

Table S1. Summary statistics of 16S rRNA sequencing

Segments	N	Clean reads	Coverage range	Average of reads per sample
Cloaca	43	2587887	32689 ~ 73206	60183
Vagina	55	3289560	38803 ~ 74390	59810
Uterus	54	3247856	34225 ~ 73746	60145
Isthmus	55	3114377	30858 ~ 71968	56625
Magnum	37	2172421	31774 ~ 74824	58714
Infundibulum	48	2865423	33766 ~ 74648	59696
Total	292	17277524	30858 ~ 74824	59170

Table S2. Relative abundance of the dominant microbial phyla in different segments

Phylum	Infundibulum	Magnum	Isthmus	Uterus	Vagina	Cloaca
Actinobacteria	0.56%	2.71%	0.21%	0.14%	0.52%	17.11%
Bacteroidetes	3.86%	3.79%	0.13%	0.15%	0.52%	1.32%
Firmicutes	16.19%	21.56%	28.67%	38.16%	28.97%	57.35%
Fusobacteria	0.00%	0.00%	0.02%	1.18%	3.84%	1.38%
Proteobacteria	79.21%	71.85%	70.93%	60.24%	66.04%	22.70%
Others	0.18%	0.09%	0.05%	0.14%	0.12%	0.14%

Table S3. Relative abundance of microbial genera at the six anatomical segments

Genus	Infundibulum	Magnum	Isthmus	Uterus	Vagina	Cloaca
<i>c_Actinobacteria;o_Actinomycetales;f_Actinomycetaceae;g_Actinomyces</i>	0	0	0	0	0.02%	3.49%
<i>c_Actinobacteria;o_Corynebacteriales;f_Corynebacteriaceae;g_Corynebacterium</i>	0.09%	0	0.03%	0.03%	0.16%	9.77%
<i>c_Bacteroidia;o_Flavobacteriales;f_Weeksellaceae;g_Chryseobacterium</i>	3.78%	3.45%	0.11%	0	0.08%	0.01%
<i>c_Bacilli;o_Bacillales;f_Planococcaceae;g_Kurthia</i>	4.74%	0	1.10%	2.10%	0.01%	1.57%
<i>c_Bacilli;o_Lactobacillales;f_Aerococcaceae;g_Aerococcus</i>	0.42%	3.10%	0.13%	2.81%	1.08%	1.08%
<i>c_Bacilli;o_Lactobacillales;f_Enterococcaceae;g_Enterococcus</i>	5.25%	7.70%	15.43%	23.13%	16.18%	28.61%
<i>c_Bacilli;o_Lactobacillales;f_Vagococcaceae;g_Vagococcus</i>	1.17%	6.64%	1.27%	1.64%	1.41%	0.10%
<i>c_Bacilli;o_Staphylococcales;f_Staphylococcaceae;g_Staphylococcus</i>	4.08%	3.53%	9.50%	6.56%	4.28%	1.87%
<i>c_Clostridia;o_Peptostreptococcales-Tissierellales;f_Peptostreptococcales-Tissierellales;g_Gallicola</i>	0	0	0	0.56%	1.92%	15.86%
<i>c_Fusobacteriia;o_Fusobacteriales;f_Fusobacteriaceae;g_Fusobacterium</i>	0	0	0.02%	1.18%	3.84%	1.38%
<i>c_Alphaproteobacteria;o_Caulobacterales;f_Caulobacteraceae;g_Brevundimonas</i>	1.83%	6.47%	1.84%	1.45%	0.93%	0.89%
<i>c_Gammaproteobacteria;o_Aeromonadales;f_Aeromonadaceae;g_Aeromonas</i>	8.92%	2.66%	1.60%	0	3.15%	0.38%
<i>c_Gammaproteobacteria;o_Burkholderiales;f_Comamonadaceae;_</i>	0.95%	0.49%	0.43%	2.66%	4.02%	0.71%
<i>c_Gammaproteobacteria;o_Burkholderiales;f_Comamonadaceae;g_Comamonas</i>	3.59%	0	0.04%	0.35%	1.62%	1.51%
<i>c_Gammaproteobacteria;o_Enterobacteriales;f_Enterobacteriaceae;_</i>	5.94%	10.08%	7.16%	1.52%	4.16%	0.59%
<i>c_Gammaproteobacteria;o_Enterobacteriales;f_Enterobacteriaceae;g_Escherichia-Shigella</i>	24.53%	25.39%	40.18%	43.79%	28.90%	13.34%
<i>c_Gammaproteobacteria;o_Enterobacteriales;f_Erwiniaaceae;g_Pantoea</i>	0.01%	2.56%	1.43%	0.63%	0.12%	0
<i>c_Gammaproteobacteria;o_Pseudomonadales;f_Moraxellaceae;g_Acinetobacter</i>	9.02%	3.34%	5.57%	7.05%	3.53%	1.34%
<i>c_Gammaproteobacteria;o_Pseudomonadales;f_Pseudomonadaceae;g_Pseudomonas</i>	11.13%	11.77%	7.77%	1.90%	13.10%	0.92%
<i>c_Gammaproteobacteria;o_Xanthomonadales;f_Xanthomonadaceae;g_Stenotrophomonas</i>	12.89%	8.39%	4.22%	0.34%	4.63%	0.81%
Others	1.64%	4.41%	2.20%	2.28%	6.87%	15.77%

