

The assessment of a modified protocol for rabbit semen cryopreservation

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INTRODUCTION & AIM

Cryopreservation allows sperm storage for long periods, facilitating genetic improvement¹. However, in rabbits, there are no standardized protocols ensuring adequate sperm quality upon thawing². Thus, the objective was to assess a modified protocol for rabbit semen cryopreservation from the University of Molise (Italy)³.

METHOD

Semen collection from five New Zealand rabbits by artificial vagina (43°C), 10 ejaculates per buck

Pool formation; ejaculates with Progressive Motility $\geq 70\%$ and Membrane Functionality $\geq 80\%$ (3 ejaculates per pool)

Diluted pool was split in two parts that were cooled to 5°C (90 minutes)

Pool adjusted to 200×10^6 sperm per ml with Tris-Citric Acid-Glucose extender (TCG)

TCG plus DMSO (16%) and Sucrose (0.1 M) was added (1:1 v/v) in two different ways:

One single step
Final concentration
 100×10^6 sperm/ml

Semen packaged in 0.25 ml straws, equilibrated for 45 min

In three fractions
10 min interval between each
Final concentration
 100×10^6 sperm/ml

Semen packaged in 0.25 ml straws, equilibrated for 15 min

Freezing: straws exposed to nitrogen vapors (-121 °C) for 10 min and immersed in LN₂ (-196 °C)

Thawing: 10s immersion in water bath at 50°C after 2 weeks, 3 straws per treatment

Progressive motility (visual)

Membrane functionality (HOST)

Viability (Eosin/Nigrosin)

Morphology (Eosin/Nigrosin)

Acrosomal integrity (Phase contrast)

Membrane integrity (SYBR14/PI)



Straws exposed to LN₂ vapors



Viability (Eosin/nigrosin):
L, Live sperm. D, Dead sperm.

RESULTS & DISCUSSION

Variables obtained in fresh semen were similar to others previously reported for New Zealand rabbits in Mexico⁴.

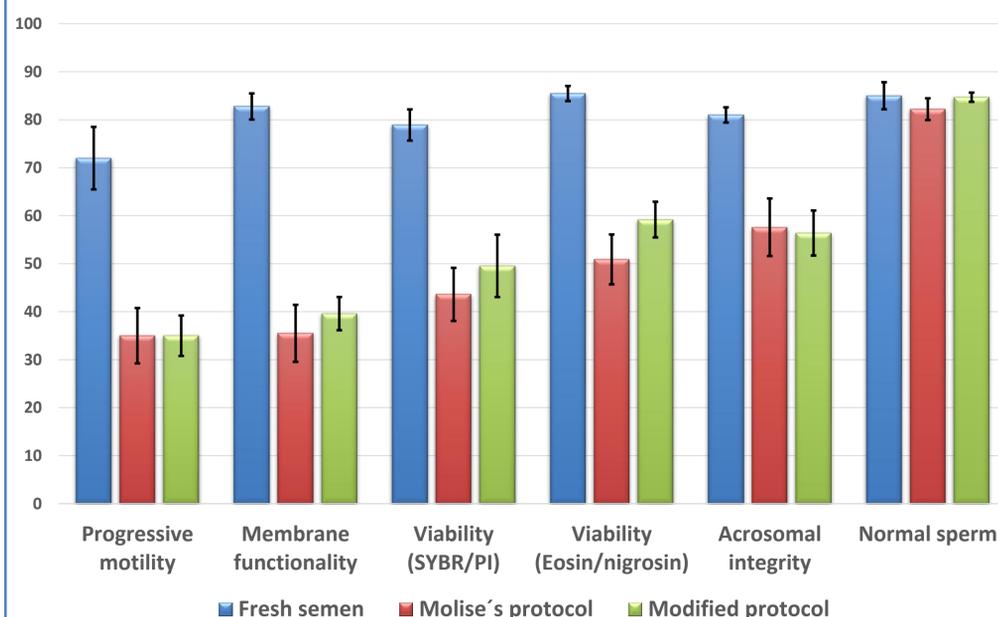


Figure 1. Frozen thawed sperm characteristics (10 pools, 3 ejaculates in each one). Values are Means \pm S.E.M.). No significant differences were found between treatments in any variable ($P > 0.05$).

In the other hand, there were not found differences between treatments in any variable of frozen/thawed semen ($P > 0.05$). In other species, the addition of the second fraction of diluent in three intervals, during semen cryopreservation protocols, is a common practice that enhances semen quality upon thawing⁵; however, in rabbit semen it seems that this practice does not affect the quality of thawed semen.

CONCLUSION

The addition of freezing medium as either one single step or in multiple fractions produced the same results. However, the next step is to perform *in vivo* tests to assess the fertility and prolificacy of both protocols.

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