



Benefits of Androhep® Plus and Androstar® Plus

Long-term extenders for boar semen

Minitüb GmbH

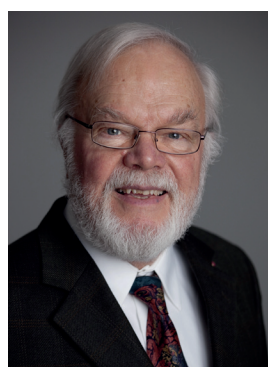
Introduction

By definition, a semen extender or a semen diluent is an aqueous solution that is used to increase the volume of the ejaculate to produce a certain number of doses. The solution should further protect the fertility of the spermatozoa during storage by providing nutrients and protective substances. The metabolic activity of the spermatozoa needs to be reduced during storage to save energy for the later fertilization process. This reduction of metabolic activity is obtained by lowering the storage temperature of the diluted semen (Gadea, 2003).

Given their particular features, boar spermatozoa are extremely sensitive to cold shock (Pursel et al. 1973) which alters sperm viability. Specifically, this sensitivity seems to be related to the lipid content of the sperm cell membranes. Thus, when the temperature drops, lateral movements of membrane phospholipids are reduced causing a separation of the lipid phases, which is associated with irreversible alterations in the membrane proteins. This inevitably leads to a change in the function of the sperm membrane, thus compromising cell viability (reviewed in White, 1993). In practice, this reaction to cold shock means that extended semen samples need to be kept at +15 to +20°C, since a further reduction in temperature limits their viability (Paulenz et al., 2000).

It has recently been demonstrated that this long proposed paradigm can be abolished. The long term boar semen extenders Androstar® Plus and Androhep® Plus contain a special cell membrane protection ingredient (MPI™) that allows for a wider range of storage temperatures, because it prevents membrane changes that lead to lower viability.

Over the course of the last 10 years, Prof. Dr. Karl-Fritz "Charlie" Weitze, Unit of Reproductive Medicine of the Clinics, University of Veterinary Medicine Hannover, Germany has carried out laboratory and field studies in cooperation with Minitüb GmbH (Tiefenbach, Germany) related to methods to protect sperm membranes from damage during cool storage and under varying storage conditions. These studies, which were carried out in his lab in Germany and then on large sow farms, resulted in the development of the 'membrane protection ingredient' (MPI™), which was in the first publications referred to as cold-shock protecting agent (Waberski et al, 2008). It is the key component of this latest generation of boar semen extenders produced and marketed by Minitube International AG, Germany [MTI]. These extenders carry the "Plus" designation in the name. The component is of non-animal origin and protects the highly sensitive membranes of boar sperm cells during all steps of semen processing and storage, from the first dilution to semen storage under various temperature protocols down to 5°C (Weitze, unpublished). The protective action of this defensive factor against sperm damage originates from its modulating interaction between sperm organelles, for example the plasma membrane, the acrosome membranes and the mitochondria. Therefore, the negative effects of temperature-dependent changes in the lipid architecture of the bilayer systems are minimized and sperm functions relevant for the fertilization process are maintained. The superior capability of the "Plus" preserved sperm cells to bind to the oviductal epithelium of the sperm reservoir in the utero-tubal junction underlines the improved protection of sperm functionality.



