TECHNICAL REPORT
03 | 2024

Antibiotic free boar semen storage at 5°C

R. Grossfeld PhD, Dr. C. Simmet DVM, Dr. K. Simmet (Minitüb GmbH), J. Quackenbush (Minitube USA Inc.)

Sperm quality and fertility of antibiotic-free preserved boar semen stored in Androstar® Premium at 5°C

Adding antibiotics to semen extenders is a standard procedure in artificial insemination (AI) of livestock to avoid bacterial growth in semen doses during the preservation period. The necessity for antimicrobial control results from the unavoidable presence of bacteria in raw semen naturally stemming from the male and, to a smaller extent, from the environment. With adaptation of the World Health Organization's one-health concept, researchers and the animal breeding industry are seeking strategies to reduce or replace antibiotics in semen extenders.

Hypothermic preservation of boar sperm at +5°C allows to omit the addition of antibiotics to boar semen extenders as bacteria will remain in a static state at this temperature[1,2]. Therefore, bacteria that are possibly present in diluted boar semen do not proliferate and will not harm the sperm cells[2].

The main challenge of hypothermic storage without antibiotics is the prevention of chilling injuries to spermatozoa and bacterial growth during the cooling of the semen doses to 5°C. Androstar® Premium boar semen extender allows hypothermic and antibiotic-free storage of boar semen by protecting the integrity of the sperm cells during cooling and by containing antimicrobially active substances[1].

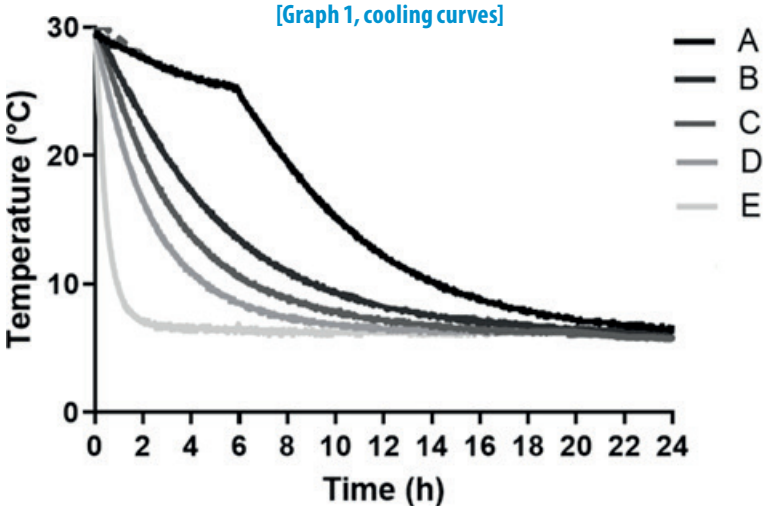
Storage of boar semen without antibiotics at 5°C in practice

The cooling of the diluted boar semen to +5°C requires a controlled cooling curve over several hours to allow an adaption of the spermatozoa to lower temperatures. However, cold damage to boar spermatozoa is an important concern during storage at such low temperatures[3].

For further development and incorporation of the hypothermic storage concept into AI practice, determining a cooling-rate frame that concurrently is tolerated by spermatozoa and inhibits bacterial growth is required. Therefore, several cooling regimes of diluted boar sperm were tested for their suitability in hypothermic storage, as demonstrated in Graph 1[3].

Split sample ejaculates from different boars (n=8), diluted in Androstar® Premium were cooled to +5°C according to cooling curves A-E. Sperm analyses for total motility were performed after 24, 72 and 144 h of storage a +5°C. All semen samples were evaluated with the AndroVision® CASA system for motility after 30 minutes of incubation at 38°C.

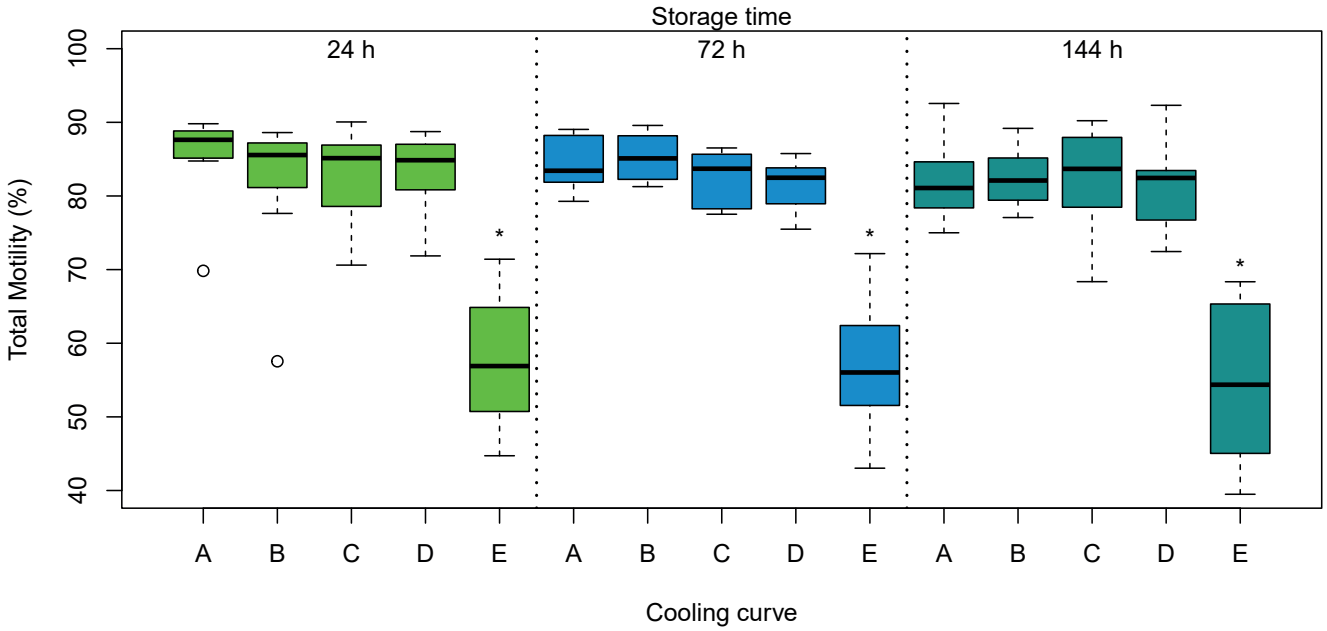
[Graph 1, cooling curves]



