

Supplementary Information

Table S1. Head-Tail separation rate of bovine sperm freeze-dried in various basal media

Groups	No. of sperm observed	Normal sperm (%)	Head-only sperm (%)
Freeze-thawed	3407	3387 (99) ^b	20 (1) ^a
FD – HTF	3938	3739 (95) ^a	199 (5) ^b
FD – TALP	4439	4297 (97) ^a	142 (3) ^b
FD – Tris-EGTA	4347	4028 (93) ^a	319 (7) ^b

Table S2. Relative comet tail length of bovine sperm freeze-dried in various basal media

Groups	No. of sperm observed	Tail length (mean ± SEM)
Freeze-thawed	1390	1.00 ± 0.00 ^a
FD – HTF	1819	1.03 ± 0.00 ^b
FD – TALP	1959	1.04 ± 0.00 ^c
FD – Tris-EGTA	1613	1.04 ± 0.00 ^c

Table S3. Developmental competency of bovine sperm freeze-dried in various basal media

Groups	Observations	No. of oocytes survived	No. of fertilized (%)	2-cell (%)
Freeze-thawed	DNA damage	42	39 (94)	-
	Chromosome segregation	49	33 (65)	31 (92)
FD – HTF	DNA damage	33	29 (88)	-
	Chromosome segregation	34	30 (90)	25 (81)
FD – TALP	DNA damage	27	23 (84)	-
	Chromosome segregation	38	37 (98)	29 (82)
FD – Tris-EGTA	DNA damage	41	37 (83)	-
	Chromosome segregation	34	31 (92)	26 (88)

Table S4. Intensity of γH2Ax at male pronuclei (mPN) following ICSI with bovine sperm freeze-dried in various basal media

Groups	No. of zygote observed	Intensity (mean ± SEM)
Freeze-thawed	26	3.278 ± 0.26 ^a
FD – HTF	35	1.499 ± 0.26 ^b
FD – TALP	19	3.370 ± 0.47 ^a
FD – Tris-EGTA	25	1.138 ± 0.19 ^b

Table S5. Chromosome segregation during the 1st mitosis of early embryo development following ICSI with bovine sperm freeze-dried in various basal media

Groups	No. of embryos observed	Normal (%)	Abnormal (%)			
			Light	Moderate	Heavy	Lethal
Freeze-thawed	30	11 (37)	1 (3)	14 (47)	4 (13) ^a	0 (0) ^a
FD – HTF	24	5 (21)	2 (8)	8 (33)	9 (38) ^{ab}	0 (0) ^a
FD – TALP	28	2 (7)	0 (0)	7 (25)	14 (50) ^b	5 (18) ^b
FD – Tris-EGTA	33	9 (27)	1 (3)	14 (42)	6 (18) ^{ab}	3 (9) ^{ab}

Table S6. Head-Tail separation rate of bovine sperm freeze-dried with different protective agents

Groups	No. of sperm observed	Normal sperm (%)	Head-only sperm (%)
Fresh mouse	3023	2880 (95)	143 (5)
Freeze-thawed	3073	3048 (99) ^b	25 (1) ^a
FD – 0	2753	2645 (96) ^a	108 (4) ^b
FD – Trehalose	3049	2997 (98) ^a	52 (2) ^b
FD – MSG	2701	2671 (99) ^a	30 (1) ^b

Table S7. Relative comet tail length of bovine sperm freeze-dried with different protective agents

Groups	No. of sperm observed	Tail length (mean ± SEM)
Fresh mouse	690	1.00 ± 0.01
Freeze-thawed	931	1.00 ± 0.00 ^a
FD – 0	958	0.98 ± 0.01 ^b
FD – Trehalose	881	0.92 ± 0.00 ^c
FD – MSG	798	0.96 ± 0.01 ^b

Table S8. Developmental competency of bovine sperm freeze-dried with different protective agents

Groups	Observations	No. of oocytes survived	No. of fertilized (%)	2-cell (%)
Fresh mouse	DNA damage	41	28 (66)	-
	Chromosome segregation	26	24 (93)	22 (93)
Freeze-thawed	DNA damage	38	22 (58)	-
	Chromosome segregation	36	23 (65)	17 (70)
FD – 0	DNA damage	45	42 (91)	-
	Chromosome segregation	63	57 (92)	35 (63)
FD – Trehalose	DNA damage	45	31 (66)	-
	Chromosome segregation	63	55 (85)	36 (66)
FD – MSG	DNA damage	54	47 (89)	-
	Chromosome segregation	75	64 (83)	50 (76)

