

Supplementary Material

The antioxidant effects of soybean lecithin- or low-density lipoprotein-based extenders for the cryopreservation of brown-bear (*Ursus arctos*) spermatozoa

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1) RE-CALCULATE THE SUBSETS DATA FROM FLOW CYTOMETRY EXPERIMENTS

1.1) PNA/IP analysis

TABLE S1.

	Pre-freezing				Post-thawing			
	EY20	S2	S3.5	S5	EY20	S2	S35	S5
VIAB	79.9±2.2 ^a	2.4±1.2 ^b	24.3±4.6 ^c	58.2±6.1 ^d	51.7±3.7 ^a	3.4±2.1 ^b	16.8±2.9 ^c	21.6±4.2 ^c
dACR	2.8±0.4 ^a	26.0±5.2 ^b	21.0±2.9 ^{bc}	8.5±2.6 ^{a,c}	11.6±1.6 ^a	43.4±8.2 ^b	29.7±2.6 ^{bc}	23.7±3.0 ^{ac}

TABLE S2.

	Pre-freezing			Post-thawing		
	EY20	S5-A	S5-B	EY20	S5-A	S5-B
VIAB	78.5±4.4	79.7±6.3	73.4±11.6	40.8±3.2 ^a	27.5±5.9 ^b	25.6±5.2 ^b
dACR	2.8±0.5	3.9±0.5	7.4±4.8	6.2±1	11.2±2	9.8±2.6

TABLE S3.

	Pre-freezing				Post-thawing			
	EY20	LDL5	LDL10	LDL15	EY20	LDL5	LDL10	LDL15
VIAB	73.2±4.9	81.3±2.8	83.4±2.4	80±5.2	41.0±6.8 ^a	46.1±3.3 ^a	67.8±3 ^b	76.3±3.7 ^b
dACR	3.8±0.8	6.3±1.9	2.8±0.3	4.5±2.7	6.6±1.1 ^{ab}	9.5±2.6 ^b	4.6±0.6 ^a	4.9±0.9 ^a

1.2) SYBR-14/IP analysis: % of DEBRIS

Results obtained after the elimination of the portion included inside the FSC/SSC gate. The SYBR-14/IP plot discriminates the % of non-DNA-containing alien particles. We divided the SYBR-14/IP plot into three different regions: SYBR-14+/IP-, SYBR-14+/IP+ and SYBR-14-/IP+. The non-DNA-containing alien particles correspond to the SYBR-14-/IP- region. Mean±S.E.M.

TABLE S4.

Experiment		Pre-freezing	Post-thawing
Soybean concentration	EY20 (Control)	2.4±0.5	2.9±0.8
	S2	2.1±0.7	1.9±0.3
	S3.5	1.8±0.5	2.0±0.4
	S5	2.7±0.6	2.6±0.3
Soybean source	EY20 (Control)	1.3±0.2	2.1±0.4
	S5-A	2.5±0.4	2.9±0.3
	S5-B	3.2±0.4	2.1±0.3
LDL concentration	EY20 (Control)	1.4±0.4	1.1±0.2
	LDL5	1.4±0.4	1.4±0.2
	LDL10	1.8±0.3	2.4±0.5
	LDL15	2.9±0.7	3.1±0.9

1.3) Re-calculate

The misestimation of percentages is corrected applying mathematical formulae and equations from Theriogenology 73 (2010) 839-847.

TABLE S1. Corrected

	Pre-freezing				Post-thawing			
	EY20	S2	S3.5	S5	EY20	S2	S35	S5
VIAB	77.7±2.3 ^a	2.3±1.1 ^b	23.7±4.4 ^c	56.5±6.0 ^d	50.1±3.3 ^a	3.3±2.0 ^b	16.4±2.8 ^c	21.0±4.1 ^c
dACR	2.8±0.4 ^a	26.1±5.2 ^b	21.1±2.9 ^{bc}	8.6±2.6 ^{a,c}	11.7±1.8 ^a	43.4±8.3 ^b	29.7±2.6 ^{bc}	23.7±3.0 ^{ac}

TABLE S2. Corrected

	Pre-freezing			Post-thawing		
	EY20	S5-A	S5-B	EY20	S5-A	S5-B
VIAB	77.5±4.3	77.6±6.0	70.8±11.2	39.9±2.1 ^a	26.6±3.9 ^b	25.0±3.5 ^b
dACR	2.9±0.5	3.9±0.5	7.5±4.8	6.4±0.9	12.6±2.4	10.5±3.5

