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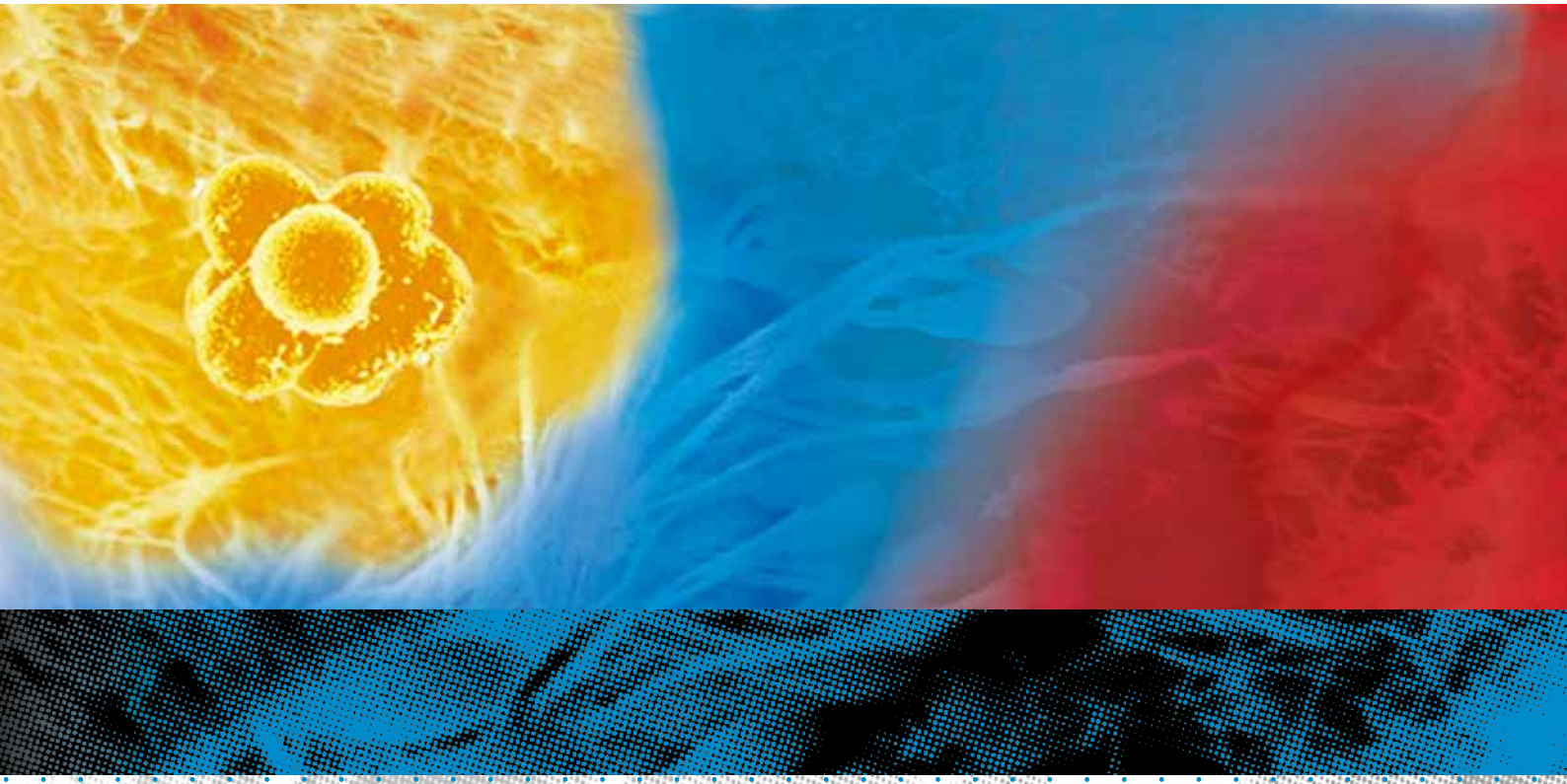

Sperm Notes

Special Edition on Temperature Management of Boar Semen

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Why is identic temperature at ejaculate collection and examination so important?