Table S4. Spearman correlation between cloacal microbial genera

Genus1	Genus2	Abundance1	Abundance2	Phylum1	Phylum2	correlation	adj_P	Direction
<i>g_Corynebacterium</i>	<i>g_Actinomyces</i>	10.12%	3.70%	Actinobacteria	Actinobacteria	0.41	4.70E-02	positive
<i>g_Brevibacterium</i>	<i>g_Actinomyces</i>	0.07%	3.70%	Actinobacteria	Actinobacteria	0.42	4.15E-02	positive
<i>f_Aerococcaceae._</i>	<i>g_Actinomyces</i>	0.16%	3.70%	Firmicutes	Actinobacteria	0.63	1.42E-04	positive
<i>f_Aerococcaceae._</i>	<i>g_Brevibacterium</i>	0.16%	0.07%	Firmicutes	Actinobacteria	0.46	2.03E-02	positive
<i>f_Aerococcaceae._</i>	<i>g_Corynebacterium</i>	0.16%	10.12%	Firmicutes	Actinobacteria	0.45	2.71E-02	positive
<i>g_Aerococcus</i>	<i>g_Kocuria</i>	0.99%	1.20%	Firmicutes	Actinobacteria	0.59	6.07E-04	positive
<i>g_Facklamia</i>	<i>g_Actinomyces</i>	0.76%	3.70%	Firmicutes	Actinobacteria	-0.45	2.57E-02	negative
<i>g_Facklamia</i>	<i>g_Turicibacter</i>	0.76%	1.29%	Firmicutes	Firmicutes	0.43	3.31E-02	positive
<i>g_Facklamia</i>	<i>g_Kocuria</i>	0.76%	1.20%	Firmicutes	Actinobacteria	0.54	3.55E-03	positive
<i>g_Facklamia</i>	<i>g_Aerococcus</i>	0.76%	0.99%	Firmicutes	Firmicutes	0.56	2.09E-03	positive
<i>g_Globicatella</i>	<i>g_Actinomyces</i>	0.06%	3.70%	Firmicutes	Actinobacteria	0.50	1.00E-02	positive
<i>g_Globicatella</i>	<i>f_Aerococcaceae._</i>	0.06%	0.16%	Firmicutes	Firmicutes	0.53	4.24E-03	positive
<i>g_Lactobacillus</i>	<i>g_Turicibacter</i>	1.26%	1.29%	Firmicutes	Firmicutes	0.44	3.25E-02	positive
<i>g_Streptococcus</i>	<i>g_Brevibacterium</i>	0.87%	0.07%	Firmicutes	Actinobacteria	-0.50	1.00E-02	negative
<i>g_Streptococcus</i>	<i>g_Turicibacter</i>	0.87%	1.29%	Firmicutes	Firmicutes	0.50	9.70E-03	positive
<i>g_Streptococcus</i>	<i>g_Actinomyces</i>	0.87%	3.70%	Firmicutes	Actinobacteria	-0.41	4.57E-02	negative
<i>g_Streptococcus</i>	<i>f_Aerococcaceae._</i>	0.87%	0.16%	Firmicutes	Firmicutes	-0.41	4.39E-02	negative
<i>g_Staphylococcus</i>	<i>g_Kocuria</i>	2.10%	1.20%	Firmicutes	Actinobacteria	0.44	2.80E-02	positive
<i>g_Clostridium_sensu_stricto_1</i>	<i>g_Turicibacter</i>	0.02%	1.29%	Firmicutes	Firmicutes	0.47	1.73E-02	positive
<i>g_Clostridium_sensu_stricto_1</i>	<i>g_Romboutsia</i>	0.02%	1.15%	Firmicutes	Firmicutes	0.49	1.00E-02	positive
<i>g_Romboutsia</i>	<i>g_Lactobacillus</i>	1.15%	1.26%	Firmicutes	Firmicutes	0.50	1.00E-02	positive
<i>g_Romboutsia</i>	<i>g_Turicibacter</i>	1.15%	1.29%	Firmicutes	Firmicutes	0.94	1.82E-19	positive
<i>g_Romboutsia</i>	<i>g_Facklamia</i>	1.15%	0.76%	Firmicutes	Firmicutes	0.50	9.97E-03	positive
<i>g_Romboutsia</i>	<i>g_Streptococcus</i>	1.15%	0.87%	Firmicutes	Firmicutes	0.44	3.05E-02	positive

