SUPPLEMENTARY MATERIAL

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The effect of mixed honeybee drone semen on sperm quality characteristics: A preliminary study

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Supplementary Table 1. Effect of mixed drone semen on sperm motility as determined by the manual motility index score

Motility index score	Individual (*n* = 31)	Two-drone samples (*n* = 17)	Three-drone samples (*n* = 12)	*P* value	Test value
T0	5 (3.25, 5)	5 (4.75, 5)	5 (3, 5)	0.401	1.310
T60	5 (3, 5)	5 (4.75, 5)	5 (4, 5)	0.225	2.122

Data is presented as median and 25,75% tiles. T0, baseline; T60, 60 minutes.

Supplementary Table 2. Effect of mixed drone semen on sperm motility parameters over time within groups.

Parameter	Individual (*n* = 31)			Two-drone samples (*n* = 17)			Three-drone samples (*n* = 12)		
	T0	**T60**	***P* value**	**T0**	**T60**	***P* value**	**T0**	**T60**	***P* value**
TM (%)	88.1(73.0,97.2)	83.3(63.5,93.3)	0.147 (*F*=2.217)	86.9(67.9,95.2)[a]	71.6(51.4,83.2)[a]	0.004 (*F*=11.52)	79.1(55.1,94.9)	78.9(65.7,96.8)	0.586 (*F*=0.314)
IM (%)	11.9(2.76,27.0)	16.7(6.70,36.5)	0.147 (*F*=2.217)	13.1(4.76,32.1)[a]	28.5(16.8,48.6)[a]	0.004 (*F*=11.52)	20.9(5.07,35.0)	21.2(7.25,34.3)	0.586 (*F*=0.314)
PR (%)	1.09(0.00,16.4)	1.05(0.26,4.34)	0.856 (*F*=0.033)	1.76(0.40,3.10)	1.95(0.00,4.45)	0.299 (*F*=1.153)	1.30(0.52,2.83)	2.81(0.81,9.13)	0.266 (*F*=1.375)
RP (%)	0.00(0.00,1.49)	0.00(0.00,0.48)	0.645 (*F*=0.217)	0.00(0.00,0.09)	0.00(0.00,0.37)	0.431 (*F*=0.653)	0.00(0.00,0.56)	0.16(0.00,0.95)	0.166 (*F*=2.200)
MP (%)	1.09(0.00,15.3)	1.05(0.07,3.79)	0.712 (*F*=0.139)	1.76(0.40,2.71)	1.56(0.00,3.99)	0.608 (*F*=0.274)	1.01(0.52,2.39)	1.97(0.81,8.62)	0.266 (*F*=1.375)
NP (%)	78.7(63.6,84.2)	77.7(62.4,83.9)	1.000 (*F*=0.000)	81.9(67.8,90.4)[a]	66.4(50.5,79.0)[a]	<0.001 (*F*=22.53)	78.4(64.1,88.7)	75.5(57.1,80.3)	0.082 (*F*=3.667)
Rapid (%)	0.63(0.00,5.89)	0.76(0.00,2.33)	0.702 (*F*=0.150)	0.34(0.00,1.19)	0.32(0.00,1.79)	0.382 (*F*=0.809)	0.46(0.00,1.21)	0.76(0.00,1.42)	0.551 (*F*=0.379)
Medium (%)	3.57(0.44,26)	2.86(0.62,8.82)	0.712 (*F*=0.139)	3.17(1.60,7.31)	2.17(0.48,7.52)	0.632 (*F*=0.239)	2.38(1.09,7.11)	4.24(1.20,19.1)	0.266 (*F*=1.375)
Slow (%)	67.6(45.6,78.6)	67.6(50.4,79.1)	1.000 (*F*=0.000)	78.7(67.5,85.1)[a]	64.7(43.2,74.9)[a]	<0.001 (*F*=22.53)	73.3(59.7,83.5)	70.3(53.6,74.2)	0.082 (*F*=3.667)

Non-parametric data has been reported as median and 25,75% tiles. [a] Medians with the same letter in a row differed significantly. T0, baseline; T60, 60 minutes. TM, total motility; IM, immotile; PR, progressive; RP, rapid progressive; MP, medium progressive; NP, non-progressive.

Supplementary Table 3. Effect of mixed drone semen on sperm kinematic parameters over time within groups.

