

## Supplementary Files

**Supplementary Table S1.** Sperm motility parameters evaluated by CASA System of sperm treated at different time with different concentration of EVs, compared to CTR.

EV concentration (x10 <sup>6</sup> )/ml	VCL (μm/s)					VSL (μm/s)				
	0h	1h	2h	3h	4h	0h	1h	2h	3h	4h
CTR	76.34 ± 70.15	64.25 ± 3.19 <sup>aA</sup>	49.76 ± 4.21 <sup>aB</sup>	49.05 ± 4.77 <sup>aB</sup>	49.50 ± 5.80 <sup>aB</sup>	34.12 ± 39.47	29.31 ± 2.75 <sup>aA</sup>	20.05 ± 1.66 <sup>aB</sup>	20.57 ± 2.43 <sup>aB</sup>	19.46 ± 1.32 <sup>aB</sup>
50		64.89 ± 4.50 <sup>aA</sup>	52.88 ± 5.35 <sup>aB</sup>	49.74 ± 5.65 <sup>aC</sup>	47.02 ± 4.13 <sup>aC</sup>		10.23 ± 1.08 <sup>bA</sup>	25.44 ± 2.60 <sup>bB</sup>	24.95 ± 2.02 <sup>bB</sup>	19.02 ± 2.18 <sup>aC</sup>
100		60.66 ± 6.54 <sup>aA</sup>	52.15 ± 5.82 <sup>aB</sup>	74.89 ± 7.02 <sup>bC</sup>	36.64 ± 3.45 <sup>bD</sup>		26.14 ± 2.81 <sup>cA</sup>	22.49 ± 2.58 <sup>aA</sup>	35.18 ± 3.10 <sup>bB</sup>	16.10 ± 1.63 <sup>bC</sup>
150		56.36 ± 5.42 <sup>aA</sup>	83.22 ± 7.19 <sup>bB</sup>	101.95 ± 8.29 <sup>cC</sup>	65.70 ± 6.74 <sup>bD</sup>		18.74 ± 2.45 <sup>dA</sup>	37.96 ± 3.69 <sup>bB</sup>	36.28 ± 3.69 <sup>dB</sup>	23.12 ± 2.52 <sup>cA</sup>
200		66.01 ± 6.95 <sup>aA</sup>	69.11 ± 6.82 <sup>cA</sup>	47.54 ± 4.05 <sup>aB</sup>	42.64 ± 5.19 <sup>bB</sup>		19.55 ± 2.71 <sup>dA</sup>	25.18 ± 2.86 <sup>bB</sup>	21.27 ± 2.38 <sup>aA</sup>	17.51 ± 1.05 <sup>aA</sup>
250		52.55 ± 5.20 <sup>aA</sup>	71.14 ± 6.06 <sup>cB</sup>	50.96 ± 5.98 <sup>aA</sup>	42.26 ± 4.90 <sup>bC</sup>		19.21 ± 2.21 <sup>dA</sup>	30.82 ± 3.09 <sup>dB</sup>	27.14 ± 2.77 <sup>bB</sup>	14.65 ± 1.90 <sup>bC</sup>
300		75.94 ± 6.03 <sup>bA</sup>	40.55 ± 4.74 <sup>dB</sup>	56.05 ± 6.21 <sup>aC</sup>	69.83 ± 6.12 <sup>cD</sup>		31.61 ± 3.12 <sup>aA</sup>	14.21 ± 1.16 <sup>eB</sup>	27.05 ± 2.85 <sup>bA</sup>	24.53 ± 2.49 <sup>cC</sup>
350		76.06 ± 8.96 <sup>bA</sup>	43.83 ± 4.56 <sup>dB</sup>	64.47 ± 6.88 <sup>dC</sup>	43.00 ± 4.59 <sup>cB</sup>		28.66 ± 3.46 <sup>aA</sup>	15.42 ± 1.32 <sup>eB</sup>	36.11 ± 3.14 <sup>dC</sup>	21.19 ± 1.25 <sup>cD</sup>
400		63.44 ± 6.43 <sup>aA</sup>	48.40 ± 4.03 <sup>aB</sup>	51.31 ± 5.58 <sup>aB</sup>	54.69 ± 5.86 <sup>dB</sup>		29.05 ± 1.48 <sup>aA</sup>	22.19 ± 1.31 <sup>aB</sup>	22.15 ± 2.75 <sup>aB</sup>	23.74 ± 2.47 <sup>cB</sup>
450		65.60 ± 6.63 <sup>aA</sup>	48.63 ± 4.63 <sup>aB</sup>	46.28 ± 4.29 <sup>aB</sup>	49.41 ± 4.34 <sup>aB</sup>		28.45 ± 3.07 <sup>aA</sup>	21.83 ± 1.75 <sup>aB</sup>	16.36 ± 1.91 <sup>cC</sup>	25.06 ± 2.90 <sup>cD</sup>
500		39.50 ± 4.25 <sup>cA</sup>	63.06 ± 6.64 <sup>cB</sup>	92.54 ± 8.49 <sup>cC</sup>	38.79 ± 3.77 <sup>dA</sup>		18.46 ± 1.57 <sup>dA</sup>	25.01 ± 2.80 <sup>bB</sup>	29.37 ± 2.32 <sup>bC</sup>	17.30 ± 1.71 <sup>bA</sup>