Genus1	Genus2	Abundance1	Abundance2	Phylum1	Phylum2	correlation	adj_P	Direction
<i>g_Gallicola</i>	<i>g_Enterococcus</i>	16.34%	29.37%	Firmicutes	Firmicutes	-0.46	2.20E-02	negative
<i>g_Gallicola</i>	<i>g_Lactobacillus</i>	16.34%	1.26%	Firmicutes	Firmicutes	0.42	4.31E-02	positive
<i>g_Gallicola</i>	<i>g_Streptococcus</i>	16.34%	0.87%	Firmicutes	Firmicutes	0.50	1.00E-02	positive
<i>g_Helcococcus</i>	<i>f_Aerococcaceae_</i>	0.52%	0.16%	Firmicutes	Firmicutes	0.68	1.02E-05	positive
<i>g_Helcococcus</i>	<i>g_Corynebacterium</i>	0.52%	10.12%	Firmicutes	Actinobacteria	0.43	3.31E-02	positive
<i>g_Helcococcus</i>	<i>g_Actinomyces</i>	0.52%	3.70%	Firmicutes	Actinobacteria	0.92	9.02E-17	positive
<i>g_Helcococcus</i>	<i>g_Bacteroides</i>	0.52%	0.84%	Firmicutes	Bacteroidetes	0.42	3.75E-02	positive
<i>g_Helcococcus</i>	<i>g_Globicatella</i>	0.52%	0.06%	Firmicutes	Firmicutes	0.50	1.00E-02	positive
<i>g_W5053</i>	<i>g_Actinomyces</i>	0.08%	3.70%	Firmicutes	Actinobacteria	0.42	3.83E-02	positive
<i>g_W5053</i>	<i>f_Aerococcaceae_</i>	0.08%	0.16%	Firmicutes	Firmicutes	0.41	4.39E-02	positive
<i>g_W5053</i>	<i>g_Bacteroides</i>	0.08%	0.84%	Firmicutes	Bacteroidetes	0.53	3.97E-03	positive
<i>g_W5053</i>	<i>g_Helcococcus</i>	0.08%	0.52%	Firmicutes	Firmicutes	0.48	1.27E-02	positive
<i>g_Phascolarctobacterium</i>	<i>g_Enterococcus</i>	0.09%	29.37%	Firmicutes	Firmicutes	-0.41	4.39E-02	negative
<i>g_Phascolarctobacterium</i>	<i>g_Gallicola</i>	0.09%	16.34%	Firmicutes	Firmicutes	0.43	3.31E-02	positive
<i>g_Phascolarctobacterium</i>	<i>g_W5053</i>	0.09%	0.08%	Firmicutes	Firmicutes	0.49	1.00E-02	positive
<i>g_Phascolarctobacterium</i>	<i>g_Bacteroides</i>	0.09%	0.84%	Firmicutes	Bacteroidetes	0.82	5.13E-10	positive
<i>g_Veillonella</i>	<i>g_Bacteroides</i>	0.05%	0.84%	Firmicutes	Bacteroidetes	0.47	1.73E-02	positive
<i>g_Veillonella</i>	<i>g_Lactobacillus</i>	0.05%	1.26%	Firmicutes	Firmicutes	0.56	1.77E-03	positive
<i>g_Veillonella</i>	<i>g_Phascolarctobacterium</i>	0.05%	0.09%	Firmicutes	Firmicutes	0.41	4.39E-02	positive
<i>g_Fusobacterium</i>	<i>g_Bacteroides</i>	1.48%	0.84%	Fusobacteria	Bacteroidetes	0.48	1.45E-02	positive
<i>g_Fusobacterium</i>	<i>g_Gallicola</i>	1.48%	16.34%	Fusobacteria	Firmicutes	0.41	4.75E-02	positive
<i>g_Fusobacterium</i>	<i>g_Phascolarctobacterium</i>	1.48%	0.09%	Fusobacteria	Firmicutes	0.44	3.03E-02	positive
<i>g_Escherichia.Shigella</i>	<i>g_Ralstonia</i>	13.15%	0.02%	Proteobacteria	Proteobacteria	-0.43	3.52E-02	negative
<i>g_Gallibacterium</i>	<i>g_Helcococcus</i>	0.12%	0.52%	Proteobacteria	Firmicutes	0.44	2.89E-02	positive
<i>g_Gallibacterium</i>	<i>g_Actinomyces</i>	0.12%	3.70%	Proteobacteria	Actinobacteria	0.46	1.93E-02	positive

