



# EquiPro® CryoGuard®

## New Minitüb media for the cryopreservation of stallion semen

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When regarding today's worldwide applied freezing protocols for stallion semen and the achieved results in motility and fertility, a significant variability can be observed in practice. You will find remarkable differences,

- when comparing the average results of different stallion studs or deep-freeze service providers
- when comparing the results achieved with different stallions; up to the point of absolute uselessness for cryopreservation
- when comparing different ejaculates of the same stallion.

All in all the freezing result of many ejaculates is not satisfying and a reduction of motility exceeding 50% after thawing compared with the original ejaculate is not unusual. This is on the one hand an indication of the understandably missing selection of fertility of the stallions, and on the other hand, however, an indication of the necessity to improve freezing protocols and to individualize them up to a certain degree. Amongst other things this results in the desire to provide a certain spectrum of cryopreservation media, in order to be able to satisfy the different requirements as well as possible. In addition it is necessary to formulate the media to be user-friendly: meaning to transport the media preferably without a cold chain and with only few preparation steps prior to the use in practice.

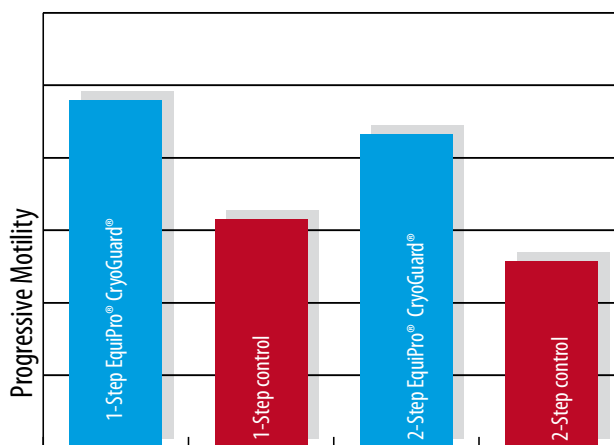
In recent years, several results in research and in cryopreservation practice indicated that the existing standard protocols for freezing should be revised. Better results were observed amongst others with the following changes:

1. Reduction of the egg yolk fraction without completely abandoning it
2. Reduction of the glycerol fraction, particularly individual adaptation to special stallions, depending on previous experiences
3. In addition to that, several laboratories achieved good results when the pre-diluted semen was slowly cooled to +5°C at first and only then the glycerol containing fraction was added. In that case the straw filling is also performed at +5°.

In cooperation with the UNIVERSITY OF VETERINARY MEDICINE HANNOVER, Minitüb performed several tests which dealt especially with semen of substandard original quality. Media with different amides (alternative cryoprotectiva; amongst others dimethylformamide) were applied as well as several combinations of different glycerol- and egg yolk fractions. The results of the media types containing amides did not meet with the expectations. In the same way those with a high fraction of egg yolk and/or glycerol achieved inferior results. Types with a low fraction of both components and without amides however achieved significantly better results and lead to the following conclusions:

- the optimum final concentration of egg yolk in a cryopreservation medium is 2%
- the glycerol concentration should not exceed 4%
- amides do not achieve an additional improvement under these preconditions.

Graphic: Progressive motility of sperm cells after cryopreservation of stallion ejaculates with EquiPro® CryoGuard® 1-Step and 2-Step.



In the context of these studies semen of several stallions was extended with the new EquiPro® CryoGuard® media and cryopreserved. The results of EquiPro® CryoGuard® 1-Step as well as 2-Step showed a higher motility after thawing compared to the control media.



On the basis of these and further scientific results, Minitüb has now developed a series of new media for the cryopreservation of stallion semen. The product family EquiPro® CryoGuard®.

### **EquiPro® CryoGuard® is available as 1-Step and 2-Step type:**

When using the 1-Step protocol, the glycerol containing cryopreservation medium is added to the semen before it is cooled down to +5°C. The 2-Step type means that the glycerol is only added after cooling to +5°C and all further preparation steps until cryopreservation are performed at +5°C. This procedure has proved itself advantageous for many stallions.