Table S9. Intensity of γH2Ax at male pronuclei (mPN) following ICSI with bovine sperm freeze-dried with different protective agents

Groups	No. of zygote observed	Intensity (mean ± SEM)
Fresh mouse	16	0.377 ± 0.15
Freeze-thawed	27	0.343 ± 0.04 ^{ab}
FD – 0	18	0.640 ± 0.12 ^a
FD – Trehalose	23	0.505 ± 0.18 ^{ab}
FD – MSG	26	0.326 ± 0.06 ^b

Table S10. Chromosome segregation during the 1st mitosis of early embryo development following ICSI with bovine sperm freeze-dried with different protective agents

Groups	No. of embryos observed	Normal (%)	Abnormal (%)			
			Light	Moderate	Heavy	Lethal
Fresh mouse	19	13 (68) ^a	0 (0)	3 (16)	1 (5)	2 (11)
Freeze-thawed	15	7 (47) ^{ab}	1 (7)	6 (40)	1 (7)	0 (0)
FD – 0	33	4 (12) ^b	0 (0)	13 (39)	10 (30)	6 (18)
FD – Trehalose	34	5 (15) ^b	2 (6)	12 (35)	8 (24)	7 (21)
FD – MSG	48	15 (31) ^b	5 (10)	15 (31)	9 (19)	4 (8)

Table S11. Head-Tail separation rate of bovine sperm freeze-dried with single and dual protectant

Groups	No. of sperm observed	Normal sperm (%)	Head-only sperm (%)
FD – W/O	3678	3452 (94) ^b	226 (6) ^a
FD – Single Protectant	3431	3372 (98) ^a	59 (2) ^b
FD – Dual Protectant	3379	3327 (98) ^a	52 (2) ^b

Table S12. Relative comet tail length of bovine sperm freeze-dried with single and dual protectant

Groups	No. of sperm observed	Tail length (mean ± SEM)
FD – W/O	846	1.00 ± 0.00 ^a
FD – Single Protectant	881	0.99 ± 0.01 ^a
FD – Dual Protectant	1227	0.96 ± 0.00 ^b

Table S13. Developmental competency of bovine sperm freeze-dried with single and dual protectant

Groups	Observations	No. of oocytes survived	No. of fertilized (%)	2-cell (%)
FD – 0	DNA damage	24	22 (93)	-
	Chromosome segregation	48	40 (81)	38 (94)
FD – Single Protectant	DNA damage	42	39 (92)	-
	Chromosome segregation	56	53 (93)	48 (94)
FD – Dual Protectant	DNA damage	37	36 (98)	-
	Chromosome segregation	66	59 (89)	51 (85)

Table S14. Intensity of γ H2Ax at male pronuclei (mPN) following ICSI with bovine sperm freeze-dried with single and dual protectant

Groups	No. of zygote observed	Intensity (mean $\pm$ SEM)
FD – 0	19	2.016 $\pm$ 0.20 ^a
FD – Single Protectant	36	0.799 $\pm$ 0.18 ^b
FD – Dual Protectant	31	1.403 $\pm$ 0.21 ^{ab}

Table S15. Chromosome segregation during the 1st mitosis of early embryo development following ICSI with bovine sperm freeze-dried with single and dual protectant

Groups	No. of embryos observed	Normal (%)	Abnormal (%)			
			Light	Moderate	Heavy	Lethal
FD – 0	34	12 (35) ^b	1 (3)	9 (26)	6 (18)	6 (18)
FD – Single Protectant	48	20 (42) ^b	1 (2)	15 (31)	9 (19)	3 (6)
FD – Double Protectant	48	5 (10) ^b	2 (4)	21 (44)	13 (27)	7 (15)

Table S16. Acrosome status of FD bovine sperm in various condition

Groups	No. of sperm observed	Lectin positive (%)	Lectin negative (%)
Freeze-thawed (Tx+)	4924	3184 (63) ^b	1740 (37) ^b
Freeze-thawed	6709	3061 (35) ^c	3648 (65) ^a
FD – 0	1288	1084 (83) ^a	204 (17) ^c
FD – Trehalose	803	301 (35) ^c	502 (65) ^a
FD – MSG	1671	1173 (68) ^{ab}	498 (32) ^{bc}
FD – Dual Protectant	1149	492 (36) ^c	657 (64) ^a