TABLE S3. Corrected

	Pre-freezing				Post-thawing			
	EY20	LDL5	LDL10	LDL15	EY20	LDL5	LDL10	LDL15
VIAB	72.5±4.6	80.2±2.8	81.9±2.4	77.3±5.2	40.7±6.5 ^a	45.5±3.3 ^a	66.2±3.0 ^b	73.4±3.9 ^b
dACR	3.8±0.7	6.3±1.9	1.8±0.3	4.5±2.8	6.6±1.1 ^{ab}	9.6±2.6 ^b	4.6±0.6 ^a	4.9±1.0 ^a

2) FLOW CYTOMETRIC DOT PLOTS OF SYBR-14/IP ANALYSIS DATA

Figure S1. CONTROL. R4=2.7%.

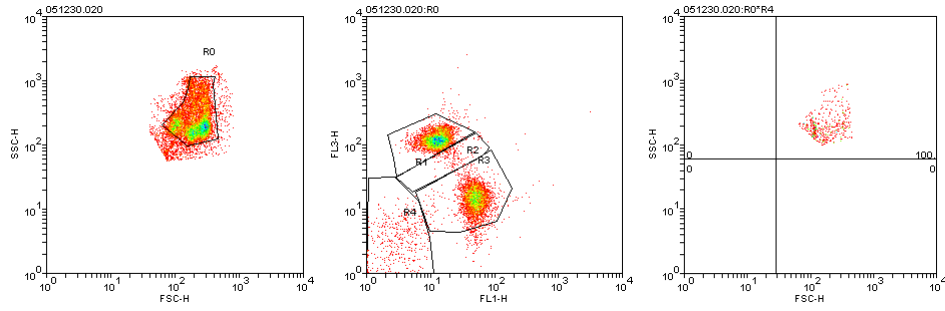


Figure S2. S2. R4=1.96%.

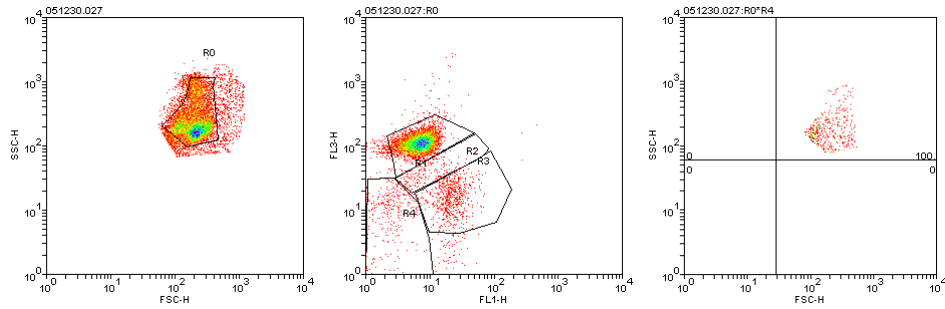


Figure S3. S3.5. R4=1.98%.

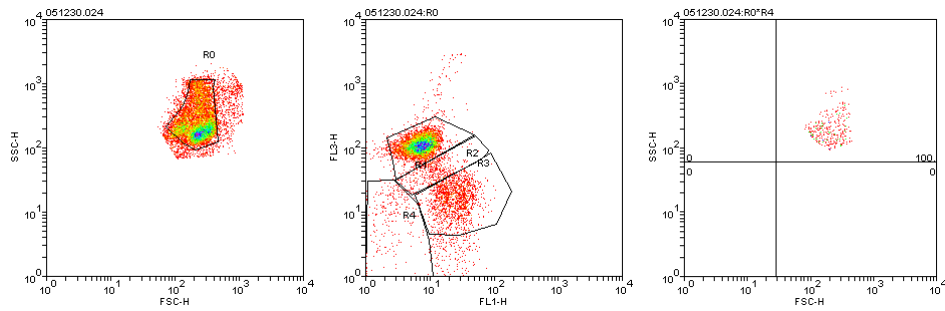


Figure S4. S5 (S5-A). R4=3.03%.