Legend: EV: amniotic derived extracellular vesicle; CTR: control; VCL: curvilinear-line velocity; VSL: straight-line velocity. Different capital letters (A–D) in superscript indicate statistically significant differences (P < 0.05) between the time 0 and 4 hours incubation. Different lowercase letter (a–e) in superscript indicate statistically significant differences (P < 0.05) between different EV concentrations.

**Supplementary Table S2.** Sperm motility parameters evaluated by CASA System of sperm treated at different time with different concentration of AMSC-EVs, compared to CTR.

EV concentration (x10 <sup>6</sup> )/ml	VAP (μm/s)					AHL (μm)				
	0h	1h	2h	3h	4h	0h	1h	2h	3h	4h
CTR	53.85 ± 55.92	41.91 ± 4.08 <sup>aA</sup>	33.26 ± 3.27 <sup>aA</sup>	31.20 ± 3.69 <sup>aA</sup>	30.95 ± 3.12 <sup>aA</sup>	4.35 ± 3.73	3.87 ± 0.72 <sup>aA</sup>	3.70 ± 0.35 <sup>aA</sup>	3.79 ± 0.43 <sup>aA</sup>	3.62 ± 3.51 <sup>aA</sup>
50		17.79 ± 1.90 <sup>bB</sup>	36.07 ± 3.49 <sup>aAB</sup>	33.26 ± 4.66 <sup>aA</sup>	29.54 ± 3.26 <sup>aA</sup>		3.74 ± 0.95 <sup>aA</sup>	3.93 ± 0.68 <sup>aA</sup>	2.84 ± 0.97 <sup>aA</sup>	4.02 ± 2.83 <sup>aA</sup>
100		33.05 ± 3.86 <sup>cC</sup>	40.23 ± 4.40 <sup>aB</sup>	32.94 ± 3.46 <sup>aA</sup>	29.45 ± 3.43 <sup>aA</sup>		4.40 ± 0.48 <sup>aA</sup>	4.56 ± 0.77 <sup>bA</sup>	3.89 ± 0.94 <sup>aA</sup>	3.96 ± 3.35 <sup>aA</sup>
150		28.36 ± 1.96 <sup>dC</sup>	40.18 ± 4.70 <sup>aB</sup>	37.92 ± 3.45 <sup>bA</sup>	30.96 ± 3.52 <sup>aA</sup>		4.59 ± 0.94 <sup>aA</sup>	4.21 ± 0.72 <sup>bA</sup>	3.53 ± 0.98 <sup>aA</sup>	3.03 ± 4.08 <sup>a</sup>
200		28.50 ± 2.14 <sup>dC</sup>	40.54 ± 4.38 <sup>aB</sup>	33.76 ± 3.72 <sup>aA</sup>	29.21 ± 3.62 <sup>aA</sup>		4.70 ± 0.27 <sup>aA</sup>	4.72 ± 0.02 <sup>bA</sup>	3.32 ± 0.83 <sup>aA</sup>	3.76 ± 2.74 <sup>aA</sup>
250		29.17 ± 2.05 <sup>dC</sup>	48.61 ± 4.30 <sup>bC</sup>	35.44 ± 3.53 <sup>aA</sup>	29.08 ± 3.40 <sup>aA</sup>		3.16 ± 0.36 <sup>aA</sup>	4.06 ± 0.44 <sup>bA</sup>	3.41 ± 0.54 <sup>aA</sup>	3.08 ± 3.84 <sup>aA</sup>
300		43.81 ± 4.48 <sup>eA</sup>	40.65 ± 4.65 <sup>aB</sup>	35.10 ± 4.24 <sup>aA</sup>	30.55 ± 3.07 <sup>aA</sup>		5.69 ± 0.83 <sup>bB</sup>	3.34 ± 0.68 <sup>aA</sup>	3.60 ± 0.24 <sup>aA</sup>	3.07 ± 3.62 <sup>aA</sup>
350		48.75 ± 4.01 <sup>eD</sup>	40.95 ± 3.53 <sup>aB</sup>	35.06 ± 7.71 <sup>aA</sup>	30.50 ± 3.72 <sup>aA</sup>		3.75 ± 0.98 <sup>aA</sup>	3.40 ± 0.36 <sup>aA</sup>	2.95 ± 0.14 <sup>aB</sup>	3.59 ± 2.61 <sup>aA</sup>
400		39.98 ± 3.88 <sup>cA</sup>	41.94 ± 3.79 <sup>aB</sup>	35.79 ± 3.69 <sup>aA</sup>	30.10 ± 3.85 <sup>aA</sup>		3.23 ± 0.14 <sup>aA</sup>	3.60 ± 0.60 <sup>aA</sup>	3.41 ± 0.56 <sup>aA</sup>	3.30 ± 3.35 <sup>aA</sup>
450		44.75 ± 4.83 <sup>eA</sup>	36.32 ± 3.17 <sup>aB</sup>	35.75 ± 3.51 <sup>aA</sup>	30.15 ± 3.26 <sup>aA</sup>		3.84 ± 0.43 <sup>aA</sup>	3.80 ± 0.80 <sup>aA</sup>	3.21 ± 0.26 <sup>aA</sup>	3.96 ± 2.62 <sup>aA</sup>
500		64.07 ± 6.78 <sup>fE</sup>	36.18 ± 3.88 <sup>aB</sup>	32.62 ± 2.94 <sup>aA</sup>	30.71 ± 3.26 <sup>aA</sup>		4.65 ± 0.02 <sup>aB</sup>	3.65 ± 0.60 <sup>aA</sup>	5.86 ± 1.02 <sup>bB</sup>	3.50 ± 2.70 <sup>aA</sup>

