

Supplementary Material

In vitro sperm-ovum binding assays show no evidence of species-specific zona pellucida binding between wild-derived strains of *Mus musculus musculus*, *M. m. domesticus* and *M. spretus*

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Table S1. Results of linear mixed effect model of the number of sperm bound on ova for four possible sperm-ovum interactions: *M. m. domesticus* sperm and *M. m. domesticus* ova (the conspecific Mmd-Mmd interaction), *M. m. domesticus* sperm and *M. m. musculus* ova (Mmd-Mmm interaction), *M. m. musculus* sperm and *M. m. domesticus* ova (Mmm-Mmd interaction) and *M. m. musculus* sperm and *M. m. musculus* ova (Mmm-Mmm interaction). Male and female IDs were used as random grouping variables. Mmd-Mmd interaction is the reference level (Intercept). For each model, the estimates $\pm$ S.E., and t-values are presented.

	$\beta \pm \text{SE}$	df	t-test	P
(Intercept)	3.14 $\pm$ 0.14	101.32	22.77	< 0.001
Mmd-Mmm	0.20 $\pm$ 0.14	97.40	1.40	0.164
Mmm-Mmd	-0.90 $\pm$ 0.16	69.49	-5.56	< 0.001
Mmm-Mmm	-0.59 $\pm$ 0.18	91.28	-3.21	0.002

Table S2. Mean numbers of sperm bound to ova (ln scale) with associated upper and lower 95% confidence intervals, estimated from a linear mixed effect model of the number of sperm bound to ova for four possible sperm-ovum interactions: *M. m. domesticus* sperm and *M. m. domesticus* ova (Mmd-Mmd), *M. m. domesticus* sperm and *M. m. musculus* ova (Mmd-Mmm), *M. m. musculus* sperm and *M. m. domesticus* ova (Mmm-Mmd) and *M. m. musculus* sperm and *M. m. musculus* ova (Mmm-Mmm). Male and female IDs were used as random grouping variables.

Combination	lsmean $\pm$ SE	df	lower CL	upper CL
Mmd-Mmd	3.14 $\pm$ 0.14	101.55	2.87	3.42
Mmd-Mmm	3.34 $\pm$ 0.12	82.73	3.10	3.58
Mmm-Mmd	2.24 $\pm$ 0.13	103.97	1.98	2.49
Mmm-Mmm	2.55 $\pm$ 0.11	88.92	2.33	2.78

Table S3. Results of linear mixed effect model of the number of sperm bound to ova for nine possible sperm-ovum interactions: *M. m. domesticus* sperm and *M. m. domesticus* ova (Mmd-Mmd), *M. m. domesticus* sperm and *M. m. musculus* ova (Mmd-Mmm), *M. m. domesticus* sperm and *M. spretus* ova (Mmd-Msp), *M. m. musculus* sperm and *M. m. domesticus* ova (Mmm-Mmd), *M. m. musculus* sperm and *M. m. musculus* ova (Mmm-Mmm), *M. m. musculus* sperm and *M. spretus* ova (Mmm-Msp), *M. spretus* sperm and *M. m. domesticus* ova (Msp-Mmd), *M. spretus* sperm and *M. m. musculus* ova (Msp-Mmm) and *M. spretus* sperm and *M. spretus* ova (Msp-Msp). Mmd-Mmd interaction is the reference level (Intercept). For each contrast, the estimates $\pm$ S.E., and t-values are presented.

Interaction	$\beta \pm \text{SE}$	df	t-test	P
(Intercept)	3.10 $\pm$ 0.13	138.75	23.78	< 0.001
Mmd-Mmm	0.21 $\pm$ 0.13	126.98	1.52	0.130
Mmd-Msp	0.01 $\pm$ 0.17	136.63	0.03	0.975
Mmm-Mmd	-0.82 $\pm$ 0.17	109.93	-4.95	< 0.001
Mmm-Mmm	-0.56 $\pm$ 0.17	124.99	-3.30	< 0.001
Mmm-Msp	-0.66 $\pm$ 0.20	153.42	-3.30	< 0.001
Msp-Mmd	0.97 $\pm$ 0.27	116.85	3.65	< 0.001
Msp-Mmm	1.25 $\pm$ 0.24	108.20	5.12	< 0.001
Msp-Msp	1.51 $\pm$ 0.29	143.19	5.15	< 0.001

