

# PRACTICAL CRYOPRESERVATION OF GANDER SPERM USING VARIOUS COMBINATIONS OF CRYO- AND OSMOPROTECTANTS

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## Aim

to find an effective, simple and cheap freezing protocol in practical circumstances.  
Two basic freezing methods with various *cryo- and osmoprotectants* were compared:

- *programmable protocol* with a freezing rate of  $-40^{\circ}\text{C}/\text{min}$  (A)
- *freezing in nitrogen vapor* (B)

## Materials and methods

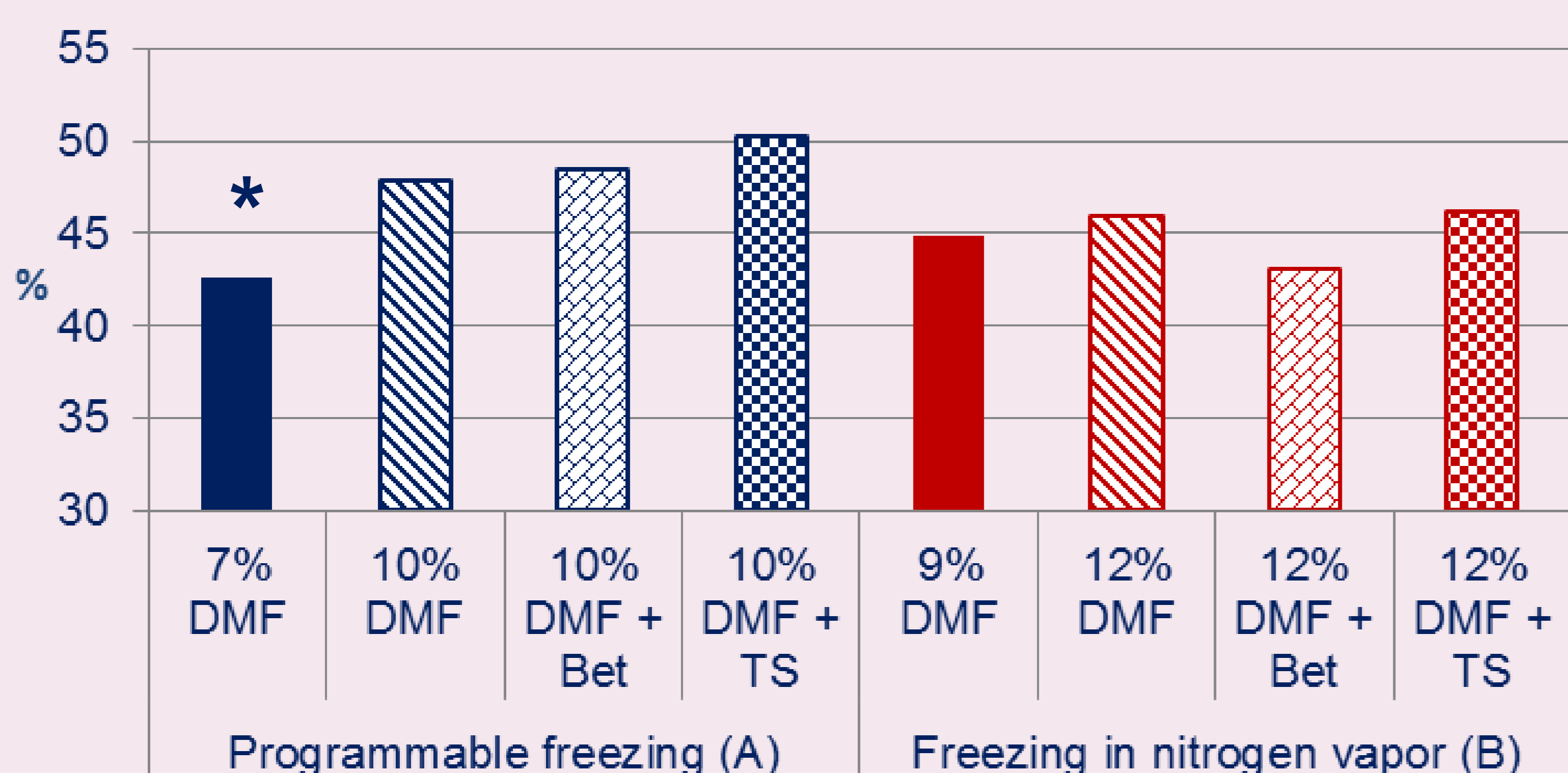
- **Animals:** 30 two year old Landes ganders
- **Semen collection:** twice a week by dorso-abdominal massage
- **Sperm qualification:** before freezing and after thawing
  - motility - subjective scoring,
  - concentration – spectrophotometer (Accucell, IMV)
  - morphological and membrane-integrity analysis: eosin-aniline blue staining
- **Sperm preparation:** pooled samples diluted 1:1 with *Lukaszewicz-extender* in 2 steps and equilibration at  $5^{\circ}\text{C}$  for 5 min
- **Sperm freezing:** A and B methods (see *Table*)
- **Thawing:** at  $40^{\circ}\text{C}$  in water bath
- **Statistical analysis:** ANOVA, Fisher LSD test (Statistica, Version 10.0)

