

# Session 7

## Determination of optimum stallion semen freezing regimes

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# Statistics of horse breeding in Lithuania (January 1, 2008)

- Registered breeds – 54
- Overall horses – 55.9 thousand
- Registered in AVIS – 36.5 thousand
- Horse stock farm owners – 2159

# Material and Methods

- Farm – joint-stock company "Vilniaus žirgynas"
- Equipment for sperm freezing:
  - a metal perforated shield fitted in the biostorage
  - Minicool AS-25
- Form of semen package – polypropilene straw 0.25 cm<sup>3</sup> (ø2.0x0.25x100 mm)

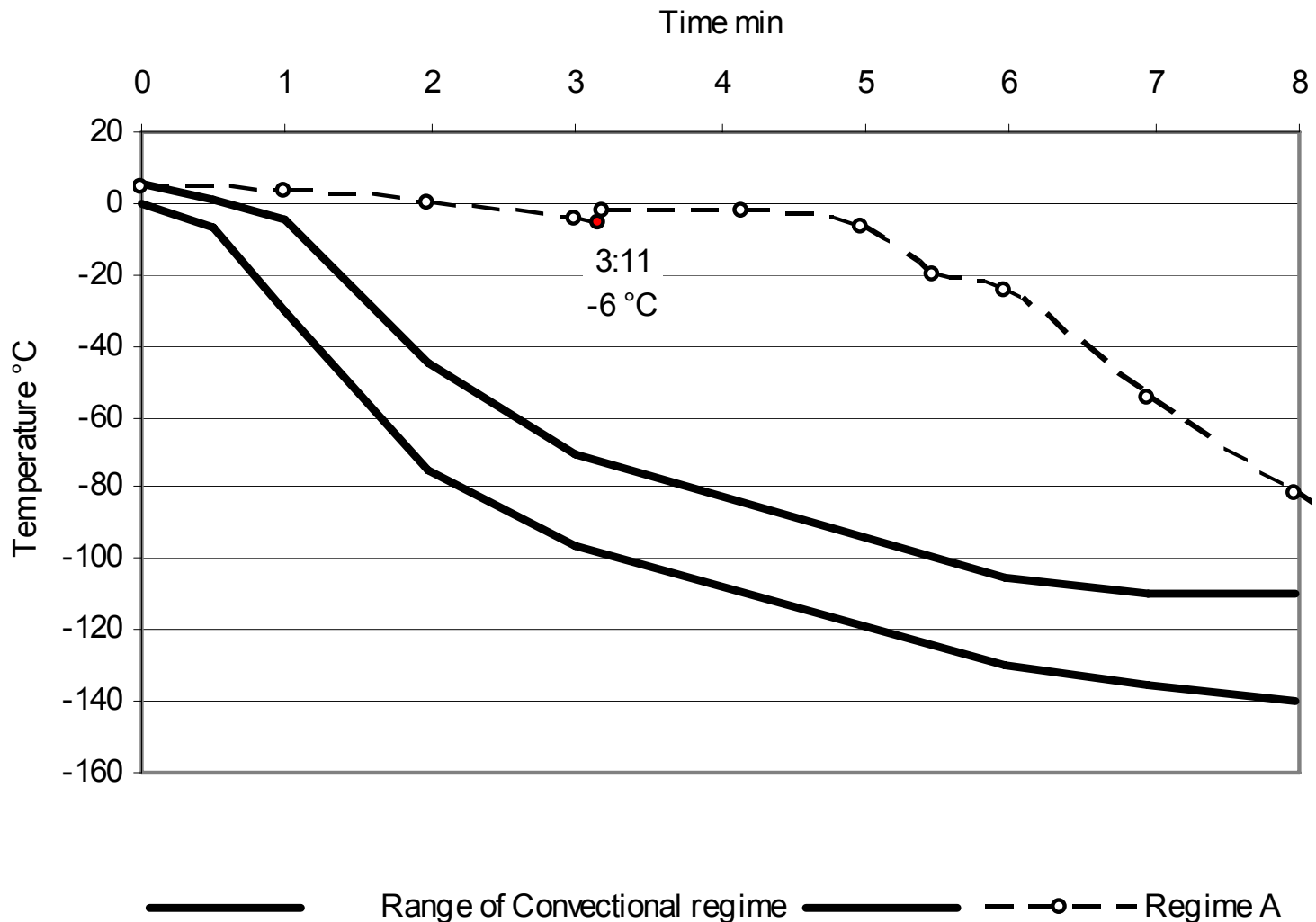
# Fresh stallion semen minimal requirements