Prof. Dr. Karl-Fritz Weitze

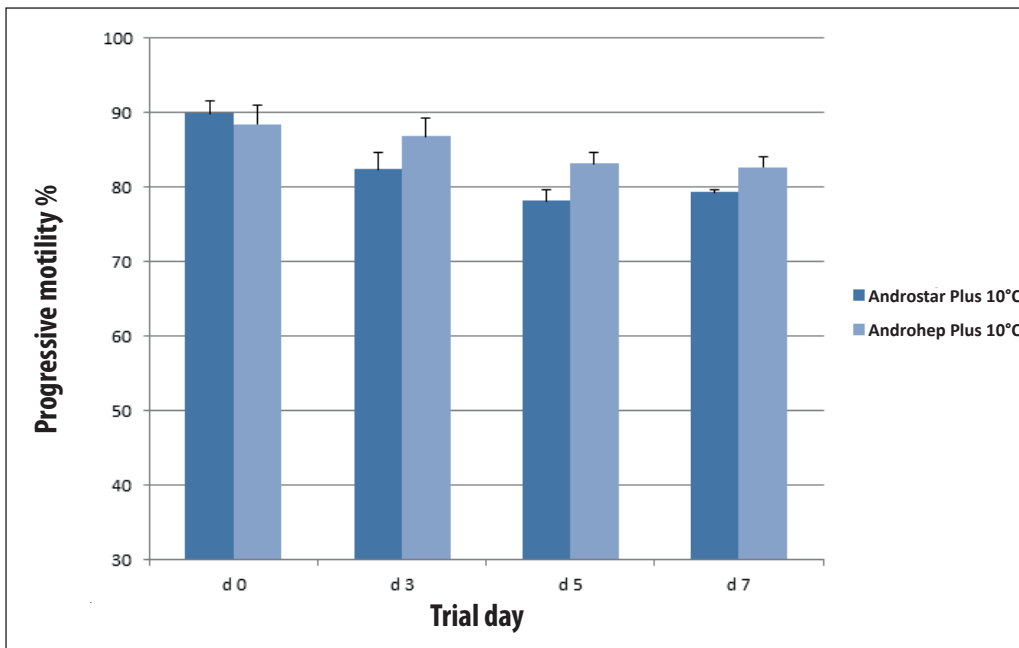


Semen storage at +10°C with Androhep® Plus and Androstar® Plus

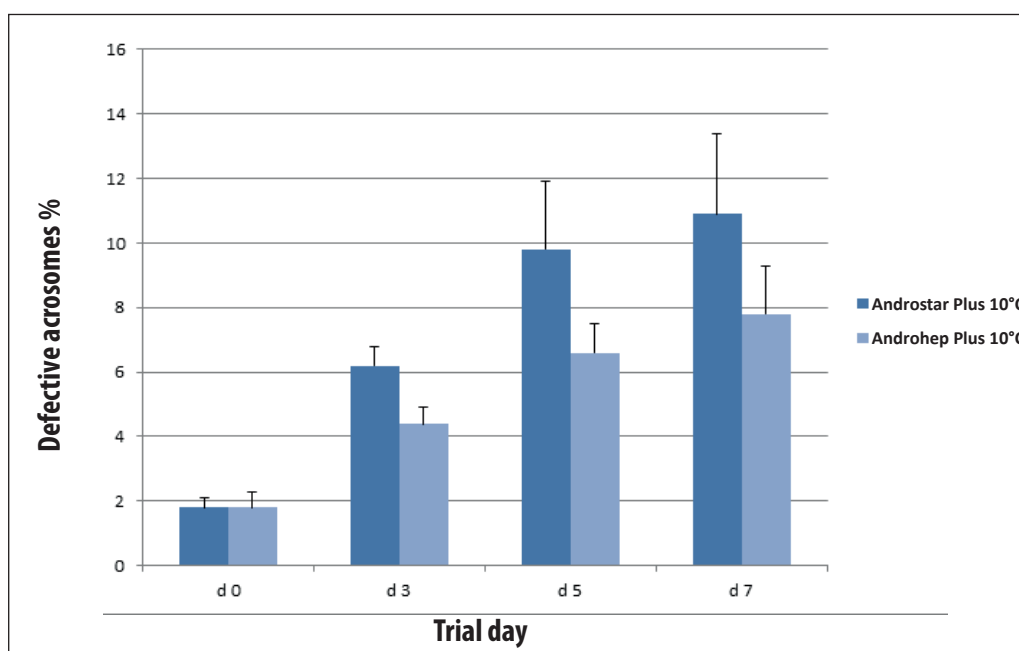
Several trials, conducted by Dr. Weitze, have shown, that semen diluted in Androstar® Plus can successfully be stored at +10°C up to 7 days without compromising semen quality (Waberski et al. 2008, Schmid et al. 2011, Weitze et al. 2011). Further, Androhep® Plus has additional protecting agents to complement the cell membrane protection ingredient. Androhep® Plus contains HEPES as a highly efficient and temperature independent pH buffering system (Gadea, 2003). This semen extender also includes BSA (bovine serum albumin) to compensate for the dilution effect on seminal plasma protein. BSA adds further protection against cold shock for the spermatozoa diluted in Androhep® Plus.

Comparisons of the semen storage capabilities of Androstar® Plus and Androhep® Plus have proven the high quality of the Androstar® Plus extender, and have also demonstrated that Androhep® Plus can offer even better protection of boar semen quality.

Graph 1 and 2, respectively, show the percentage of progressive motile spermatozoa and the percentage of damaged acrosomes on different days of semen storage at +10°C. Split sample ejaculates of 6 boars were isothermally diluted, either in Androstar® Plus or Androhep® Plus, then cooled to +10°C within 3 hours and stored up to 7 days.



