

Assessment of the post-thaw measurable parameters of equine spermatozoa taken for a comparison between amides and glycerol as cryoprotectants



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1. Background

- Variability has been observed in the post-thaw quality of spermatozoa between stallions.
- Glycerol is commercially used as a cryoprotectant but can have toxic effects on spermatozoa if added at high concentrations.

2. Aim

To assess amides as alternative cryoprotectants to glycerol for freezing stallion semen. Specifically, to evaluate whether the utilization of amides could improve the freezability of stallion spermatozoa compared to glycerol.

3. Method

• Ejaculates from 10 stallions were split and frozen in an egg based extender which contained one of the following treatments:

- Glycerol (GLY) (5%)
- Methyl formamide (MF) (5%)
- Dimethyl formamide (DF) (5%)
- MF + DF (2.5% + 2.5%)
- GLY + DF (1% + 4%)

• Data was analysed via a 2-way ANOVA.



4. Results

- No significant difference ($P < 0.05$) was observed between treatments for motility at 1hr.
- The percentage of live normal cells was significantly higher using GLY (71.2 ± 5.5) compared to MF (44.7 ± 10.2).
- The percentage of abnormal cells was significantly lower ($P < 0.05$) using GLY + DF (1.9 ± 0.7) compared to GLY (9.7 ± 4.7).
- Further results are displayed in Table 1.

Table 1. Differences of the measurable parameters between the cryoprotectant treatments (mean $\pm$ standard error).

Parameter	(%)	Motility 0hr	Motility 1hr	Live Acceptable	Live	Live Normal	Live Distal	Live Proximal	Live Bent Tail	Other Abnormal
Cryoprotectant	GLY	35.0 ± 5.1	20.2 ± 5.0	46.4 ± 3.5^a	59.8 ± 2.6	71.2 ± 5.5^a	5.7 ± 2.0	7.2 ± 2.0	6.2 ± 1.8	9.7 ± 4.7^a
	MF	24.5 ± 4.7^a	10.2 ± 2.8	31.4 ± 7.5^b	40.6 ± 9.2	44.7 ± 10.2^b	9.0 ± 3.1	9.3 ± 2.9	4.2 ± 1.7	2.8 ± 1.1^b
	DF	29.0 ± 4.6	13.5 ± 3.7	36.0 ± 7.1	46.0 ± 8.9	60.1 ± 8.1	9.3 ± 2.0	12.4 ± 4.2	4.5 ± 1.6	3.7 ± 1.0
	MF+DF	42.5 ± 4.5^b	21.0 ± 4.6	39.5 ± 5.4	52.5 ± 6.7	67.0 ± 3.4	9.9 ± 3.7	10.8 ± 3.0	6.4 ± 2.3	5.9 ± 1.3^c
	GLY+DF	30.0 ± 6.8	17.0 ± 4.7	32.7 ± 7.5^b	41.1 ± 9.6	50.9 ± 11.7	5.6 ± 1.8	8.5 ± 3.0	3.1 ± 1.3	1.9 ± 0.7^b

Different letters (a,b,c) in the same column differ significantly, highlighting the pairwise comparisons (ANOVA – Tukey test at $P < 0.05$).

5. Conclusions

- This study indicated that dimethyl formamide consistently performed as well as glycerol.
- Dimethyl formamide could replace glycerol without a significant ($P < 0.05$) decline in measurable post-thaw parameters.
- The use of alternative cryoprotectants could help remedy problems associated with 'poor freezers'.