Table S5. Spearman correlation between vaginal microbial genera

Genus1	Genus2	Abundance1	Abundance2	Phylum1	Phylum2	Correlation	adj_P	Direction
<i>g_Turicibacter</i>	<i>g_Corynebacterium</i>	0.21%	0.22%	Firmicutes	Actinobacteria	0.41	1.30E-02	positive
<i>g_Aerococcus</i>	<i>g_Bacteroides</i>	1.06%	0.39%	Firmicutes	Bacteroidetes	-0.42	9.65E-03	negative
<i>g_Enterococcus</i>	<i>g_Corynebacterium</i>	17.48%	0.22%	Firmicutes	Actinobacteria	0.48	1.81E-03	positive
<i>g_Enterococcus</i>	<i>g_Rhodococcus</i>	17.48%	0.04%	Firmicutes	Actinobacteria	0.38	2.36E-02	positive
<i>g_Lactobacillus</i>	<i>g_Turicibacter</i>	1.02%	0.21%	Firmicutes	Firmicutes	0.56	1.22E-04	positive
<i>g_Lactobacillus</i>	<i>g_Corynebacterium</i>	1.02%	0.22%	Firmicutes	Actinobacteria	0.49	1.57E-03	positive
<i>g_Streptococcus</i>	<i>g_Lactobacillus</i>	0.43%	1.02%	Firmicutes	Firmicutes	0.56	1.21E-04	positive
<i>g_Streptococcus</i>	<i>g_Turicibacter</i>	0.43%	0.21%	Firmicutes	Firmicutes	0.58	5.38E-05	positive
<i>g_Staphylococcus</i>	<i>g_Aerococcus</i>	3.82%	1.06%	Firmicutes	Firmicutes	0.56	1.17E-04	positive
<i>g_Clostridium_sensu_stricto_1</i>	<i>g_Streptococcus</i>	0.14%	0.43%	Firmicutes	Firmicutes	0.41	1.24E-02	positive
<i>g_Clostridium_sensu_stricto_1</i>	<i>g_Lactobacillus</i>	0.14%	1.02%	Firmicutes	Firmicutes	0.37	2.83E-02	positive
<i>g_Clostridium_sensu_stricto_1</i>	<i>g_Turicibacter</i>	0.14%	0.21%	Firmicutes	Firmicutes	0.56	1.02E-04	positive
<i>g_Clostridium_sensu_stricto_1</i>	<i>g_Romboutsia</i>	0.14%	0.36%	Firmicutes	Firmicutes	0.50	9.46E-04	positive
<i>g_Clostridium_sensu_stricto_1</i>	<i>g_Gallicola</i>	0.14%	2.11%	Firmicutes	Firmicutes	0.39	2.12E-02	positive
<i>g_Romboutsia</i>	<i>g_Streptococcus</i>	0.36%	0.43%	Firmicutes	Firmicutes	0.58	4.51E-05	positive
<i>g_Romboutsia</i>	<i>g_Corynebacterium</i>	0.36%	0.22%	Firmicutes	Actinobacteria	0.41	1.38E-02	positive
<i>g_Romboutsia</i>	<i>g_Turicibacter</i>	0.36%	0.21%	Firmicutes	Firmicutes	0.92	4.84E-22	positive
<i>g_Romboutsia</i>	<i>g_Lactobacillus</i>	0.36%	1.02%	Firmicutes	Firmicutes	0.62	9.34E-06	positive
<i>g_Gallicola</i>	<i>g_Bacteroides</i>	2.11%	0.39%	Firmicutes	Bacteroidetes	0.37	3.33E-02	positive
<i>g_Gallicola</i>	<i>g_Corynebacterium</i>	2.11%	0.22%	Firmicutes	Actinobacteria	0.41	1.25E-02	positive
<i>g_Fusobacterium</i>	<i>g_Bacteroides</i>	4.62%	0.39%	Fusobacteria	Bacteroidetes	0.41	1.30E-02	positive
<i>g_Fusobacterium</i>	<i>g_Gallicola</i>	4.62%	2.11%	Fusobacteria	Firmicutes	0.41	1.38E-02	positive
<i>g_Fusobacterium</i>	<i>g_Corynebacterium</i>	4.62%	0.22%	Fusobacteria	Actinobacteria	0.37	3.34E-02	positive