Freshly ejaculated boar spermatozoa are highly sensitive against cooling and quick dilution. Especially during the first 10 to 15 minutes after semen collection the spermatozoa are busy with adapting to the new seminal plasma environment. Any change of temperature in this time lapse can be ruinous for their fertilizing capacity, even though a decline in motility or membrane integrity might become visible only after some days of storage.

Take home message:

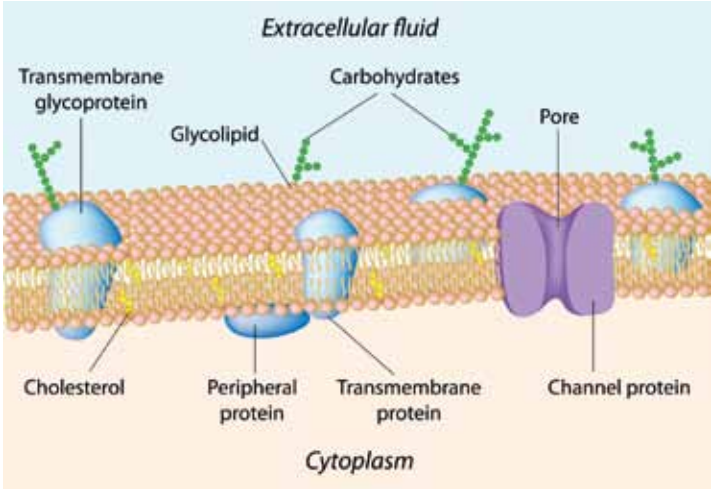
- Collect boar semen into insulated collection vessels pre-warmed to +38°C.
- Prepare samples for analysis with isothermal extender.
- Let the sample rest a few minutes before measuring motility.

What happens during the first 15 minutes that we have to be so careful with temperature?

During ejaculation, the epididymal sperm cells experience their first dilution when they are extended by the secretions of the accessory glands which in the boar represent a large volume. Beside the dilution stress, the epididymal sperm cells have to put up also with a first temperature change when the „cool" sperm rich fraction of epididymal semen is mixed up with the warmer secretions of the accessory glands.

The ejaculated sperm cells are also exposed to dozens of seminal plasma components which stimulate motility and prepare them for the race to the egg. During this process the formerly immotile epididymal sperm undergo a cascade of motility stimulating reactions rendering them highly susceptible to any additional stress. Particularly seminal plasma proteins trigger changes in the sperm membranes in order to establish the final functionality of the plasma membranes.

This process of reorganization of the membrane structure must not be disturbed and has to be completed before the sperm cells can withstand further stress situations such as dilution and temperature changes. Recent research results indicate that the sperm plasma membrane needs some minutes to reorganize itself after a stress caused by temperature change or dilution.

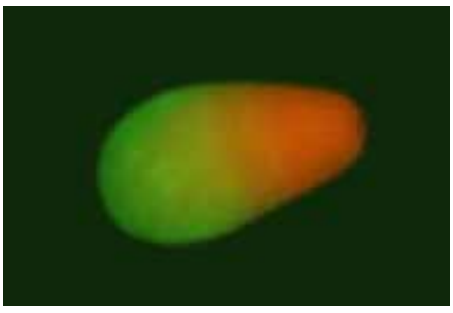


Picture 1: Sketch of plasma membrane

Furthermore, it is also well known that undue chilling creates alterations in the most diverse aspects of the sperm cells' household as there is for example intracellular pH or energy metabolism. These disturbances are more likely to happen in freshly collected ejaculates and can be observed as alterations of Ca^{2+}- equilibrium in the living cells.

In addition, temperature changes alter also the membrane permeability for ions like sodium (Na) and potassium (K) by affecting their highly temperature sensitive transport mechanism, the so-called Na/K-pump. This results in a continuous leakage of potassium, putting cell survival into danger due to a lack of intracellular potassium.

