

Effect of Two Freezing Extenders on Characteristic of Fresh and Frozen-Thawed Semen in Endangered Old Kladruber Stallions – A Pilot Study

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Supplementary Online Material (SOM)

Table S1. Effect of Gent and Lact extenders on parameters of extended ejaculate in individual stallions (mean $\pm$ SEM)

Stallion	Extender	TMOT	PMOT	Live sp.	Normal sp.
1	Fresh G	57.2 $\pm$ 2.4	48.4 $\pm$ 3.6	83.4 $\pm$ 4.3	82.6 $\pm$ 2.2
	F-T G	27.3 $\pm$ 2.4 ¹	21.3 $\pm$ 1.9	38.0 $\pm$ 3.7	76.5 $\pm$ 2.4
	Fresh L	63.6 $\pm$ 2.4	48.5 $\pm$ 3.6	78.5 $\pm$ 4.3	84.0 $\pm$ 2.0
	F-T L	12.7 $\pm$ 2.8 ²	9.3 $\pm$ 2.3	29.2 $\pm$ 3.9	74.5 $\pm$ 2.4
2	Fresh G	86.8 $\pm$ 2.4	44.3 $\pm$ 3.6	88.6 $\pm$ 4.3	82.6 $\pm$ 2.6
	F-T G	48.8 $\pm$ 2.6 ¹	39.8 $\pm$ 2.1 ¹	53.8 $\pm$ 4.1	81.8 $\pm$ 2.7
	Fresh L	93.7 $\pm$ 2.4	54.7 $\pm$ 3.6	89.7 $\pm$ 4.3	86.6 $\pm$ 2.2
	F-T L	25.2 $\pm$ 3.1 ²	18.8 $\pm$ 2.6 ²	43.5 $\pm$ 3.6	81.1 $\pm$ 2.2
3	Fresh G	61.9 $\pm$ 2.4 ^a	51.9 $\pm$ 3.6	87.1 $\pm$ 4.3	78.0 $\pm$ 2.5
	F-T G	31.8 $\pm$ 2.3	25.8 $\pm$ 1.9	46.0 $\pm$ 4.1 ¹	73.8 $\pm$ 2.5
	Fresh L	82.9 $\pm$ 2.4 ^b	55.6 $\pm$ 3.6	83.6 $\pm$ 4.3	80.4 $\pm$ 2.2
	F-T L	26.0 $\pm$ 3.0	19.7 $\pm$ 2.3	33.9 $\pm$ 3.6 ²	73.2 $\pm$ 2.3
4	Fresh G	89.3 $\pm$ 2.4	74.0 $\pm$ 3.6	89.0 $\pm$ 4.3	74.8 $\pm$ 1.8 ^a
	F-T G	19.7 $\pm$ 3.1	15.5 $\pm$ 2.6	35.5 $\pm$ 4.5	68.1 $\pm$ 6.1
	Fresh L	93.2 $\pm$ 2.4	75.4 $\pm$ 3.6	89.7 $\pm$ 4.3	83.7 $\pm$ 2.2 ^b
	F-T L	15.6 $\pm$ 3.1	13.3 $\pm$ 2.6	31.8 $\pm$ 4.5	72.1 $\pm$ 5.0
5	Fresh G	37.7 $\pm$ 2.8 ^a	25.2 $\pm$ 4.2	80.7 $\pm$ 5.0 ^a	61.6 $\pm$ 2.2
	F-T G	7.2 $\pm$ 3.1	5.4 $\pm$ 2.6	50.0 $\pm$ 4.5	65.1 $\pm$ 6.1
	Fresh L	51.9 $\pm$ 2.8 ^b	33.1 $\pm$ 4.2	62.2 $\pm$ 5.0 ^b	62.4 $\pm$ 2.6
	F-T L	7.5 $\pm$ 3.1	6.0 $\pm$ 2.6	48.8 $\pm$ 4.5	67.1 $\pm$ 4.9
6	Fresh G	84.0 $\pm$ 2.8	74.7 $\pm$ 4.2	90.3 $\pm$ 5.0	78.1 $\pm$ 2.6
	F-T G	28.2 $\pm$ 3.6 ¹	22.4 $\pm$ 2.9 ¹	55.3 $\pm$ 5.1	74.7 $\pm$ 3.3
	Fresh L	89.6 $\pm$ 2.8	77.2 $\pm$ 4.2	89.4 $\pm$ 5.0	77.7 $\pm$ 2.6
	F-T L	13.6 $\pm$ 3.3 ²	11.4 $\pm$ 2.7 ²	47.1 $\pm$ 4.8	74.6 $\pm$ 3.0

TMOT = total motility, PMOT = progressive motility

^{a,b}different superscripts in a column indicate differences within an individual stallion between fresh Gent (Fresh G) and fresh Lact (Fresh L) samples at a level of $P < 0.05$

^{1,2}different superscripts in a column indicate differences within an individual stallion between frozen-thawed Gent (F-T G) and frozen-thawed Lact (F-T L) samples at a level of $P < 0.05$

Table S2. Kinematic characteristics and distribution of sperm subpopulations in fresh and frozen-thawed Gent and Lact extenders in individual stallions (mean $\pm$ SEM)