Genus1	Genus2	Abundance1	Abundance2	Phylum1	Phylum2	Correlation	adj_P	Direction
<i>g_Fusobacterium</i>	<i>g_Lactobacillus</i>	4.62%	1.02%	Fusobacteria	Firmicutes	0.51	7.82E-04	positive
<i>g_Brevundimonas</i>	<i>g_Corynebacterium</i>	0.77%	0.22%	Proteobacteria	Actinobacteria	0.38	2.76E-02	positive
<i>g_Brevundimonas</i>	<i>g_Lactobacillus</i>	0.77%	1.02%	Proteobacteria	Firmicutes	0.43	9.65E-03	positive
<i>g_Ralstonia</i>	<i>g_Corynebacterium</i>	0.01%	0.22%	Proteobacteria	Actinobacteria	0.52	4.53E-04	positive
<i>g_Ralstonia</i>	<i>g_Rhodococcus</i>	0.01%	0.04%	Proteobacteria	Actinobacteria	0.67	3.23E-07	positive
<i>g_Escherichia.Shigella</i>	<i>f_Enterobacteriaceae._</i>	30.10%	4.12%	Proteobacteria	Proteobacteria	-0.60	2.17E-05	negative
<i>g_Acinetobacter</i>	<i>g_Escherichia.Shigella</i>	4.20%	30.10%	Proteobacteria	Proteobacteria	-0.48	1.83E-03	negative
<i>g_Acinetobacter</i>	<i>f_Enterobacteriaceae._</i>	4.20%	4.12%	Proteobacteria	Proteobacteria	0.45	5.23E-03	positive
<i>g_Pseudomonas</i>	<i>g_Escherichia.Shigella</i>	12.91%	30.10%	Proteobacteria	Proteobacteria	-0.51	7.54E-04	negative

Table S6. The difference of eggshell color-L value between the birds with the highest and lowest vaginal *Staphylococcus* abundance.

Age (weeks)	The highest abundance	The lowest abundance	adj_ <i>P</i>
36	56.89 ± 1.76	59.31 ± 2.23	0.015
56	58.00 ± 1.63	60.72 ± 1.90	0.003
72	58.85 ± 2.70	61.91 ± 2.86	0.029
80	60.55 ± 2.81	61.42 ± 3.23	0.528
90	54.47 ± 2.59	57.86 ± 3.70	0.039

Table S7. The difference of eggshell color-L value between the birds with the highest and lowest vaginal *Ralstonia* abundance.

Age (weeks)	The highest abundance	The lowest abundance	adj_ <i>P</i>
36	56.95 ± 3.01	59.13 ± 1.56	0.057
56	58.49 ± 2.58	60.89 ± 2.09	0.035
72	59.23 ± 2.65	62.06 ± 1.94	0.014
80	58.41 ± 2.38	61.51 ± 2.43	0.010
90	53.62 ± 3.35	57.95 ± 3.79	0.015

Table S8. The difference of egg number between the birds with the highest and lowest vaginal *Romboutsia* abundance.