1: Progressive motility of boar semen diluted in Androstar® Plus or Androhep® Plus after several days of storage at +10°C.



2: Defective acrosomes of boar semen diluted in Androstar® Plus or Androhep® Plus after several days of storage at +10°C.

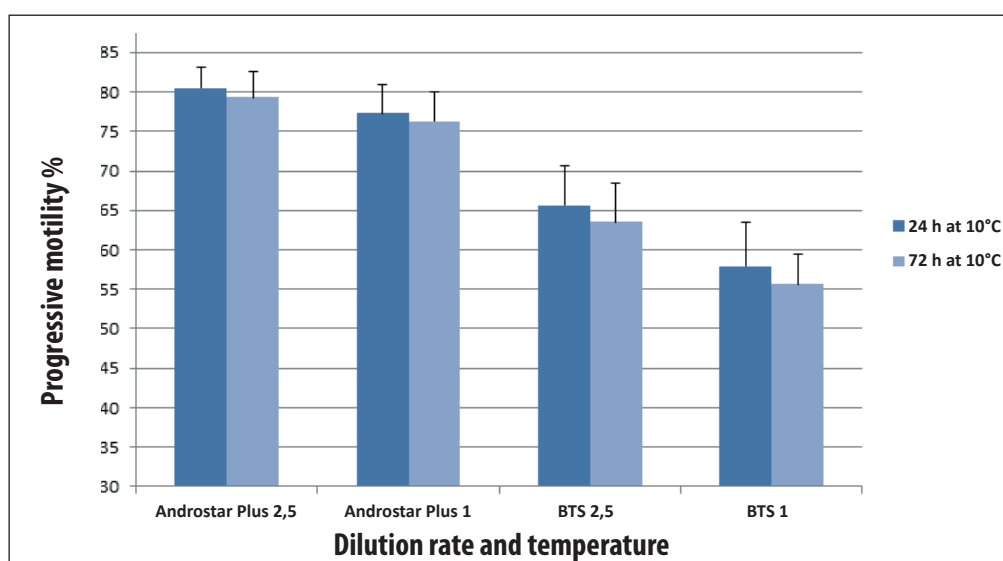
Both extenders demonstrate excellent protection of the sperm cells at +10°C storage temperatures. Androhep® Plus has superior semen preservation capabilities due to the synergistic effects of the MPI™ and BSA, and the more complex HEPES buffer.

Further tests provided evidence that Androstar® Plus and Androhep® Plus can also protect boar sperm for several days at storage temperatures up to +25°C. Moreover, the extenders have also proven their ability to maintain semen quality during oscillating storage temperatures between +10°C and +25°C (Weitze, 2013).