Table S4. Results of a *post hoc* analysis of variation in the number of sperm bound to ova in all combinations. Analysis is based on a mixed linear model involving nine possible sperm-ovum interactions: *M. m. domesticus* sperm and *M. m. domesticus* ova (Mmd-Mmd), *M. m. domesticus* sperm and *M. m. musculus* ova (Mmd-Mmm), *M. m. domesticus* sperm and *M. spretus* ova (Mmd-Msp), *M. m. musculus* sperm and *M. m. domesticus* ova (Mmm-Mmd), *M. m. musculus* sperm and *M. m. musculus* ova (Mmm-Mmm), *M. m. musculus* sperm and *M. spretus* ova (Mmm-Msp), *M. spretus* sperm and *M. m. domesticus* ova (Msp-Mmd), *M. spretus* sperm and *M. m. musculus* ova (Msp-Mmm) and *M. spretus* sperm and *M. spretus* ova (Msp-Msp). For each comparison, the estimates $\pm$ S.E., and t-values are presented.

Interaction comparison	Estimate $\pm$ SE	df	t-test	P
Mmd/Mmd-Mmd/Mmm	-0.21 $\pm$ 0.14	131.3	-1.50	0.854
Mmd/Mmd-Mmd/Msp	-0.01 $\pm$ 0.17	138.8	-0.03	1.000
Mmd/Mmd-Mmm/Mmd	0.82 $\pm$ 0.17	117.0	4.89	< 0.001
Mmd/Mmd-Mmm/Mmm	0.56 $\pm$ 0.17	126.1	3.28	0.035
Mmd/Mmd-Mmm/Msp	0.66 $\pm$ 0.20	155.0	3.26	0.036
Mmd/Mmd-Msp/Mmd	-0.97 $\pm$ 0.27	118.3	-3.61	0.013
Mmd/Mmd-Msp/Mmm	-1.25 $\pm$ 0.25	109.0	-5.09	< 0.001
Mmd/Mmd-Msp/Msp	-1.51 $\pm$ 0.30	148.2	-5.10	< 0.001
Mmd/Mmm-Mmd/Msp	0.20 $\pm$ 0.16	144.1	1.21	0.952
Mmd/Mmm-Mmm/Mmd	1.03 $\pm$ 0.17	121.0	6.04	< 0.001
Mmd/Mmm-Mmm/Mmm	0.77 $\pm$ 0.15	96.4	5.13	< 0.001
Mmd/Mmm-Mmm/Msp	0.87 $\pm$ 0.20	147.2	4.43	0.001
Mmd/Mmm-Msp/Mmd	-0.77 $\pm$ 0.27	116.4	-2.88	0.104
Mmd/Mmm-Msp/Mmm	-1.05 $\pm$ 0.23	94.9	-4.49	0.001
Mmd/Mmm-Msp/Msp	-1.31 $\pm$ 0.30	149.7	-4.41	0.001
Mmd/Msp-Mmm/Mmd	0.83 $\pm$ 0.20	150.1	4.11	0.002
Mmd/Msp-Mmm/Mmm	0.57 $\pm$ 0.19	145.2	2.94	0.087
Mmd/Msp-Mmm/Msp	0.67 $\pm$ 0.21	153.8	3.12	0.054
Mmd/Msp-Msp/Mmd	-0.97 $\pm$ 0.28	131.6	-3.45	0.021
Mmd/Msp-Msp/Mmm	-1.25 $\pm$ 0.27	121.9	-4.64	< 0.001
Mmd/Msp-Msp/Msp	-1.51 $\pm$ 0.29	119.3	-5.17	< 0.001
Mmm/Mmd-Mmm/Mmm	-0.26 $\pm$ 0.14	144.7	-1.90	0.613
Mmm/Mmd-Mmm/Msp	-0.16 $\pm$ 0.17	113.6	-0.97	0.988
Mmm/Mmd-Msp/Mmd	-1.80 $\pm$ 0.26	115.0	-6.88	< 0.001
Mmm/Mmd-Msp/Mmm	-2.07 $\pm$ 0.24	106.8	-8.50	< 0.001
Mmm/Mmd-Msp/Msp	-2.33 $\pm$ 0.30	151.7	-7.87	< 0.001
Mmm/Mmm-Mmm/Msp	0.10 $\pm$ 0.16	127.7	0.61	0.100
Mmm/Mmm-Msp/Mmd	-1.53 $\pm$ 0.26	117.0	-5.91	< 0.001
Mmm/Mmm-Msp/Mmm	-1.81 $\pm$ 0.23	97.0	-7.77	< 0.001
Mmm/Mmm-Msp/Msp	-2.07 $\pm$ 0.29	146.6	-7.22	< 0.001
Mmm/Msp-Msp/Mmd	-1.63 $\pm$ 0.29	136.5	-5.66	< 0.001
Mmm/Msp-Msp/Mmm	-1.91 $\pm$ 0.26	126.4	-7.37	< 0.001
Mmm/Msp-Msp/Msp	-2.17 $\pm$ 0.30	130.6	-7.33	< 0.001
Msp/Mmd-Msp/Mmm	-0.28 $\pm$ 0.26	157.0	-1.08	0.976
Msp/Mmd-Msp/Msp	-0.54 $\pm$ 0.26	107.2	-2.05	0.511
Msp/Mmm-Msp/Msp	-0.26 $\pm$ 0.25	103.4	-1.02	0.983

