

Table S1.

Effect of staining with MitoTracker® dyes on CASA kinematic parameters of boar spermatozoa.

Comparison of CASA parameters for semen samples extended in Beltsville Thawing Solution (a) or Androstar® Plus (b). Samples were treated with either MitoTracker® Green FM, MitoTracker® Red FM or DMSO, respectively, and stored at 17°C or 5°C. The staining of the samples had no influence on the CASA parameters ($p > 0.05$). Data are shown as mean and standard deviation.

(a)	BTS 5 °C			BTS 17 °C		
	MitoTracker® Red	MitoTracker® Green	DMSO	MitoTracker® Red	MitoTracker® Green	DMSO
Total motility [%]	48.1 ± 18.3	48.6 ± 19.9	53.2 ± 18.1	79.4 ± 16.2	80.5 ± 11.9	82.8 ± 10.9
Progressive motility [%]	34.4 ± 20.0	32.5 ± 19.0	38.3 ± 18.2	65.3 ± 21.7	66.4 ± 12.6	70.8 ± 10.0
VAP [µm/s]	44.3 ± 15.6	49.1 ± 15.8	49.1 ± 11.7	51.6 ± 11.0	53.4 ± 8.2	58.8 ± 6.9
VCL [µm/s]	73.2 ± 32.9	66.3 ± 34.3	81.7 ± 26.3	83.5 ± 21.6	90.3 ± 19.0	101.3 ± 20.9
VSL [µm/s]	32.8 ± 9.3	30.2 ± 10.2	36.4 ± 8.0	40.3 ± 7.8	40.7 ± 6.1	43.6 ± 5.2
STR	0.75 ± 0.09	0.76 ± 0.11	0.74 ± 0.07	0.78 ± 0.05	0.76 ± 0.07	0.74 ± 0.09
LIN	0.50 ± 0.15	0.46 ± 0.10	0.49 ± 0.09	0.49 ± 0.08	0.46 ± 0.08	0.44 ± 0.11
WOB	0.62 ± 0.10	0.64 ± 0.11	0.61 ± 0.08	0.59 ± 0.07	0.59 ± 0.06	0.62 ± 0.07
ALH [µm]	1.79 ± 0.75	1.64 ± 0.79	1.90 ± 0.67	1.93 ± 0.48	2.13 ± 0.59	2.29 ± 0.58
BCF [Hz]	26.1 ± 8.5	23.1 ± 8.4	28.3 ± 6.4	32.0 ± 5.7	33.2 ± 3.1	35.6 ± 2.1

VAP = average path velocity

VCL = curvilinear velocity

VSL = straight-line velocity

STR = straightness (VSL/VAP)

LIN = linearity (VSL/VCL)

WOB = wobble (VAP/VCL)