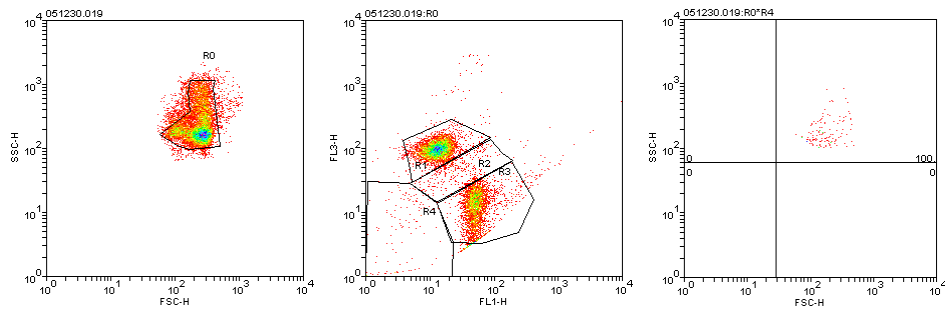


Figure S5. S5-B. R4=2.03%.

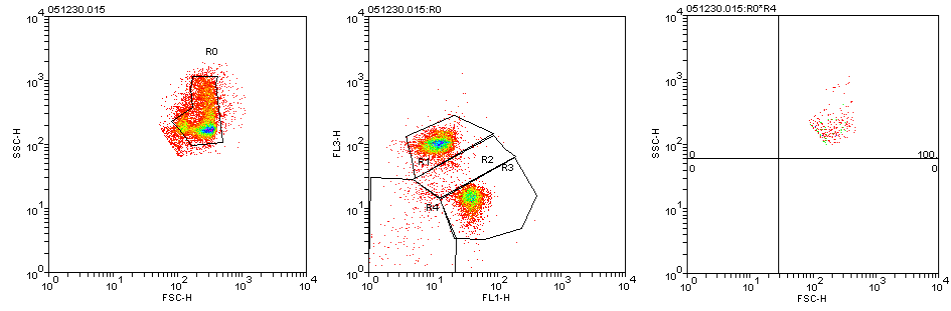


Figure S6. LDL5. R4=1.58%.

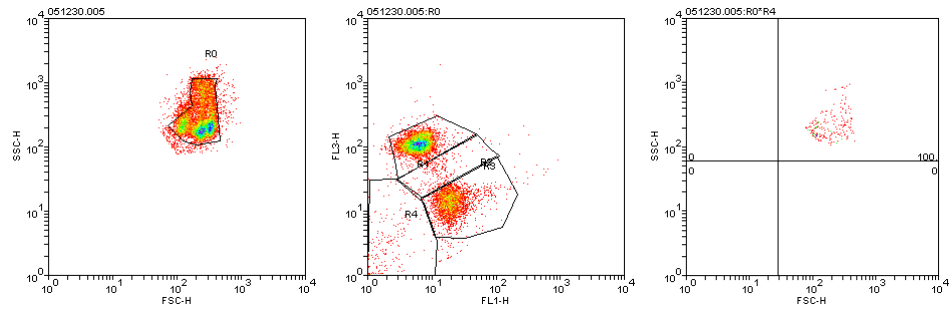


Figure S7. LDL10. R4=2.78%.

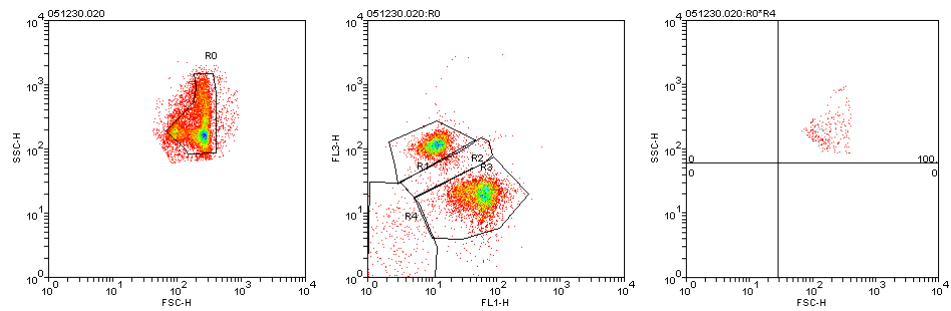


Figure S8. LDL15. 3.08%