As a consequence of the above mentioned events during and immediately after ejaculation, isothermal conditions during collection and the first 15 minutes after ejaculation are fundamental to preserve the full fertilizing capacity of the semen. That´s why boar semen must be collected into insulated collection vessels which are pre-warmed to +38°C prior to collection.
Once the ejaculate has arrived in the lab, sample preparation for the semen analysis must be done with isothermal extender. At this step the sperm cells suffer their second dilution shock which becomes more visible with increasing dilution rates. It is therefore also advisable to let the sample rest a few minutes before measuring motility in order to visualize the full potential of the sample.



Picture 2: Sperm cell with defect plasma mem-brane and acrosome (stained with PI-FTC-PNA)

Practical management of temperature during semen collection and dilution

Introduction

The success of artificial insemination (AI) depends mainly on the quality of the semen and insemination procedure. The quality of the semen dose is based upon:

1. Exclusive use of semen from healthy boars
2. Semen fertility
3. Procedures used for semen production

Because of the particular lipid composition of the porcine sperm cell membrane, boar semen is especially affected by temperature changes and exposure to low temperatures. Low temperatures cause an increase in membrane permeability leading to impaired sperm functionality.

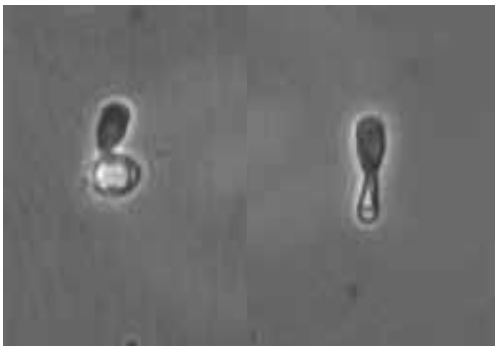
How should we work in practice to maintain sperm viability during semen production?

Temperature during semen collection

Optimal results in artificial insemination can only be obtained if the initial semen quality meets or exceeds the minimum quality standards established. When we collect, process and store the semen, it is important to avoid temperature fluctuations.

During semen collection, measures must be taken to ensure that both the materials for collection as well as the ejaculate itself are kept within safe limits. Low ambient temperature or disturbances during semen collection may cause a fast drop of the freshly collected semen causing the so called „cold shock". When we analyze a sample of cold shocked semen under the microscope, it is typical to observe a high percentage of sperm cells with the tail around or below the head (coiled tail) (Picture 1).

During the collection, semen should get in contact only with isothermal material. Sperm viability and longevity are affected by temperature variations as little as ± 2°C. Therefore, it is recommended to use insulated and pre-heated collection cups (+38°C). Considering that 50% of the sperm cells in an ejaculate are found in the first 20 ml of the sperm rich fraction, preheating the collection vessel is of great importance for the first contact of the semen with its new environment.



Picture 1: Sperm cells with coiled tails

Take home message:

- The use of extender in the collection cup is a risk for seminal quality
- Know your working temperature profile and implement the correct cooling rate
- Apply 1:1 minimum predilution rate when quick dilution is not possible
- The dilution of the ejaculate in two steps allows earlier shipping of semen doses on high volume production days.

The duration of the ejaculation is associated with good libido but not necessarily with good semen quality. The longer semen collection takes the longer the sperm cells are exposed to temperature fluctuations and bacterial contamination originating from the environment. Automatic collection systems like the BoarMatic (Picture 2) minimize the impact of these potential causes of quality loss, providing a closed system for semen collection with a plastic sheath that isolates the semen from the environment.