COMBINATIONS OF CRYO-AND OSMOPROTECTANTS		
Methods	Programmable freezing (A)	Freezing in nitrogen vapor (B)
1	7% DMF	9% DMF
2	10% DMF	12% DMF
3	10% DMF + 0,1 M betaine	12% DMF + 0,1 M betaine
4	10% DMF + 3% trehalose +3% sucrose	12% DMF + 3% trehalose +3% sucrose



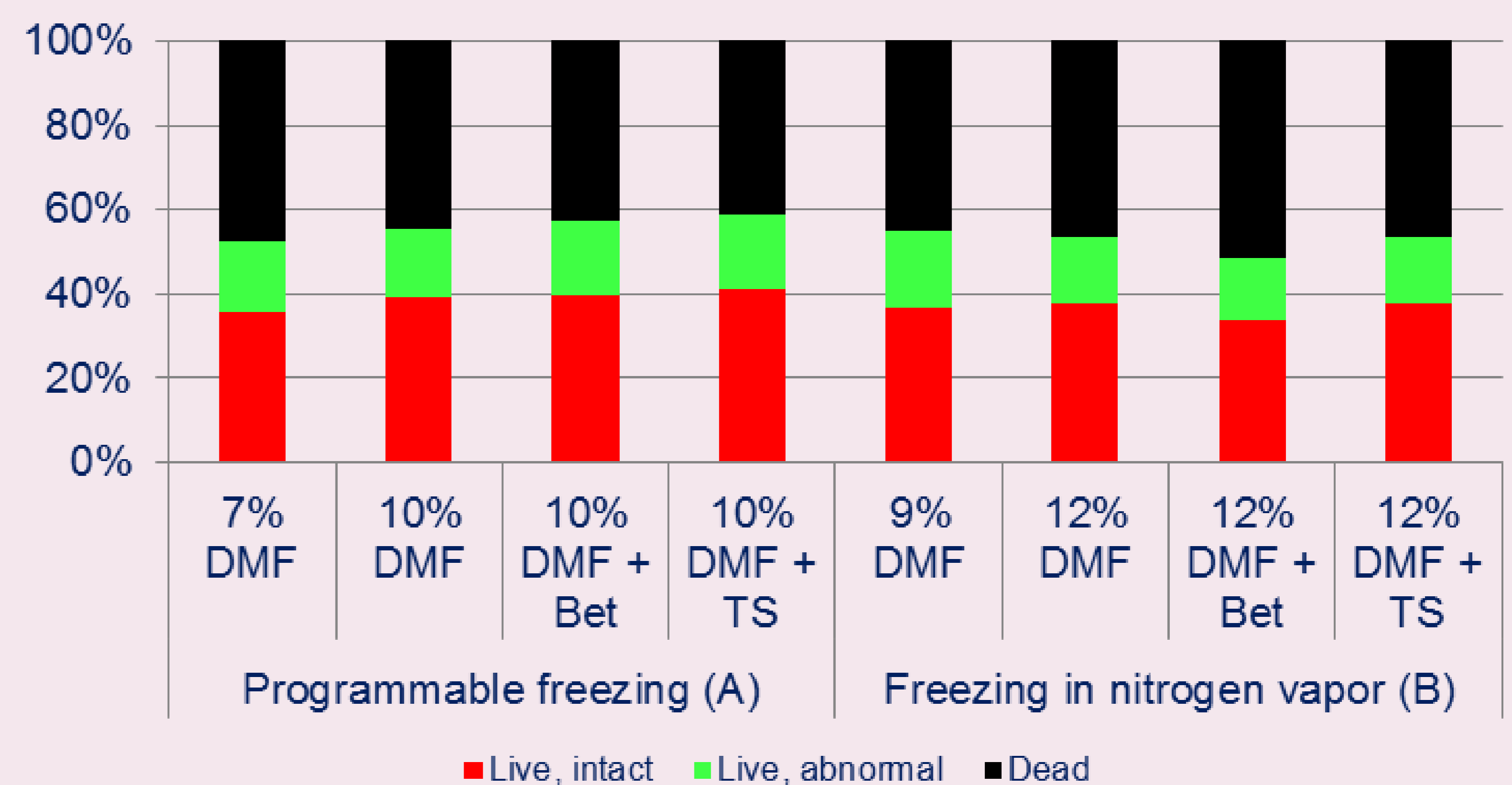
## Results

Survival rate of live, intact spermatozoa after freezing/thawing



\*  $p \leq 0.01$

Changes in sperm quality after freezing/thawing



## Conclusions

- 10-12% DMF is necessary for acceptable cryo protective effect.
- Irrespective of the protocols neither betaine nor trehalose-sucrose combination could improve *significantly* the survival of frozen gander sperm.
- Since both methods could produce more than 40% sperm survival, the simple nitrogen vapor protocol seems to be promising tool in the practice.

## Acknowledgements

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