GRAPH 1: Samples cooled by curve A, B, C, D and E had an initial cooling rate of 0.01, 0.06, 0.09, 0.14 and 0.65°C min^{-1}, respectively, until reaching 25°C. Then, samples were cooled to 10°C at the rates of 0.03, 0.03, 0.05, 0.06 and 0.31°C min^{-1}, respectively. Finally, samples were cooled to 5°C at a rate of 0.01°C min^{-1} (curves A, B, C and D) and at 0.02°C min^{-1} (curve E) (adapted from Paschoal et al.[3]).

Graph 2 shows the results of the motility analysis after different cooling curves at the respective time points of analysis.

[Graph 2, motility results Exp. 1]



GRAPH 2: Total sperm motility of sperm samples cooled according to different cooling regimes after 24, 72 and 144h of storage at +5°C (modified from Paschoal et al.[3]).

In samples submitted to cooling rate E, the percentages of motile spermatozoa were lower ($P < 0.01$) after 24, 72 and 144 h storage compared to samples cooled at the other cooling rates.

Hypothermic storage is also the only sustainable tool to combat multi-drug resistant strains of bacteria, as the low temperature reliably inhibits the growth of Serratia marcescens and Klebsiella oxytoca. In a study from Maaßen at al.[4] trial semen samples were spiked with ~102 CFU/ml of these bacteria species. Storage at +5°C for 144h inhibited the growth of both bacterial species and maintained the sperm quality, whereas bacterial counts increased to more than 10^{10} CFU/ml in the +17°C samples used as positive controls. This was accompanied by an increase in the sperm agglutination and the loss of motility and membrane integrity.

In-vivo fertility after hypothermic storage of boar semen

Several trials have been conducted in the past to prove the suitability of antibiotic-free conservation of diluted boar semen at 5°C [5,6]. In the study of Waberski et al.[5] pooled semen from 23 boars (3 boars per pool) were split into two groups. The semen samples in one group were cooled to and stored at 5°C while the samples in the other group were stored at 17°C as a control. The samples were subsequently transported to a sow farm and used for AI. The insemination trial resulted in similar field fertility with 14.1 ± 0.2 vs. 14.5 ± 0.2 total born piglets after AI with semen stored at 17°C and 5°C, respectively (Table 1).

Field fertility of boar semen samples stored with either one of the following temperatures and extenders:

17°C: Semen stored in Androstar® Premium with antibiotics (w/AB; 0.25 g/L gentamicin sulphate)

5°C: Semen stored in Androstar® Premium without antibiotics (w/o AB)

There were no differences in farrowing rate and number of total born piglets ($p > 0.05$).

[Table 1, fertility results]

Semen storage	Sows, n (gilts, n)	Farrowing rate (%)	Total born piglets (n)
17°C with AB	406 (71)	93.1	14.1 ± 0.2
5°C without AB	411 (87)	92.0	14.5 ± 0.2

Conclusion

Hypothermic storage of boar semen allows to omit the use of antibiotics in diluted boar semen. Several cooling curves are suitable for cooling boar sperm to +5°C. Therefore, different cooling regimes in practice are possible making the use of hypothermic storage of boar sperm usable in different environments of AI centers. In addition, equal fertility results after hypothermic storage, compared to regular AI processes, pave the way for eliminating the use of antibiotics in boar sperm production.

References

1. Luther, A. M., Nguyen, T. Q., Verspohl, J. & Waberski, D. Antimicrobially active semen extenders allow the reduction of antibiotic use in pig insemination. Antibiotics 10, (2021).
2. Menezes, T. de A. et al. Antibiotic-free extended boar semen preserved under low temperature maintains acceptable in-vitro sperm quality and reduces bacterial load. Theriogenology 149, 131–138 (2020).
3. Paschoal, A. F. L. et al. Determination of a cooling-rate frame for antibiotic-free preservation of boar semen at 5°C. PLoS One 15, 1–14 (2020).
4. Maaßen, I. K., Luther, A.-M., Verspohl, J. & Waberski, D. Storage of Extended Boar Semen at 5°C Inhibits Growth of Multi-Drug Resistant Serratia marcescens and Klebsiella oxytoca while Maintaining High Sperm Quality. Antibiotics 12, 857 (2023).
5. Waberski, D. et al. Sperm function in vitro and fertility after antibiotic-free, hypothermic storage of liquid preserved boar semen. Sci Rep 9, 1–10 (2019).
6. Reckinger, F., Riesenbeck, A., Waberski, D. & Waßmuth, R. Hypothermic preservation of boar semen at 5°C: A field test. Reproduction in Domestic Animals 58, 27–28 (2023).