



EquiPlus - Preservation extender medium for fresh and cooled stallion semen

EquiPlus is a defined and standardised extender for the preservation of fresh and cooled stallion semen. It is comprised of several tested sugars and buffers and is available without antibiotics or with antibiotics to control bacterial growth. EquiPlus's unique formula includes caseinates, derived from various fractions of milk protein, and highly purified by high-end processing techniques. Caseinates have proven to be the most effective for the preservation of stallion semen due to the protection they provide to the sperm membrane.

EquiPlus is available in powder form or as a ready to use liquid. Liquid EquiPlus is a sterile, milky-white solution that only requires a simple warming step prior to use. Powdered EquiPlus is a white powder, which must be dissolved in purified water prior to use. EquiPlus is suitable to use for centrifuged and non-centrifuged semen.

EquiPlus in scientific studies

Traditional milk and egg yolk based extenders consist of a range of biological substances that can suffer from batch-to-batch, including the risk of microbiological contamination. In contrast, EquiPlus extenders contain defined and highly purified proteins manufactured in standardised batches. The end result is a product that is superior to skim milk extenders when semen is stored at 5°C (Aurich 2006).

In a comparative study, Pagl *et al.* (2006) concluded that semen preserved with EquiPlus (DMP) showed better results for motility and membrane integrity over a storage period of 72 hours when compared to either phosphate buffered saline (PBS) or Kenney skim milk extender.

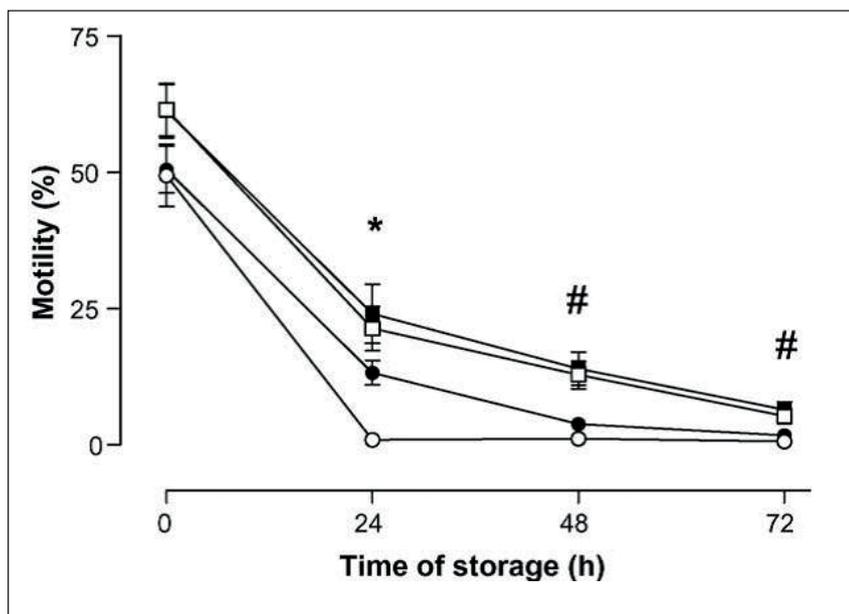


Fig. 1: Percentage of motile spermatozoa in non-centrifuged semen diluted either in PBS (○) or defined milk protein (DMP) extender (□), DMP extender with added n-acetyl cysteine (■) and Kenney extender (●) during storage at 5°C for 72 h. * PBS vs. all extenders, $p < 0.05$; # DMP and DMP plus vs. Kenney extender and PBS, $p < 0.05$. Source: Pagl *et al.* (2006).