- ejaculation volume – no less than 10 cm<sup>3</sup>
- total spermatozoa count per ejaculation – no less than 1.5 milliard
- density – not lower than 0.15 milliard/cm<sup>3</sup>
- sperm motility – not lower than 60 %

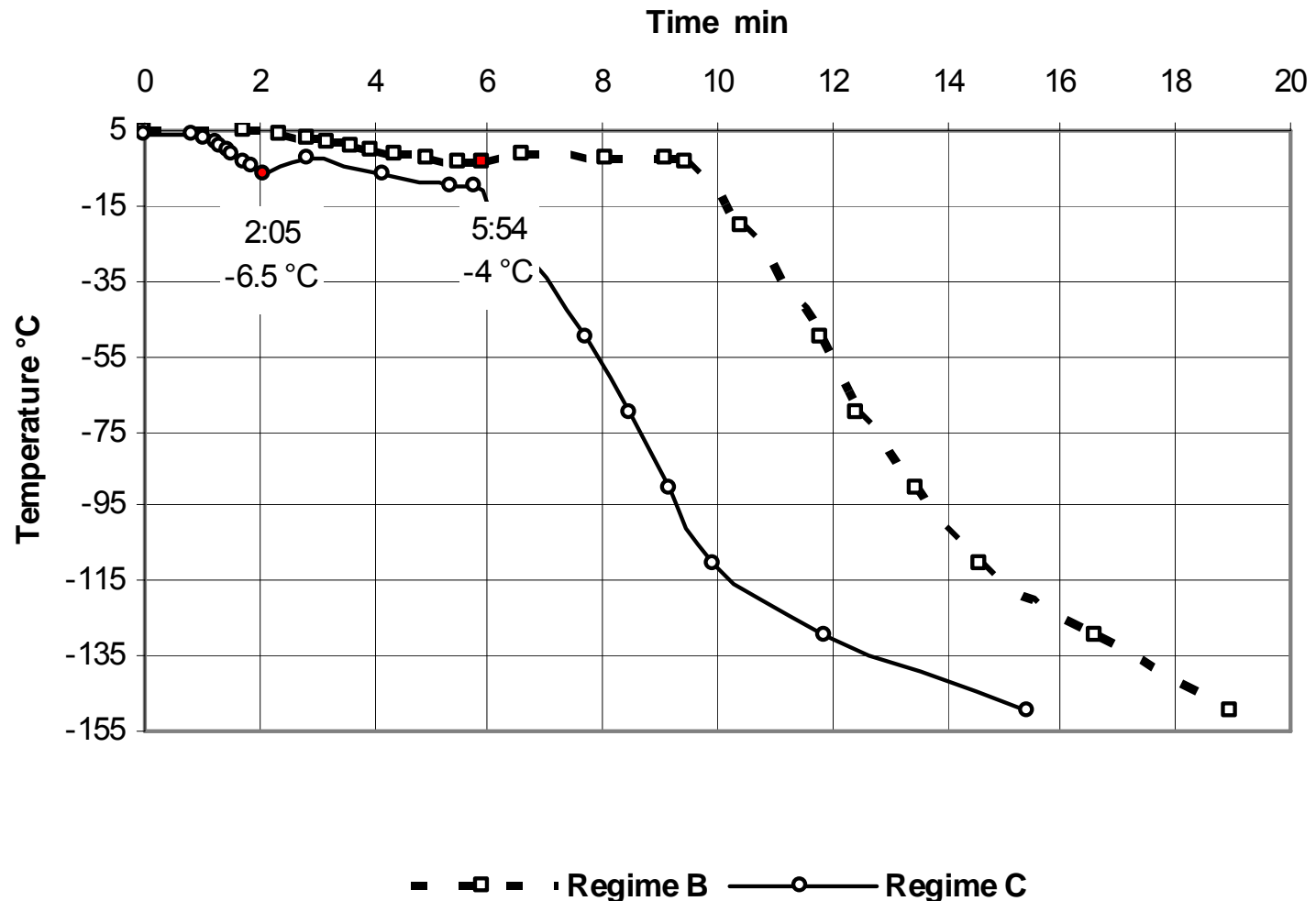
# Extender composition used for stallion semen freezing