		Kinematic parameters				Subpopulation (%)		
		STR	VAP	VCL	VSL	slow	moderate	rapid
1	Fresh G	81.1 $\pm$ 0.6	86.6 $\pm$ 1.1 ^a	146.0 $\pm$ 1.8 ^a	72.0 $\pm$ 1.1 ^a	19 ^a	53 ^a	27 ^a
	F-T G	88.1 $\pm$ 0.2 ¹	68.2 $\pm$ 0.5 ¹	116.8 $\pm$ 0.8	61.8 $\pm$ 0.5 ¹	34 ¹	37	28 ¹
	Fresh L	81.5 $\pm$ 0.4	76.5 $\pm$ 0.7 ^b	114.4 $\pm$ 1.1 ^b	63.8 $\pm$ 0.7 ^b	33 ^b	46 ^b	21 ^b
	F-T L	85.5 $\pm$ 0.5 ²	60.4 $\pm$ 1.0 ²	112.9 $\pm$ 1.8	53.5 $\pm$ 1.0 ²	44 ²	39	16 ²
2	Fresh G	53.8 $\pm$ 0.4 ^a	99.5 $\pm$ 0.8 ^a	195.0 $\pm$ 1.3	53.3 $\pm$ 0.8 ^a	11 ^a	64 ^a	25 ^a
	F-T G	83.5 $\pm$ 0.2 ¹	87.1 $\pm$ 0.5 ¹	158.5 $\pm$ 0.8 ¹	75.4 $\pm$ 0.5 ¹	25 ¹	37 ¹	38 ¹
	Fresh L	62.9 $\pm$ 0.3 ^b	108.6 $\pm$ 0.6 ^b	190.4 $\pm$ 1.0	70.6 $\pm$ 0.6 ^b	19 ^b	45 ^b	35 ^b
	F-T L	79.6 $\pm$ 0.4 ²	69.5 $\pm$ 0.8 ²	145.6 $\pm$ 1.3 ²	58.8 $\pm$ 0.8 ²	39 ²	31 ²	29 ²
3	Fresh G	80.9 $\pm$ 0.7 ^a	105.9 $\pm$ 1.2 ^a	199.5 $\pm$ 1.9 ^a	89.2 $\pm$ 1.2 ^a	16 ^a	33 ^a	52 ^a
	F-T G	85.4 $\pm$ 0.3 ¹	74.6 $\pm$ 0.6 ¹	140.6 $\pm$ 1.0 ¹	65.3 $\pm$ 0.6 ¹	34 ¹	33	33 ¹
	Fresh L	67.6 $\pm$ 0.4 ^b	95.5 $\pm$ 0.6 ^b	155.1 $\pm$ 1.1 ^b	65.8 $\pm$ 0.7 ^b	19 ^b	57 ^b	24 ^b
	F-T L	82.0 $\pm$ 0.4 ²	66.7 $\pm$ 0.7 ²	127.7 $\pm$ 1.2 ²	57.1 $\pm$ 0.7 ²	37 ²	35	27 ²
4	Fresh G	77.3 $\pm$ 0.4	101.0 $\pm$ 0.7 ^a	178.8 $\pm$ 1.2 ^a	79.9 $\pm$ 0.7 ^a	11 ^a	59 ^a	30 ^a
	F-T G	91.3 $\pm$ 0.7	65.4 $\pm$ 1.4 ¹	119.8 $\pm$ 2.4 ¹	61.5 $\pm$ 1.4 ¹	36 ¹	34	29 ¹
	Fresh L	76.4 $\pm$ 0.4	118.0 $\pm$ 0.6 ^b	195.5 $\pm$ 1.0 ^b	93.5 $\pm$ 0.6 ^b	14 ^b	53 ^b	34 ^b
	F-T L	89.3 $\pm$ 0.9	78.6 $\pm$ 1.8 ²	148.8 $\pm$ 3.2 ²	73.4 $\pm$ 1.9 ²	27 ²	36	37 ²
5	Fresh G	70.6 $\pm$ 0.7 ^a	70.0 $\pm$ 1.2 ^a	135.5 $\pm$ 1.9	53.8 $\pm$ 1.2 ^a	38 ^a	37 ^a	25 ^a
	F-T G	83.9 $\pm$ 1.2	70.7 $\pm$ 2.3	130.8 $\pm$ 3.9	63.5 $\pm$ 2.3	44 ¹	30 ¹	27 ¹
	Fresh L	74.1 $\pm$ 0.5 ^b	80.7 $\pm$ 0.8 ^b	131.0 $\pm$ 1.4	64.4 $\pm$ 0.8 ^b	33 ^b	29 ^b	38 ^b
	F-T L	88.3 $\pm$ 1.2	69.0 $\pm$ 2.3	135.4 $\pm$ 3.9	63.2 $\pm$ 2.3	32 ²	35 ²	33 ²
6	Fresh G	81.8 $\pm$ 0.5	111.2 $\pm$ 0.9 ^a	190.7 $\pm$ 1.5 ^a	93.6 $\pm$ 0.9	9 ^a	54	37
	F-T G	88.9 $\pm$ 0.6	68.7 $\pm$ 1.2 ¹	120.7 $\pm$ 2.1 ¹	63.8 $\pm$ 1.3 ¹	37 ¹	35	28 ¹
	Fresh L	81.5 $\pm$ 0.4	106.6 $\pm$ 0.7 ^b	155.6 $\pm$ 1.1 ^b	89.5 $\pm$ 0.7	13 ^b	52	36
	F-T L	90.4 $\pm$ 1.0	82.2 $\pm$ 1.9 ²	149.3 $\pm$ 3.3 ²	77.4 $\pm$ 1.9 ²	28 ²	37	34 ²

STR = straightness coefficient, VAP = average path velocity, VCL = curvilinear velocity, VSL = straight line velocity

^{a,b}different superscripts in a column indicate differences within an individual stallion between fresh Gent (Fresh G) and fresh Lact (Fresh L) samples at a level of $P < 0.05$ ^{1,2}different superscripts in a column indicate differences within an individual stallion between frozen-thawed Gent (F-T G) and frozen-thawed Lact (F-T L) samples at a level of $P < 0.05$