Legend: EV: amniotic derived extracellular vesicle; CTR: control; VAP: average path velocity; AHL: amplitude lateral head. Different capital letters (A,B) in superscript indicate statistically significant differences (P <0.05) between the time 0 and 4 hours incubation. Different lowercase letter (a,b) in superscript indicate statistically significant differences (P<0.05) between different EV concentrations.

**Supplementary Table S3.** Sperm motility parameters evaluated by CASA System of sperm treated at different time with different concentration of EVs, compared to CTR.

EV concentration (x10 <sup>6</sup> )/ml	LIN (%)					STR				
	0h	1h	2h	3h	4h	0h	1h	2h	3h	4h
CTR	44.58 ± 19.64	48.34 ± 3.01 <sup>aA</sup>	48.95 ± 2.60 <sup>aA</sup>	47.01 ± 1.21 <sup>aA</sup>	36.59 ± 1.20 <sup>aB</sup>	0.75 ± 0.28	0.86 ± 0.10 <sup>aA</sup>	0.84 ± 0.13 <sup>aB</sup>	0.86 ± 0.13 <sup>aA</sup>	0.78 ± 0.11 <sup>aC</sup>
50		44.82 ± 3.87 <sup>bA</sup>	46.46 ± 2.08 <sup>aAB</sup>	48.33 ± 1.61 <sup>aB</sup>	38.60 ± 2.43 <sup>aC</sup>		0.80 ± 0.14 <sup>bA</sup>	0.87 ± 0.11 <sup>bB</sup>	0.92 ± 0.19 <sup>bC</sup>	0.77 ± 0.11 <sup>aD</sup>
100		49.26 ± 4.58 <sup>aA</sup>	46.54 ± 4.14 <sup>aA</sup>	49.29 ± 2.22 <sup>aA</sup>	46.81 ± 1.91 <sup>bA</sup>		0.84 ± 0.11 <sup>cA</sup>	0.82 ± 0.12 <sup>aB</sup>	0.83 ± 0.11 <sup>cB</sup>	0.90 ± 0.10 <sup>bC</sup>
150		48.03 ± 4.77 <sup>aA</sup>	46.70 ± 4.96 <sup>aA</sup>	48.80 ± 2.34 <sup>aA</sup>	48.12 ± 2.21 <sup>bA</sup>		0.75 ± 0.12 <sup>dA</sup>	0.75 ± 0.19 <sup>cA</sup>	0.66 ± 0.19 <sup>dB</sup>	0.80 ± 0.10 <sup>cC</sup>
200		34.20 ± 3.67 <sup>cA</sup>	47.37 ± 4.94 <sup>aB</sup>	51.10 ± 2.64 <sup>bb</sup>	46.24 ± 1.55 <sup>bb</sup>		0.81 ± 0.13 <sup>bA</sup>	0.82 ± 0.10 <sup>aA</sup>	0.81 ± 0.16 <sup>bA</sup>	0.84 ± 0.13 <sup>dB</sup>