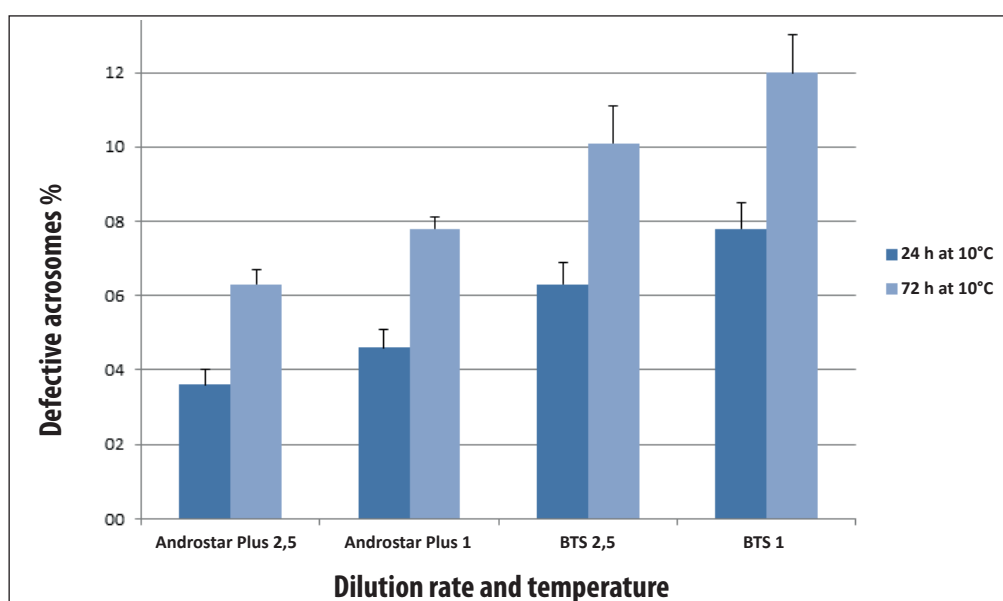
Protection against the dilution effect

During processing steps, spermatozoa can be adversely affected by high dilution, i.e. the so-called dilution effect, probably due to the decreased protective effects of substances in the seminal plasma, especially if only the sperm rich fraction is collected. The technique of TCI [also called PCAI or IUI] allows the production of a larger number of semen doses from each ejaculate without compromising conception rates. Therefore, since ejaculate efficiency is increased, fewer boars will be needed to produce the same number of AI doses, thus reducing costs. Under some production systems, the sperm numbers per dose and dose volumes used with this technique may also result in an increased dilution rate of the ejaculates, thus affecting spermatozoa adversely if they are not protected by a specialized medium. The “Plus” extenders with the MPI™ help protect against these damaging effects.

Graph 3 shows the effect of high dilution rates on boar sperm motility. Graph 4 shows the effect of dilution on acrosome integrity. In this trial, 6 boar ejaculates were diluted in either Androstar® Plus or BTS in two concentrations and stored at +10°C (Waberski et al, 2008).



3: Effect of high dilution rates and low storage temperatures on motility of boar sperm either diluted in Androstar® Plus or BTS in two concentrations (2.5 billion sperm/100 ml vs. 1.0 billion sperm/100 ml).



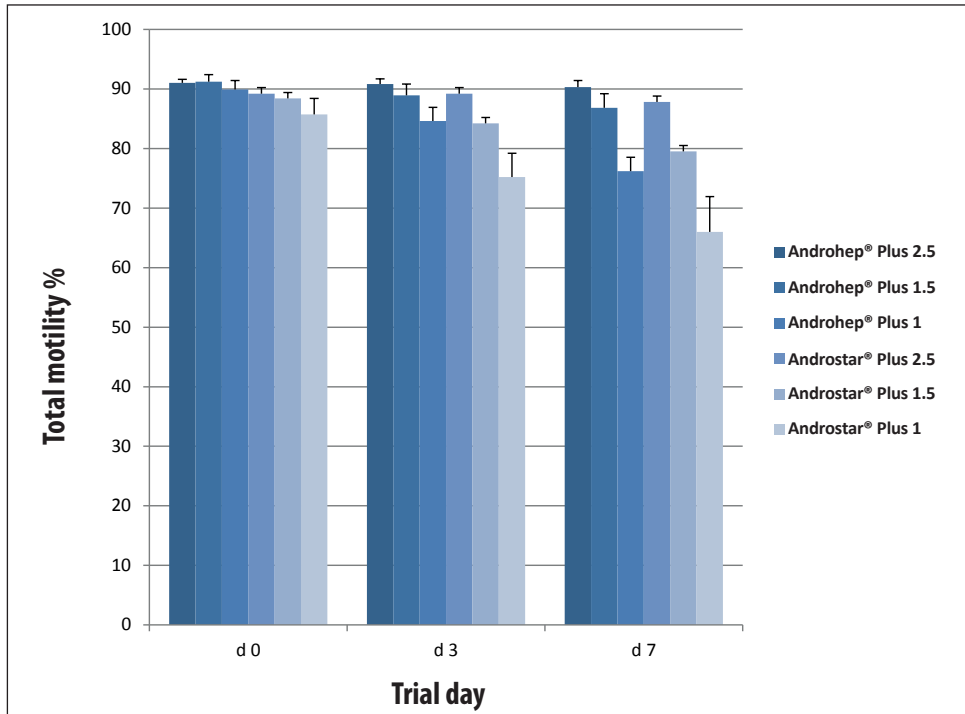
4: Effect of high dilution rates and low storage temperatures on the acrosome integrity of boar sperm either diluted in Androstar® Plus or BTS in two concentrations (2.5 billion sperm/100 ml vs. 1.0 billion sperm/100 ml).

These results demonstrate that sperm motility and especially acrosome integrity is affected by dilution and storage temperature. Androstar® Plus protects sperm quality better than the standard short term extender BTS against cold shock and high dilution.