Age (weeks)	The highest abundance	The lowest abundance	adj_ <i>P</i>
36	108.9 ± 7.34	115.8 ± 6.56	0.040
56	238.5 ± 9.88	247.6 ± 5.74	0.021
72	340.4 ± 12.44	352.2 ± 6.8	0.017
80	390.1 ± 15.02	402.5 ± 7.93	0.033
90	446.6 ± 19.84	460.5 ± 11.15	0.069

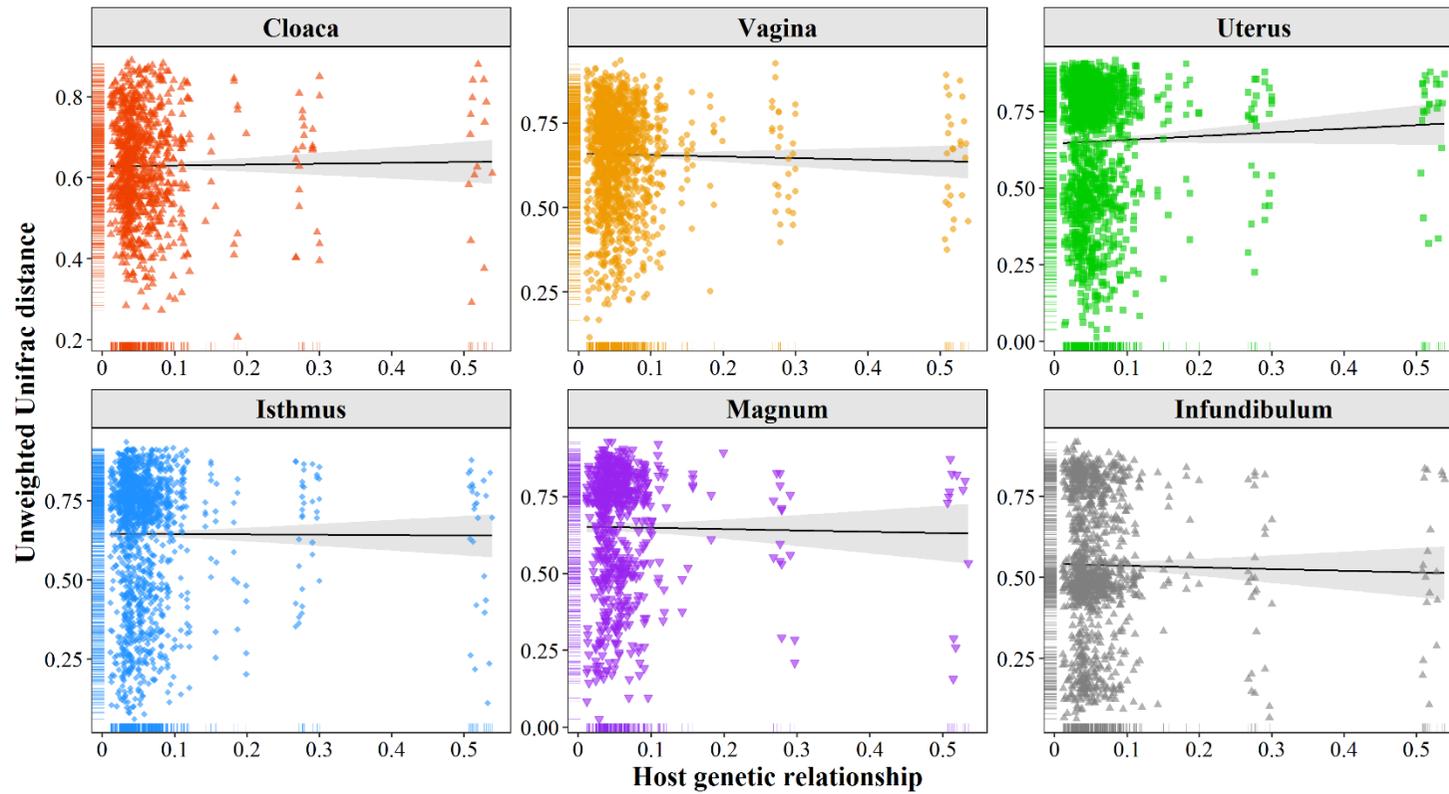


Figure S1. Relationship between host genetic relationship and unweighted UniFrac distance on the six segments of reproductive tract.