ALH = amplitude of lateral head-displacement BCF = beat cross frequency

(b)	Androstar® Plus 5 °C			Androstar® Plus 17 °C		
	MitoTracker® Red	MitoTracker® Green	DMSO	MitoTracker® Red	MitoTracker® Green	DMSO
Total motility [%]	72.9 ± 18.5	73.8 ± 17.3	73.4 ± 19.8	84.2 ± 8.2	85.4 ± 7.1	85.9 ± 2.9
Progressive motility [%]	61.4 ± 20.5	62.1 ± 18.6	63.9 ± 19.0	72.8 ± 12.8	73.3 ± 9.2	78.1 ± 3.3
VAP [µm/s]	58.9 ± 10.1	58.1 ± 8.7	59.4 ± 6.0	59.0 ± 10.5	62.1 ± 5.0	65.6 ± 7.7
VCL [µm/s]	99.6 ± 23.0	99.0 ± 21.4	98.5 ± 18.5	95.6 ± 23.1	101.9 ± 11.6	105.4 ± 18.4
VSL [µm/s]	43.0 ± 6.8	42.8 ± 5.6	43.3 ± 5.0	45.6 ± 7.2	48.2 ± 4.5	51.1 ± 5.5
STR	0.73 ± 0.07	0.73 ± 0.07	0.73 ± 0.08	0.77 ± 0.06	0.77 ± 0.05	0.78 ± 0.05
LIN	0.44 ± 0.10	0.44 ± 0.10	0.45 ± 0.10	0.49 ± 0.09	0.47 ± 0.08	0.49 ± 0.08
WOB	0.60 ± 0.08	0.59 ± 0.08	0.61 ± 0.07	0.62 ± 0.06	0.61 ± 0.06	0.63 ± 0.06
ALH [µm]	2.25 ± 0.63	2.25 ± 0.53	2.17 ± 0.47	2.21 ± 0.53	2.24 ± 0.40	2.17 ± 0.46
BCF [Hz]	33.8 ± 5.0	34.0 ± 3.7	34.0 ± 3.1	35.5 ± 4.7	37.6 ± 2.4	38.2 ± 3.0
VAP = average path velocity VCL = curvilinear velocity VSL = straight-line velocity STR = straightness (VSL/VAP) LIN = linearity (VSL/VCL) WOB = wobble (VAP/VCL) ALH = amplitude of lateral head-displacement BCF = beat cross frequency						

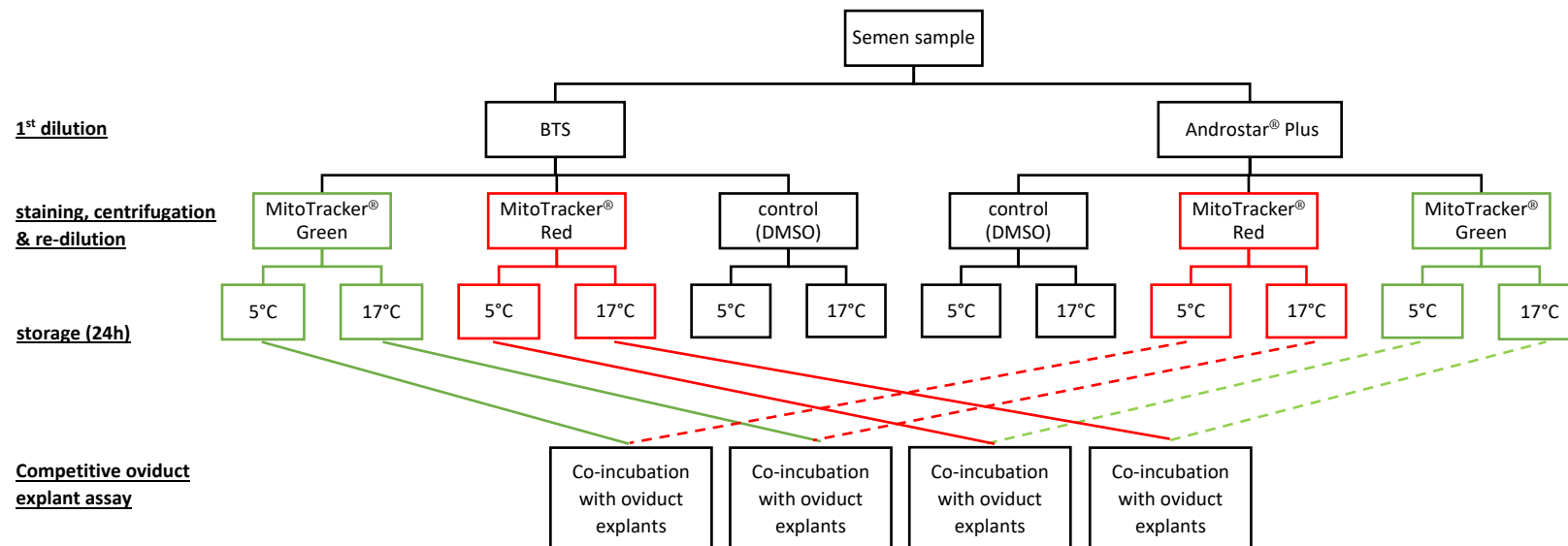


Figure S1.