Table S5. Fixed effects from the linear mixed-effects model assessing differences in sperm curvilinear velocity (VCL, $\mu\text{m/s}$) between species. The model included 'Species' as a fixed effect and 'Strain' as a random intercept. Estimates ($\mu\text{m/s}$), standard errors (SE), degrees of freedom (df), t-values, and P values are reported. The intercept corresponds to the reference species Mmd. Mmm and Msp are differences from the reference. Intercept stands for Mmd.

	$\beta \pm$ SE	t-test	P
(Intercept)	123.34 $\pm$ 4.61	26.78	< 0.001
Mmm	4.43 $\pm$ 6.01	0.74	0.463
Msp	15.31 $\pm$ 9.77	1.57	0.121

Table S6. Estimated marginal means (EMMs) of sperm curvilinear velocity (VCL, $\mu\text{m/s}$) for three species (Mmd, Mmm, Msp) from a linear mixed-effects model with 'species' as a fixed effect and 'strain' as a random intercept. SE – standard error; 95% CI – 95% confidence interval. Mmd is the reference species in the model.

Taxon	EMM VCL ($\mu\text{m/s}$)	SE	df	95% CI
Mmd	123.27	5.23	5.16	109.95-136.59
Mmm	128.27	4.47	4.54	116.41-140.12
Msp	138.65	9.56	6.65	115.79-161.51

Table S7. Fixed effects from the linear mixed-effects model testing changes in sperm curvilinear velocity (VCL) over time. The model included 'Time' (0 vs. 120 min), 'Species' (reference level = *M. m. domesticus*, Mmd), and their interaction as fixed effects, with individual ID included as a random intercept. The intercept therefore represents VCL in Mmd at time 0, while interaction terms indicate species-specific differences in temporal change relative to Mmd. Estimates (β), standard errors (SE), degrees of freedom (df), t-values, and significance levels (P) are shown.

Effect	Estimate	Std. Error	df	t-test	P
(Intercept)	173.46	6.34	53.0	27.37	< 0.001
Time (120 min)	-23.63	7.82	28.0	-3.02	0.005
Mmm	-2.59	7.69	53.0	-0.34	0.738
Msp	11.17	9.68	53.0	1.15	0.254
Time $\times$ Mmm	-15.57	9.49	28.0	-1.64	0.112
Time $\times$ Msp	-30.96	11.95	28.0	-2.59	0.015

Table S8. Fixed-effect estimates from the linear mixed-effects model testing the effects of 'Time' (0 vs. 120 min), 'Species' (reference level = *M. m. domesticus*, Mmd), and their interaction on the proportion of motile sperm. Individual identity was included as a random intercept. The intercept therefore represents the proportion of motile sperm in Mmd at time 0, while interaction terms indicate species-specific differences in temporal change relative to Mmd.

Effect	Estimate	Std. Error	df	t-test	P
(Intercept)	79.63	4.34	53.6	18.34	< 0.001
Time (120 min)	-10.25	5.46	28.0	-1.88	0.071
Mmm	-8.86	5.27	53.6	-1.68	0.098
Msp	8.71	6.63	53.6	1.31	0.195
Time (120 min) $\times$ Mmm	-20.28	6.62	28.0	-3.10	0.005
Time (120 min) $\times$ Msp	3.58	8.34	28.0	0.43	0.671