	0.6	0	0.1	6.6	0.5
Anaerococcus	0.1	0	6.1	0.3	0	0.1	9.8	0.5	0	0.1	6.6	0.4
Remaining taxa (2247)	1.9	2.4	24.3	1.8	1.4	1.9	18.2	2.8	2.1	2	18.1	1.8

(b) Showered

Coital Event	1				2				3			
Coital Status	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post
	F		M		F		M		F		M	
Lactobacillus	97.6	97.6	0.1	61	95.9	96.2	0	10.2	95.5	83.3	0.1	33.9
Corynebacterium	0.1	0.4	78.7	5.5	0.7	0.6	4.3	8.7	0.9	1	2.3	5.7
Staphylococcus	0	0.1	2.2	0.4	0.1	0.2	0.4	1.1	0.2	0.2	9.7	1.5
Streptococcus	0	0.5	1.7	7.2	0.1	0.5	12.7	4.3	0	0.3	22.7	4.7
Finegoldia	0.1	0.3	3.1	4.3	0.2	0.6	17	24.1	0.6	0.7	14.8	3.8
Atopobium	0	0	0	0.1	0	0	1.2	0.5	0	0	0.2	0.2
Prevotella	0.1	0	0.7	0.8	0.1	0.5	30.3	12.9	0.1	0.2	22.3	17.6
Bifidobacterium	0	0	0	0	0	0	0	0	0	0	0	0
Peptoniphilus	0	0.1	1.5	2.9	0.2	0.2	15.7	23.8	0.1	0.2	13	13.9
Anaerococcus	0	0.1	2.1	1	0.1	0.1	2.7	2.9	0.1	0.2	3.6	7.3
Remaining taxa (6117)	2.1	0.9	9.8	16.9	2.6	1.2	15.8	11.4	2.4	13.8	11.2	11.3

Figure 2. Representative taxonomic heatmap of bacteria taxa shown as relative abundance for (a) non-showered and (b) showered microbiomes of Couple 1. *Lactobacillus* increased to become the majority genus in post-coital penile microbiomes, regardless of post-coital showering. Bacterial diversity diminished in non-showered post-coital penile microbiomes relative to showered penile microbiomes.

REFERENCES

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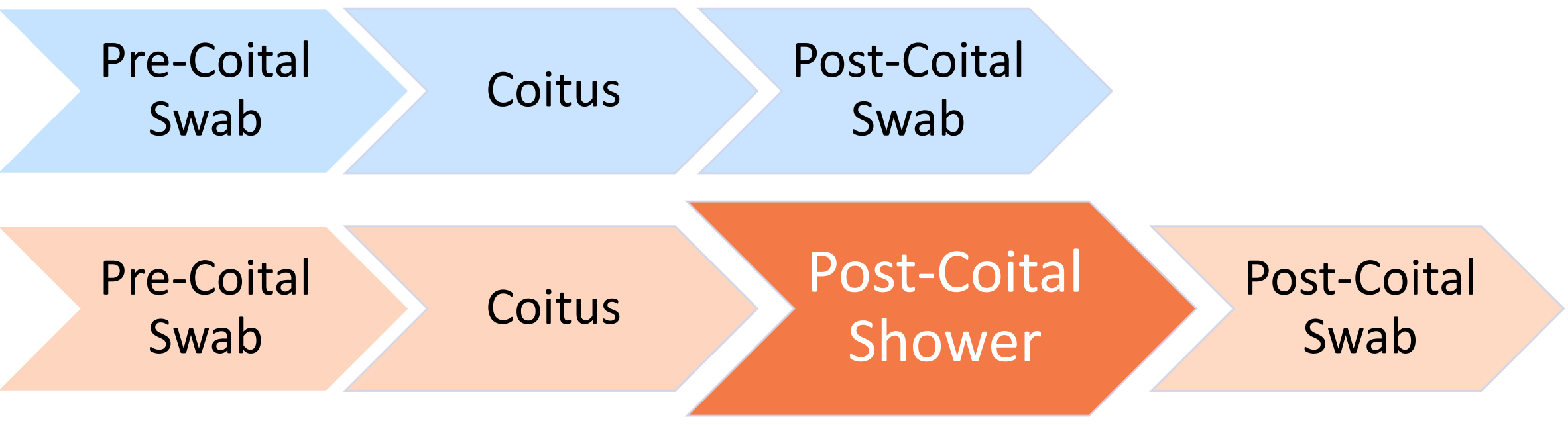
[2] T. Nguyen-Hieu, G. Aboudharam, M. Drancourt, Heat degradation of eukaryotic and bacterial DNA: an experimental model for paleomicrobiology, BMC Research Notes 5 (2012) 528. https://doi.org/10.1186/1756-0500-5-528.

MATERIALS & METHODS



Pre-Coital Swab → Coitus → Post-Coital Swab

Pre-Coital Swab → Coitus → Post-Coital Shower → Post-Coital Swab

Sampling

(n = 2 couples, 6 coital events per couple)

Minimum 72 hours abstinence prior

Microbiome Extraction

PureLink™ Microbiome DNA Purification Kit

Illumina 16S Metagenomic Sequencing Library Preparation

Sequencing

Illumina MiSeqFGx™

Bioinformatic Processing

DADA2 (SILVA v132 and NCBI BLAST)

Statistical Analysis

R (4.1.1) and RStudio (v1.4)

CONCLUSIONS

- Penile microbiomes display greater diversity than vaginal microbiomes.
- *Lactobacillus* transfer observed from vaginal to penile microbiomes.
- Penile bacterial diversity loss observed in post-coital sampling was more severe in non-showered microbiomes compared to showered microbiomes.