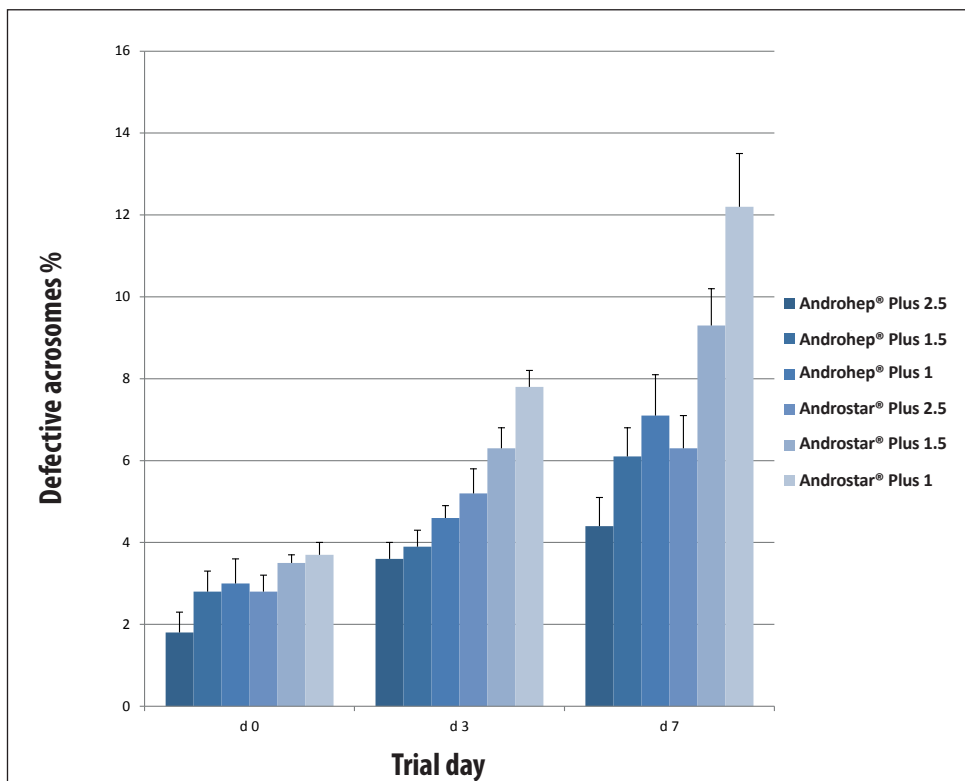
Graph 5 shows the result of another trial that compares the protection ability of Androstar® Plus and Androhep® Plus. Here, the storage temperature was +16°C. In this trial, 6 boar ejaculates were diluted in either Androstar® Plus or Androhep® Plus in three concentrations:

- 2.5 billion sperm per 80 ml dose
- 1.5 billion sperm per 80 ml dose
- 1.0 billion sperm per 80 ml dose

After dilution, the semen doses were stored up to seven days and then tested for progressive motility (Graph 5) and acrosome integrity (Graph 6).



5: Effect of high dilution rates on motility of boar sperm either diluted in Androstar® Plus or Androhep® Plus in three concentrations (2.5 billion sperm/80 ml, 1.5 billion sperm/80 ml, 1.0 billion sperm/80 ml) stored up to 7 days at +16°C.



6: Effect of high dilution rates on the acrosome integrity of boar sperm either diluted in Androstar® Plus or Androhep® Plus in three concentrations (2.5 billion sperm/80 ml, 1.5 billion sperm/80 ml, 1.0 billion sperm/80 ml) stored up to 7 days at +16°C.

Here again it can be seen that high dilution has a negative effect on sperm quality especially after several days of storage. Androhep® Plus is capable of protecting sperm better than Androstar® Plus, whereas Androstar® Plus protects sperm quality better than BTS.

Conclusion

These findings demonstrate that Minitube's long term boar semen extenders protect the spermatozoa effectively against cold shock and sub-optimal storage conditions of varying temperatures between +10°C and +25°C.

Both, Androstar® Plus and Androhep® Plus show good protection properties not only at varying storage temperatures but also at high dilution rates. If boar semen is highly diluted for use in TCAI or for other reasons, the sperm should be protected with a long term extender to assure high fertility of the semen.

Boar semen processed with the Androhep® Plus and Androstar® Plus extender is capable to better withstand low temperatures down to 5°C, as well as storage at high temperatures of up to 25°C, and it can cope better with accidental temperature fluctuations during storage. Notably, Androhep® Plus is better suited for storing highly diluted semen. Both extenders increase the stress-robustness of boar sperm even from heat stressed boars, resulting in improved sperm motility and fewer defective acrosomes on day 1 and until after several days of storage.

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