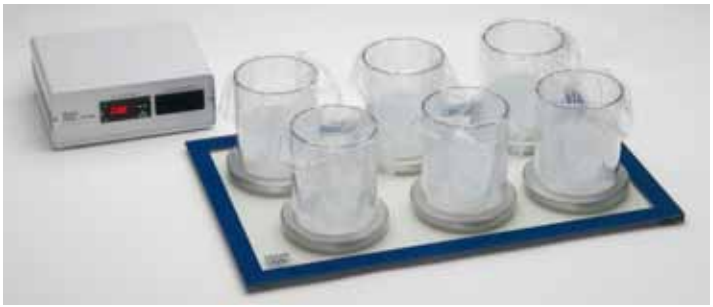
Picture 2: Detail of the penis and ejaculate protection during the collection with BoarMatic

It is not advisable to use extender in the collection cup. This practice aims to mitigate the temperature drop of the semen during ejaculation and to provide a first contact with antibiotics to stop bacterial growth. However, in practice the temperature of the extender in the collection cup is not kept at +38°C causing a thermal shock particularly for the first jets of the sperm rich fraction. Furthermore, the use of antibiotics and unavoidable spillage in this area may result in the development of resistant bacteria, facilitating the appearance of multi-resistant strains just in the boar barn.

Passing the ejaculate from the collection area to the laboratory

Once semen is collected, the ejaculate will be handed over to the laboratory taking into account hygiene and temperature. Only the collection bag enters the laboratory, leaving the collection cup and the filter in the collection area. The bag with the ejaculate is placed in an isothermal container exclusively used to maintain the semen until processing (Picture 3). Another alternative is to use heated plates provided with stands where the bag with the ejaculate is placed to maintain temperate (Picture 4).

Picture 3: The ejaculate in the lab inside the insulated cup



Picture 4: Stands on the heating plates for semen bags

Semen dilution

Semen processing must always follow two basic principles of semen handling: hygiene and temperature control. Both factors influence the functionality of the sperm after ejaculation and the semen during storage.

In practice, the first dilution must be made between 10 and 15 minutes after collection with isothermal extender, taking into account the duration of the collection and transport to the laboratory. In this context, the term „isothermal" refers to the concept of temperature equality between semen and extender and not to a specific temperature.

When the collection room and the laboratory are separated by a distance that in time is 30 to 60 minutes, you should pay attention to the temperature and dilution rate (minimum 1:1) to protect the semen until processing.

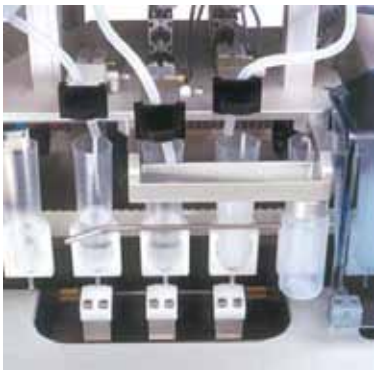
The traditional dilution protocol used is a **one-step dilution** with diluent heated to about +30°C to +32°C. The cooling rate suitable for boar semen is +1°C/10 minutes, corresponding to an equilibration time between 1-2 hours at room temperature before entering the cool storage (+17°C) to reach the final temperature.

Keep in mind that exposure of the semen to physiological temperatures can affect the integrity of the membrane and therefore the quality of semen. Therefore the storage of extended semen in water baths at body temperature is not advisable.

At present, many boar studs adapted the production protocol to their customers´ requirements being the two-step dilution method a widely used semen processing scheme.

The procedure of the **two-step dilution** allows several alternatives to cover specific stud requirements, **always exposing the semen in a first step to isothermal extender** 10 to 15 minutes after collection:

1. **Transport of semen** when the collection site is remote from the laboratory. When you have to transport the semen from one or more sites to the laboratory, extend the semen at a ratio of 1:1 at the site of collection. This will protect the sperm cells for a period of up to one hour; longer periods of transport require a higher dilution rate. It is advisable to check the temperature of the semen upon arrival in the laboratory to adjust the temperature of the extender for final dilution, usually between +25°C and +30°C depending on the season.
2. **Bottle necks** in the processing line. Sometimes the freshly collected ejaculates have to wait more than 15 minutes to pass semen analysis before being extended. To prevent damage to the ejaculate, an initial semen dilution at a ratio of 1:1 provides a buffer in the process line of up to one hour. A second dilution with isothermal extender within one hour is ideal to add the rest of the semen extender.
3. Using a **second filling station** in the tube filling machine (Picture 5). In those studs, the semen is extended to 50% of the final volume with an isothermal extender. The tubes are filled in the first filling station with the first half of the dose volume. The second filling station completes 100% of the dilution with isothermal extender. In some cases the final dilution is performed with extender at room temperature, providing a faster cooling rate and producing semen doses between +23°C to +25°C. This procedure additionally speeds up laboratory work by saving time during the dilution of the ejaculate and filling the tubes.



Picture 5: Two-step dilution

Whatever dilution procedure is chosen, three basic requirements must be met for best practice:

1. Control of semen temperature at arrival in the lab to adjust extender temperature.
2. Add the extender gently and avoid a direct jet into the semen.
3. Respect the ideal cooling rate during semen processing to deliver a product with the best possible quality. Semen doses can be cooled to +17°C during transport to the farm or on the farm of destination if there is not enough time at the boar stud for this last step.

Temperature management in semen production: slow moves are better moves

Boar studs operate under an enormous pressure. Considering that a large number of ejaculates must be processed in a few hours, under practical conditions the little time allowed in the lab for processing a given ejaculate turns out to be a critical factor at the boar stud. Optimized lab automation and workflow schemes are essential to reach a processing speed as high as 50 ejaculates per hour, but semen does not like to be rushed through the processing chain. Temperature management of the ejaculates is one of the stumbling blocks which require consideration.

The conflict lies between the need for isothermal dilution of the freshly collected ejaculate and the wish to push production and getting the semen doses to the farms at storage temperatures. Therefore, the so called two step dilution procedure was developed which starts the temperature decrease already during the dilution process.

The semen is pre-extended at a dilution ratio of 1:1 with isothermal extender, typically at +30°C to +32°C and the final dilution is done at least 15 minutes later with extender at room temperature (+21°C). The desired effect is a much faster temperature decrease in the extended semen which allows the semen doses to reach much quicker the desired temperature range of +17°C to +20°C for delivery to the customer. The advantage of the two step procedure is clear: semen doses can be delivered earlier at the day of semen production.

In contrast, extending the ejaculate in one single step completely with isothermal extender at +30°C to 32°C requires more time for the semen doses to reach the desired transport temperature in the cooling chamber before delivery.

Results of different insemination trials under field conditions confirmed the viability of the two step method by not showing differences in fertility compared to conventionally processed semen doses according to the one step dilution procedure. However, it is important to keep in mind that these trials were done with short term stored semen doses with relatively high sperm numbers. These conditions could have masked a possible detrimental effect of rapid cooling.

Research results obtained under strictly controlled experimental conditions show a different picture. These data suggest that the probability of damaging sperm cells to a degree which is relevant for their fertilizing capacity increases significantly when freshly collected and pre-extended semen is quickly chilled by adding the final volume of extender at room temperature.

The experiment compared the two step dilution procedure as described with the one step method, where the pre-extended sample (+32°C) was finally extended with isothermal extender at +32°C. Fig. 1 and 2 show CASA-motility data and the percentage of sperm cells with defect acrosomes after storage at +17°C for 24, 72 and 144 hours. To study the possible effect of a cold shock protecting extender, split-samples prepared with Androstar Plus and BTS were compared.

Take home message:

- Early chilling of boar semen works under good AI conditions.
- Suboptimal timing of AI and/or low sperm dose AI require a more careful semen temperature management.