Ingredients	Quantity
Redistilled water, cm <sup>3</sup>	100
Lactose, g	11.0
Sodium citrate, g	0.008
Egg yolk, cm <sup>3</sup>	10
Glycerol, cm <sup>3</sup>	3

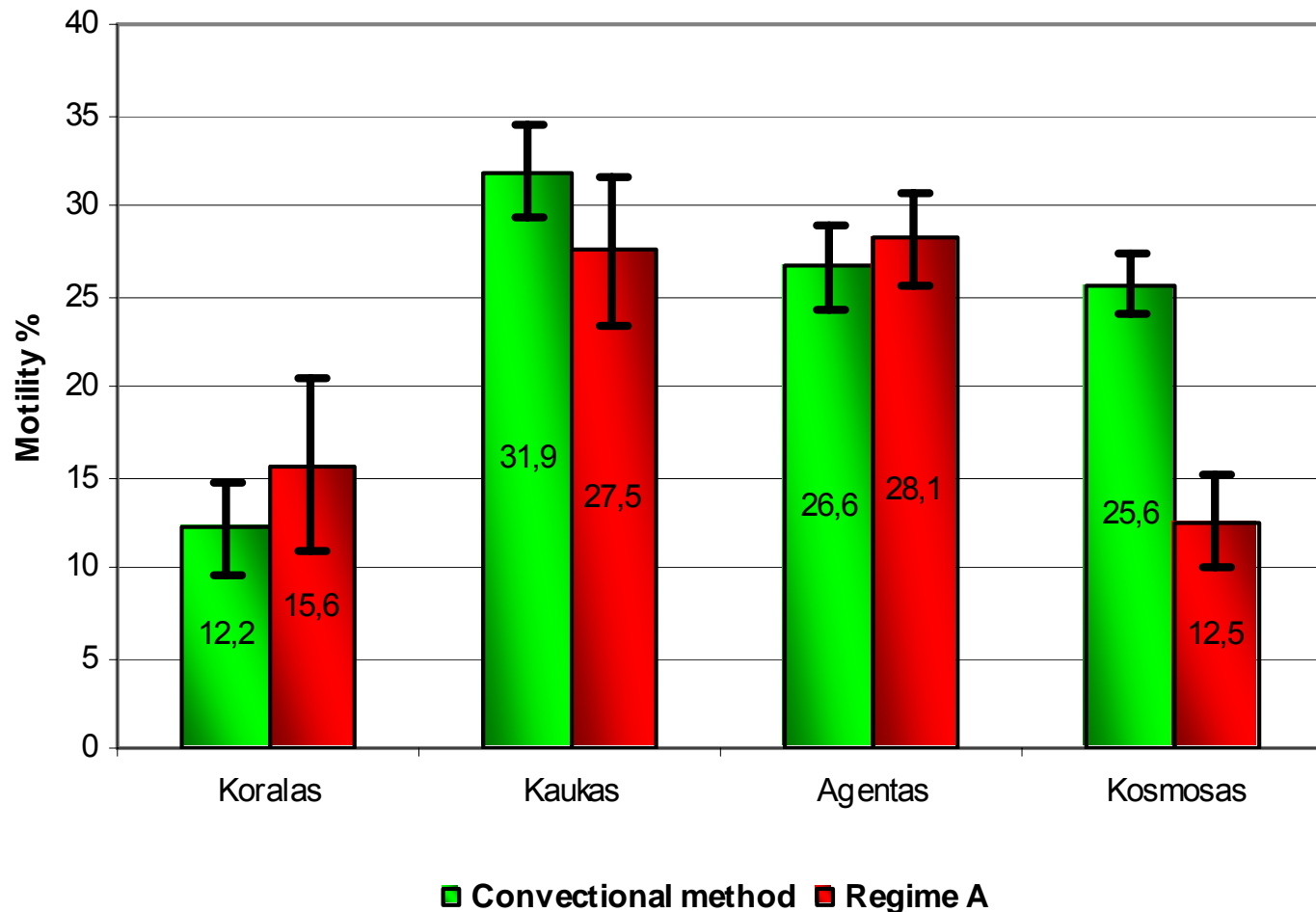
# Possible range of semen temperature changes at convectional freezing