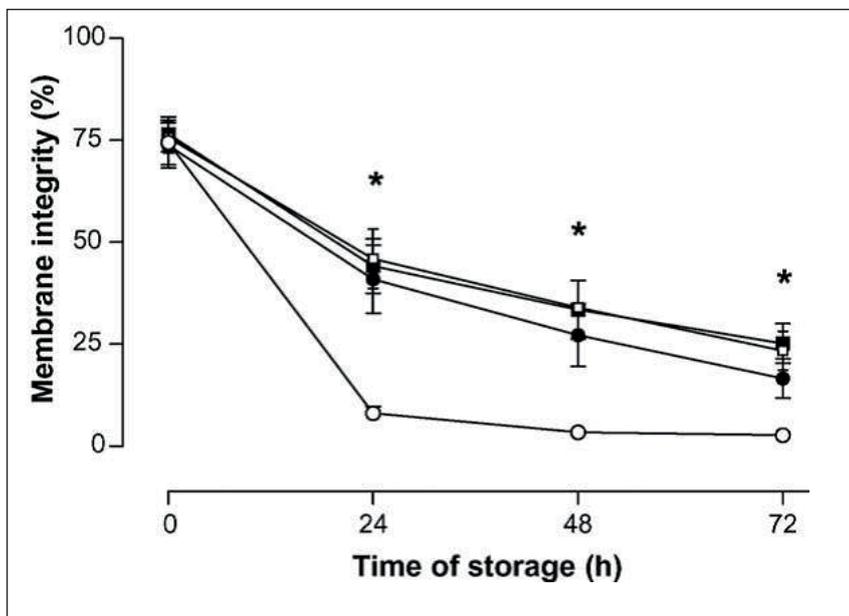


Fig. 2: Percentage of membrane-intact spermatozoa in semen diluted either in PBS (○) or defined milk protein (DMP) extender (□), DMP extender with added n-acetyl cysteine (■) and Kenney extender (●) during storage at 5°C for 72 h. * PBS vs. all extenders, $p < 0.05$. Source: Pagl *et al.* (2006)

Significant differences between extenders became very evident after semen was stored for more than 24 hours. The authors concluded that there is a potential advantage to EquiPlus when semen is stored over a period of 2 days. Semen centrifuged and extended with EquiPlus showed almost identical values for motility and membrane integrity after 24 hours and 48 hours. After 72 hours of cooled storage, motility in centrifuged semen in EquiPlus extender was significantly higher, suggesting a benefit of centrifugation when semen has to be stored for up to 3 days.

Another recent study conducted by LeFrappier *et al.* (2010) also showed that EquiPlus has excellent performance when semen was extended up to 3 days. Throughout the study EquiPlus was in the top group showing the best results for both total motility and progressive motility over a storage period of 72 hours ($P < 0.05$). Semen extended with EquiPlus was also among the top of the test groups for the CASA parameters including curvilinear velocity, straight-line velocity, and curvilinear distance ($P < 0.05$) (LeFrappier 2010).

Some facts about EquiPlus

EquiPlus is available in different formulations and package sizes:

- as powder with and without antibiotics
 - 100 ml package
 - 1 liter package
- in liquid formulation with and without antibiotics, 100 ml and 200 ml ready to use

Minitube provides detailed information about the ingredients of EquiPlus and the type and amount of antibiotics used. There is scientific evidence that not only bacteria but also some antibiotics may have harmful effects on spermatozoa (Aurich & Spengler 2006), with individual variability among stallions. It is, therefore, a good option in certain cases to choose an extender without antibiotics or with a different selection of antibiotics: e.g. for stallions that are categorized as “bad coolers”. Centrifugation can also improve the properties of cooled semen.

Strict quality control of the production and sterilization process eliminates the risk of mold growth in liquid EquiPlus. Therefore due to Minitube's manufacturing process, no fungicides are required, unlike other commercially available liquid stallion semen extenders. Fungicides can be harmful to the overall fertility of the sperm. According to Aurich *et al.* (2007), antibiotics, fungicides and generally any extender additive should be avoided unless it protects the sperm. Spermatology research shows that in other species, for example with bull and rabbit sperm (Foote, 2002), there is evidence that antifungal agents in semen extenders tend to be highly spermicidal.

EquiPlus liquid extender is packaged and delivered in a glass bottle with a secure cap. Glass is inert making it the ideal container for packaging the extender. Plastic containers on the other hand, may release plastic molecules into the extender fluid, contaminating the extender and potentially harming sperm. Once the EquiPlus liquid is opened or the EquiPlus powder is mixed with water, the extenders can be kept for up to 3 days in the refrigerator at +5°C.

In practice and in science, the temperature range of +4–6°C is considered to be the optimal storage temperature to preserve both motility and fertility of equine semen over 24 hours and more (Varner *et al.*, 1989). Shipped cooled semen has grown in popularity because semen shows almost no loss of fertility when stored at +5°C for about 24 hours (Aurich 2005). EquiPlus trials also support this statement that the best results for 24 hour old semen are generally achieved with storage temperature at +5°C (Pagl *et al.* 2006, Price *et al.* 2008).

Conclusion

EquiPlus is an extender for stallion semen and ideal for the storage of sperm at +4°C to +6°C for up to 3 days. In numerous studies as well as in the field, EquiPlus has proven to provide consistent results with a wide range of stallions.

Based on the proven EquiPlus formulation, Minitube also introduced **EquiPlus Freeze**, a series of semen extenders for the long term cryopreservation of stallion semen.

References

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