

**Table S1.** Mean  $\pm$  SEM and range of the sperm motility parameters after thawing in donkey ejaculates classified as having good (GFE,  $n = 8$ ) or poor freezability (PFE,  $n = 7$ ).

Parameter	GFE		PFE	
	Mean $\pm$ SEM	Range	Mean $\pm$ SEM	Range
TM (%)	48.76 $\pm$ 1.58	42.35–53.27	32.29 $\pm$ 3.17	16.09–40.14
PM (%)	30.06 $\pm$ 1.62	24.88–36.44	17.12 $\pm$ 3.82	5.10–30.61
VCL ( $\mu\text{m/s}$ )	71.48 $\pm$ 6.39	52.71–111.16	67.27 $\pm$ 5.68	49.54–94.73
VSL ( $\mu\text{m/s}$ )	50.21 $\pm$ 6.77	35.35–94.52	48.38 $\pm$ 5.65	30.76–70.83
VAP ( $\mu\text{m/s}$ )	55.37 $\pm$ 7.20	38.36–102.04	52.89 $\pm$ 5.82	34.01–75.03
LIN (%)	68.94 $\pm$ 2.89	58.54–85.03	70.77 $\pm$ 3.27	59.90–80.77
STR (%)	90.48 $\pm$ 0.88	87.64–93.66	91.08 $\pm$ 0.67	88.88–94.40
WOB (%)	76.13 $\pm$ 2.84	65.20–91.80	77.62 $\pm$ 3.24	67.40–88.05
ALH ( $\mu\text{m}$ )	2.51 $\pm$ 0.12	2.08–3.20	2.30 $\pm$ 0.12	1.88–2.72
BCF (Hz)	11.95 $\pm$ 0.56	9.80–14.39	12.11 $\pm$ 0.53	10.28–13.95

TM (%): total motility; PM (%): progressive motility; VCL ( $\mu\text{m/s}$ ): curvilinear velocity; VSL ( $\mu\text{m/s}$ ): straight-line velocity; VAP ( $\mu\text{m/s}$ ): average path velocity; LIN (%): linearity coefficient; STR (%): straightness coefficient; WOB (%): wobble coefficient; ALH ( $\mu\text{m}$ ): amplitude of lateral head displacement; BCF (Hz): beat-cross frequency.

**Table S2.** Mean  $\pm$  SEM and range of the sperm function parameters after thawing in donkey ejaculates classified as having good (GFE,  $n = 8$ ) or poor freezability (PFE,  $n = 7$ ).

Parameter	GFE		PFE	
	Mean $\pm$ SEM	Range	Mean $\pm$ SEM	Range
SYBR14 <sup>+</sup> /PI <sup>-</sup> (%)	48.85 $\pm$ 1.23	43.16–55.48	30.43 $\pm$ 2.64	20.89–37.40
High MMP (%)	1.81 $\pm$ 0.13	1.42–2.36	2.12 $\pm$ 0.12	1.72–2.44
Intermediate MMP (%)	39.09 $\pm$ 1.95	30.40–46.28	55.64 $\pm$ 3.49	42.90–68.52
DCF <sup>+</sup> /PI <sup>-</sup> (%)	0.07 $\pm$ 0.03	0.02–0.27	0.12 $\pm$ 0.04	0.04–0.33
E <sup>+</sup> /YO-PRO-1 <sup>-</sup> (%)	0.52 $\pm$ 0.13	0.13–1.04	0.91 $\pm$ 0.27	0.28–1.97
Fluo3 <sup>+</sup> /PI <sup>-</sup> (%)	8.08 $\pm$ 0.93	5.69–14.36	9.92 $\pm$ 1.50	4.41–16.11
M540 <sup>+</sup> /YO-PRO-1 <sup>-</sup> (%)	2.02 $\pm$ 0.28	1.12–2.91	1.32 $\pm$ 0.20	0.39–1.89

SYBR14<sup>+</sup>/Propidium iodide (PI)<sup>-</sup> (%): sperm with intact plasma membrane (viable sperm); High MMP (%): sperm with high mitochondrial membrane potential; Intermediate MMP (%): sperm with intermediate mitochondrial membrane potential; Dichlorofluorescein (DCF)<sup>+</sup>/PI<sup>-</sup> (%): viable sperm with high intracellular ROS levels; Ethidium (E)<sup>+</sup>/YO-PRO-1<sup>-</sup> (%): viable sperm with high-O<sub>2</sub><sup>-</sup> levels; Fluo3-acetomethoxyester (Fluo3)<sup>+</sup>/PI<sup>-</sup> (%): viable sperm with high levels of intracellular calcium; Merocyanine 540 (M540)<sup>+</sup>/YO-PRO-1<sup>-</sup> (%): viable sperm with high membrane lipid disorder.

**Table S3.** Mean  $\pm$  SEM and range of the levels of enzymatic and non-enzymatic antioxidants in seminal plasma (SP), as well as levels of seminal oxidative stress index (OSI) of all donkey ejaculates included in the study.

Group	Antioxidant	Mean $\pm$ SEM	Range
Enzymatic antioxidants	PON1 (IU/L)	0.25 $\pm$ 0.04	0.10–0.70
	SOD (IU/mL)	2168.80 $\pm$ 216.72	320.00–3784.00
	CAT (IU/mL)	0.26 $\pm$ 0.04	0.08–0.48
	GPX (IU/L)	101.59 $\pm$ 12.97	10.20–172.00
Non-enzymatic antioxidants	Total thiol ( $\mu\text{mol/L}$ )	81.55 $\pm$ 8.95	34.30–157.50
	CUPRAC (mmol/L)	1.67 $\pm$ 0.14	0.61–2.40
	FRAP (mmol/L)	1.71 $\pm$ 0.18	0.34–2.85
	TEAC (mmol/L)	2.30 $\pm$ 0.15	0.98–3.03
TOS ( $\mu\text{mol/L}$ )		7.41 $\pm$ 0.54	2.40–9.30
OSI (arbitrary units)		3.60 $\pm$ 0.51	0.88–9.38

PON1 (IU/L): paraoxonase type 1; SOD (IU/mL): superoxide dismutase; CAT (IU/mL): catalase-like; GPX (IU/L): glutathione peroxidase-like; CUPRAC (mmol/L): cupric reducing antioxidant capacity; FRAP (mmol/L): plasma iron-reducing capacity; TEAC (mmol/L): Trolox equivalent antioxidant capacity; TOS ( $\mu\text{mol/L}$ ): total oxidative status; OSI (arbitrary units): seminal oxidative stress index.