250		36.90 ± 2.90 <sup>cA</sup>	46.12 ± 4.93 <sup>aB</sup>	50.38 ± 2.42 <sup>bb</sup>	46.00 ± 1.68 <sup>bb</sup>		0.78 ± 0.16 <sup>dA</sup>	0.78 ± 0.15 <sup>cA</sup>	0.86 ± 0.11 <sup>eB</sup>	0.77 ± 0.14 <sup>aC</sup>
300		38.47 ± 1.41 <sup>cA</sup>	46.84 ± 3.34 <sup>aB</sup>	50.85 ± 1.47 <sup>bC</sup>	54.29 ± 2.30 <sup>cD</sup>		0.79 ± 0.18 <sup>dA</sup>	0.83 ± 0.11 <sup>aB</sup>	0.86 ± 0.10 <sup>eB</sup>	0.67 ± 0.11 <sup>cC</sup>
350		56.83 ± 2.17 <sup>dA</sup>	51.36 ± 4.36 <sup>bb</sup>	64.25 ± 2.16 <sup>cC</sup>	52.45 ± 2.41 <sup>cb</sup>		0.81 ± 0.16 <sup>bA</sup>	0.82 ± 0.16 <sup>aA</sup>	0.88 ± 0.19 <sup>eB</sup>	0.82 ± 0.18 <sup>dA</sup>
400		56.75 ± 2.75 <sup>dA</sup>	59.91 ± 2.35 <sup>cAB</sup>	54.49 ± 1.14 <sup>dB</sup>	54.37 ± 1.16 <sup>cB</sup>		0.87 ± 0.11 <sup>aA</sup>	0.87 ± 0.11 <sup>dB</sup>	0.85 ± 0.12 <sup>aC</sup>	0.88 ± 0.18 <sup>bb</sup>
450		50.32 ± 2.00 <sup>eA</sup>	52.68 ± 2.52 <sup>bA</sup>	41.07 ± 2.87 <sup>aB</sup>	47.39 ± 2.50 <sup>bA</sup>		0.83 ± 0.12 <sup>bA</sup>	0.79 ± 0.19 <sup>cB</sup>	0.82 ± 0.15 <sup>bA</sup>	0.81 ± 0.15 <sup>cA</sup>
500		44.28 ± 2.58 <sup>iA</sup>	49.13 ± 1.72 <sup>aB</sup>	37.09 ± 1.33 <sup>iC</sup>	48.82 ± 1.56 <sup>bb</sup>		0.82 ± 0.11 <sup>bA</sup>	0.82 ± 0.11 <sup>aA</sup>	0.72 ± 0.17 <sup>iB</sup>	0.89 ± 0.18 <sup>bC</sup>

Legend: EV: amniotic derived extracellular vesicle; CTR: control; LIN: linearity; STR: straightness. Different capital letters (A,B) in superscript indicate statistically significant differences (P <0.05) between the time 0 and 4 hours incubation. Different lowercase letter (a,b) in superscript indicate statistically significant differences (P<0.05) between different EV concentrations