All EquiPro® CryoGuard® media are provided by Minitüb in user friendly presentations. The media are either ready to use and simply composed, or a small quantity of egg yolk has to be added. It is not necessary to add pure water. Beyond that, the media are very stable and can be transported at ambient temperatures up to +25°C. For longer storage they should be kept in the refrigerator at approx. +5°C.

### **EquiPro® CryoGuard® 1-Step**

In the 1-Step-procedure the cryopreservation medium is added to the semen pellet after centrifugation at room temperature. Afterwards cooling to +5°C and freezing is performed. It comes as a ready to use medium which is available in two types:

#### **a) EquiPro® CryoGuard® 1-Step with egg yolk:**

The extender is delivered in the form of 2 liquid components, the first containing 95 ml of medium and the second 5 ml of an egg yolk-glycerol mixture. The egg yolk Minitüb uses for this purpose comes from a controlled stock, and has been pasteurized and sterilised with gamma radiation. For use, both components must be mixed and then brought to the correct temperature at which the medium should be mixed with the semen.

**EquiPro® CryoGuard® 1-Step with egg yolk**      [REF.: 13570/0410](#)

#### **b) EquiPro® CryoGuard® 1-Step without egg yolk:**

This type consists of only one liquid component, containing glycerol. The user adds 2 ml of freshly filtered egg yolk to the EquiPro® CryoGuard®. Afterwards the mixture is homogenized and brought to the desired temperature.

**EquiPro® CryoGuard® 1-Step without egg yolk**      [REF. : 13570/0420](#)

### **Protocol for semen processing with EquiPro® CryoGuard® 1-Step:**

The stallion ejaculate is extended 1+1 with liquid EquiPro® and centrifuged. After removing the supernatant the semen pellet is re-suspended in EquiPro CryoGuard® 1-Step to the calculated final volume. After dispensing into straws, cooling and freezing is performed.

## EquiPro® CryoGuard® 2-Step

This medium is delivered as two liquid fractions. Only the second fraction contains glycerol. The user performs the addition of the glycerol containing fraction in a second step, after the pre-diluted semen has been cooled to +5°C. Thus, the time period in which the semen is exposed to the glycerol is minimized. Again, this medium is available in two versions:

### a) EquiPro® CryoGuard® 2-Step with egg yolk:

This version consists of 3 fractions:

4. Fraction A: EquiPro® for centrifugation and bloating of pellets
5. Fraction B: EquiPro® CryoGuard®: liquid medium, to which the egg yolk-glycerol component is added before using
6. Fraction C: Egg yolk-glycerol component

**EquiPro® CryoGuard® 2-Step with egg yolk** [REF. : 13570/0510](#)

### b) EquiPro® CryoGuard® 2-Step, without egg yolk

This version consists of 2 fractions:

1. Fraction A: EquiPro® for centrifugation and bloating
2. Fraction B: EquiPro® CryoGuard®: the operator adds 4 ml of fresh egg yolk before using

**EquiPro® CryoGuard® 2-Step, without egg yolk** [REF. : 13570/0520](#)

## Protocol for the semen processing with EquiPro® CryoGuard® 2-Step:

1. Pre-dilute and centrifuge the semen with fraction A
2. Extract the supernatant and bloat the semen pellets with fraction A to half of the calculated final volume
3. Cool the pre-diluted ejaculate down to +5°C within 2.5 to 5 hours
4. Prepare the ready to use fraction B out of medium plus egg yolk-glycerol mixture (REF.: 13570/0510) or egg yolk (REF.: 13570/0520)
5. Precool fraction B and the filling- and sealing machine to +5°C
6. Add fraction B to the semen up to the final volume
7. Print the required straws
8. Fill and seal the straws at +5°C
9. Freeze the semen straws