Flow chart illustrating the sample processing before co-incubation with oviduct explants.

Boar semen samples were extended in Beltsville Thawing Solution (BTS) or Androstar® Plus extender. Details on the individual processing steps are provide in the Material and Methods section.

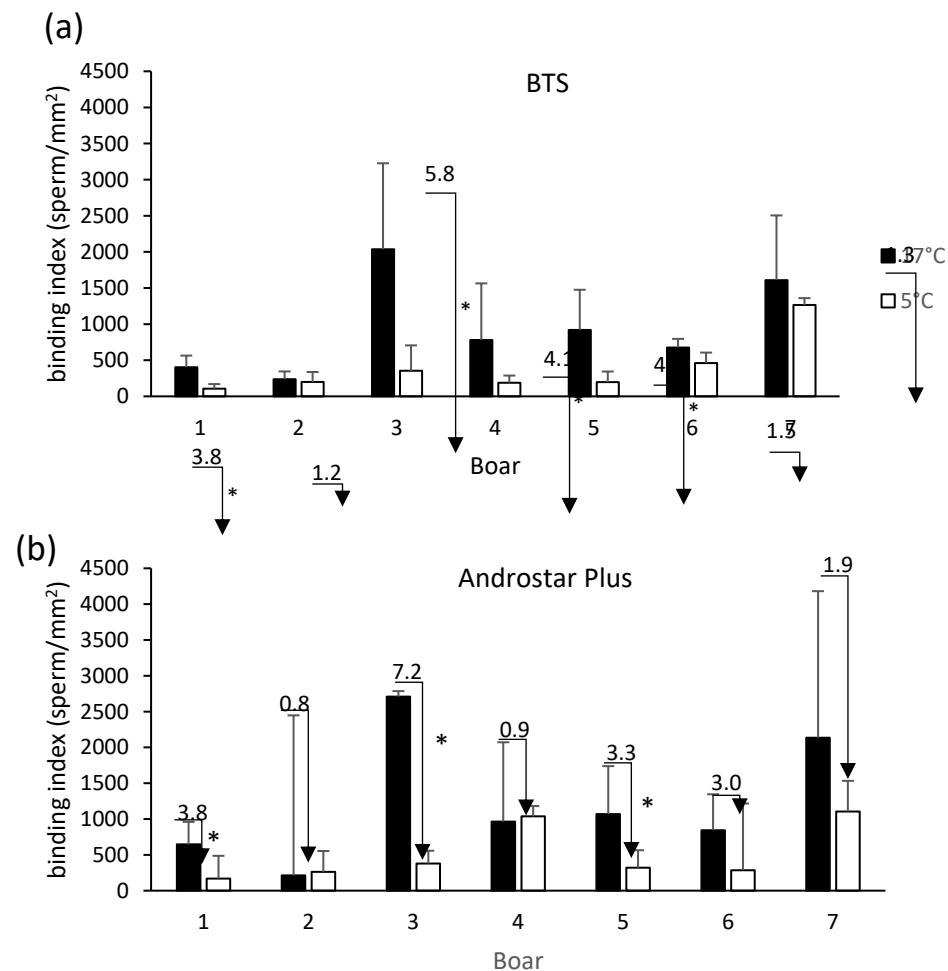


Figure S2. Effect of storage temperature on sperm binding for individual boars.

Number of bound spermatozoa per explant area (binding index) for semen samples from individual boars after storage in (a) Beltsville Thawing Solution (BTS) or (b) Androstar Plus at either 5°C or 17°C (n = 4 explants per condition). Numbers indicate the fold change in binding index. An asterisk indicates a significant effect of storage temperature on the binding index as estimated by Kruskal-Wallis test ($p < 0.05$).