# Freezing regimes realized with Minicool AS-25

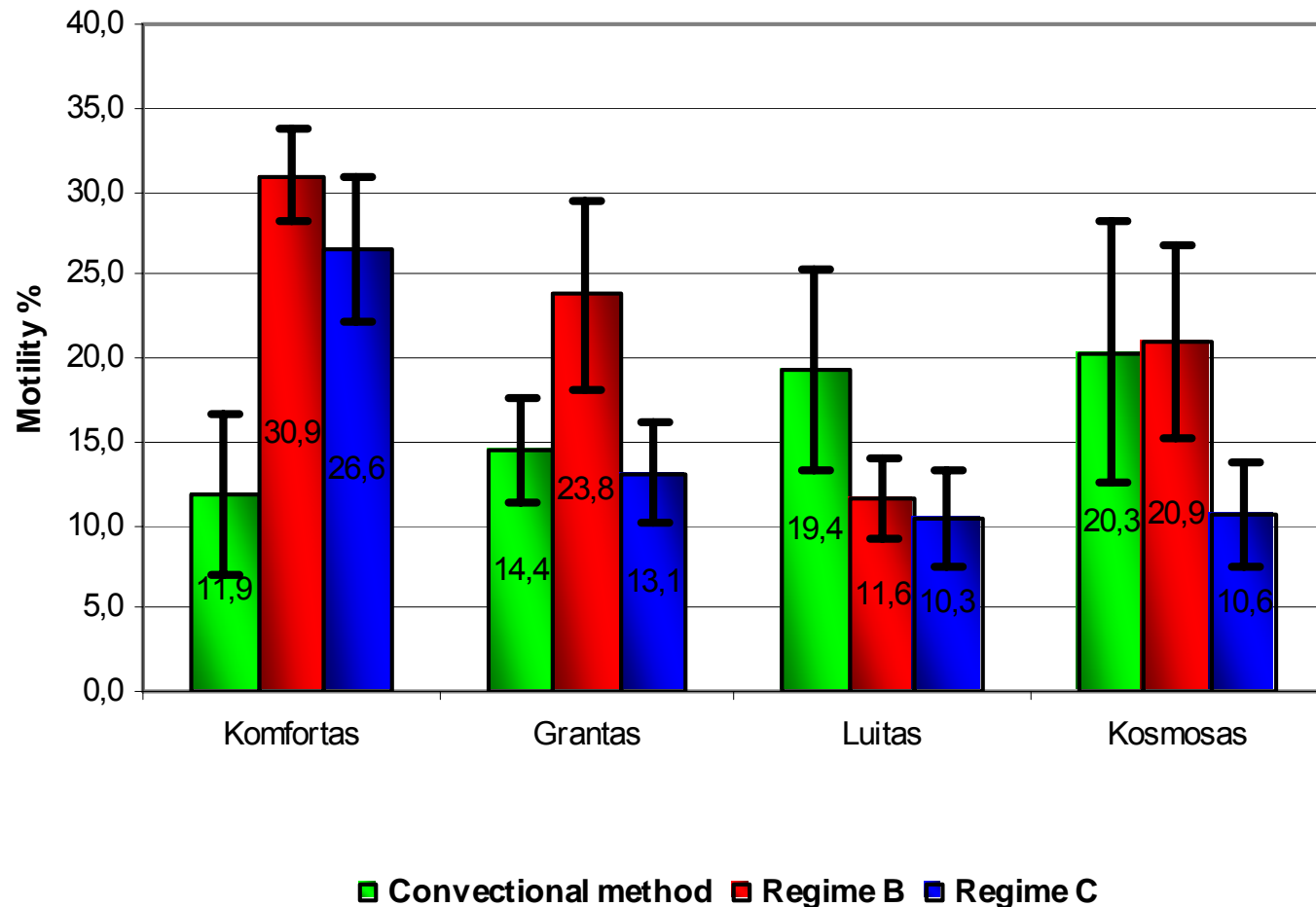


# Post-thaw motility of spermatozoa frozen using different methods





# Post-thaw motility of spermatozoa cryopreserved in three different methods



# Conclusions

1. Frozen semen quality of most stallions is invariable as regards freezing methods and regimes. Thus, the semen can be frozen by the convectional method using ordinary equipment and having lower expenditure of freezing agent.
2. Optimum freezing regime should be found for cryopreservation of the semen that is characterized as having "unstable freezing". In this case, equipment that can reproduce identical freezing regime for each individual stallion semen should be used.
3. Superfreezing temperature of the free water present in the semen can be the parameter characterizing the freezing regime, while the number of motile spermatozoa after thawing can become the optimum criteria.