Parameter	Individual samples (*n* = 31)			Two-drone samples (*n* = 17)			Three-drone samples (*n* = 12)		
	T0	**T60**	***P* value**	**T0**	**T60**	***P* Value**	**T0**	**T60**	***P* Value**
VCL (µm/s)	33.3(23.0,55.7)	33.3(24.1,39.8)	0.721 (*F*=0.130)	33.6(22.7,38.6)	28.8(22.5,36.9)	0.484 (*F*=0.514)	27.9(25.1,38.5)	30.8(27.1,46.5)	0.266 (*F*=1.375)
VSL (µm/s)	15.6(4.74,29.0)	15.3 ± 10.6	0.721 (*F*=0.130)	16.9(8.51,20.8)	14.2 ± 10.1	0.236 (*F*=1.515)	13.1(8.14,22.7)	20.3 ± 11.3	0.266 (*F*=1.375)
VAP (µm/s)	23.9(10.7,45.0)	20.5(14.1,33.8)	0.721 (*F*=0.130)	25.3(14.5,31.4)	20.6(10.3,31.2)	0.484 (*F*=0.514)	20.8(13.1,33.1)	24.1(19.6,38.4)	0.266 (*F*=1.375)
ALH (µm/s)	0.99(0.85,1.34)	0.99(0.84,1.17)	0.856 (*F*=0.033)	0.97(0.82,1.07)	0.91(0.78,1.05)	0.236 (*F*=1.515)	0.93(0.87,1.03)	1.00(0.86,1.11)	0.137 (*F*=2.570)
LIN (%)	41.3(16.3,49.8)	41.1(26.3,46.0)	0.147 (*F*=2.217)	43.3(30.8,48.9)	41.9(19.1,51.9)	0.236 (*F*=1.515)	39.7(25.8,53.7)	49.0(33.9,53.3)	0.586 (*F*=0.314)
STR (%)	55.6(35.2,59.8)	55.5(42.0,58.1)	0.147 (*F*=2.217)	56.1(46.8,60.6)	54.9(38.9,62.1)	0.0895 (*F*=3.267)	50.1(42.0,63.5)	59.9(51.9,63.3)	0.586 (*F*=0.314)
WOB (%)	65.9(42.7,76.5)	63.9(52.5,74.8)	0.474 (*F*=0.525)	68.6(54.5,74.5)	66.8(40.4,76.9)	0.236 (*F*=1.515)	68.8(48.0,80.7)	75.3(57.3,80.0)	0.586 (*F*=0.314)
BCF (Hz)	5.4 (2.58,9.02)	5.27 ± 3.06	1.000 (*F*=0.000)	6.30(3.61,7.32)	4.91 ± 2.92	0.484 (*F*=0.514)	5.46(3.44,7.86)	6.32 ± 2.58	1.000 (*F*=0.000)

Parametric data has been reported as mean and standard deviation (SD) and non-parametric data has been reported as median and 25,75% tiles. T0, baseline; T60, 60 minutes. VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average path velocity; ALH, amplitude of lateral head displacement; LIN, linearity; STR, straightness; WOB, wobble; BCF, beat cross frequency.

Supplementary Table 4. Correlations between sperm concentration and motility, and kinematic parameters over time.

Sperm concentration	**T0**		**T60**	
versus	***P* value**	**rho**	***P* value**	**rho**
TM (%)	0.001	0.417	0.004	0.369
PR (%)	0.001	0.404	0.006	0.354
RP (%)	0.053	0.251	0.262	0.147
MP (%)	0.002	0.402	0.004	0.369
Rapid (%)	0.023	0.293	0.357	0.121
Medium (%)	0.004	0.364	0.011	0.326
Slow (%)	0.062	0.242	0.069	0.237
VCL (μm/s)	<0.001	0.444	0.012	0.322
VSL (μm/s)	<0.001	0.462	0.004	0.368
VAP (μm/s)	<0.001	0.456	0.004	0.362
ALH (μm/s)	0.001	0.403	0.019	0.303
LIN (%)	<0.001	0.497	<0.001	0.477
STR (%)	<0.001	0.485	<0.001	0.450
WOB (%)	<0.001	0.522	<0.001	0.514
BCF (Hz)	<0.001	0.455	0.003	0.379

T0, baseline; T60, 60 minutes. TM, total motility; IM, immotile; PR, progressive; RP, rapid progressive; MP, medium progressive; NP, non-progressive; VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average path velocity; ALH, amplitude of lateral head displacement; LIN, linearity; STR, straightness; WOB, wobble; BCF, beat cross frequency; rho, Spearman's coefficient of rank correlation.

Supplementary Table 5. Comparison of sperm component lengths among groups of mixed drone semen.

Component	Individual	Two-drone	Three-drone	*P* value	Test statistic
Head length (µm)	9.94 ± 0.54	9.67 ± 0.30	9.66 ± 0.21	0.288	2.491
Tail length (µm)	223 ± 5.61	224 ± 9.18	226 ± 4.83	0.489	1.433
Total length (µm)	233 ± 5.91	234 ± 9.58	235 ± 4.78	0.609	0.993

Data is reported as mean and standard deviation (SD).