Fig. 1. Progressive motility after one step or two step dilution of boar semen with Androstar Plus (A) and BTS (B) dilution; final storage temperature at 17°C, n=6

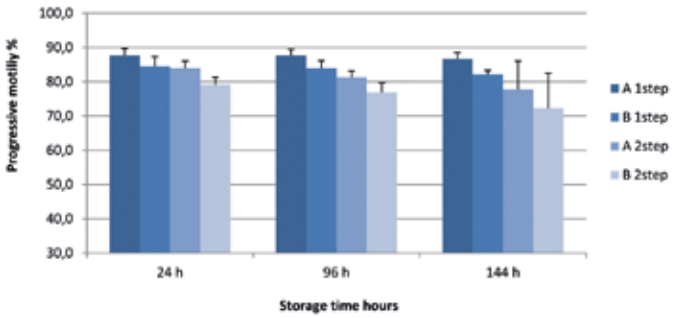
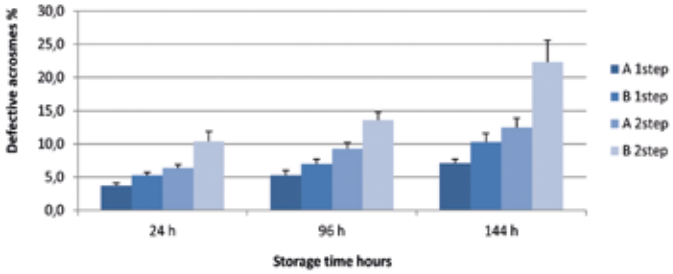


Fig. 2. Defective acrosomes after one step or two step dilution of boar semen with Androstar Plus (A) and BTS (B) dilution; final storage temperature at 17°C, n=6



The results indicate that the two step dilution procedure using extender at +21°C for the final semen dilution had a significantly negative effect upon motility and membrane integrity. This effect was seen with both extenders used, but Androstar Plus provided significantly better protection compared to BTS.

Based on the presented results and further experimental data it must be concluded, that early chilling during the dilution process bears the risk of fertility decreasing changes of sperm, which can hamper the AI success, especially under suboptimal AI management and when inseminating low sperm numbers.

Can we handle boar semen beyond the +17°C paradigm?

Shipping and storing liquid preserved boar semen is a synonym for struggling to keep the right temperature. There are a few situations which can expose boar semen to temperatures as low as 10°C and less. Popular examples are inadequate shipping conditions in winter time or semen storage units which do not work correctly. On the other side, warm climates pose the challenge of keeping semen cool enough during transport.

But there are ways to help the sperm cells to adapt to more extreme environments than the recommended standard +17°C. Key elements are the adequate temperature management and the extender choice.

Accidental drop of shipping temperature

The following experiment (Fig. 1 and 2) mimicked the temperature history of semen doses shipped overnight and getting down to +10°C for a period of 12 hours before being stored at the sow farm at +17°C. The experimental groups differ only in the temperature management between semen dilution and reaching the final storage temperature. Group 1 (25 10 17) received an adapted cooling procedure consisting of a 6 h compensatory incubation period at +25°C before an experimental overnight cooling to +10°C whereas group 2 (10 17) was cooled down to + 10°C with 1 hour holding time at room temperature followed by 1 hour holding time at 17°C. Group 3 (17) served as a control with standard storage temperature at +17°C all the time. All samples were extended with Androstar Plus as split samples to 2.5 x 10^6 sperm cells per 80 ml using the one step procedure with isothermal extender at +32°C. Progressive motility and acrosome damage were recorded as parameters for in-vitro semen quality and indicators for the preservation of fertilizing capacity.

Experiment 1 shows a significant protecting effect of a compensatory incubation period at +25°C before overnight cooling to +10°C and subsequent storage at +17°C in comparison to immediate cooling to +10°C. The samples with a compensatory incubation period at +25°C show no difference to the controls stored all the time at standard storage temperature +17°C.

The samples without a compensatory incubation period before being cooled down to +10°C are slightly but not significantly inferior after 72 h of storage. However, a further decrease in semen quality can be observed after 168 hours of storage making the sperm damaging effects of fast cooling rates more evident.

Considering the results of experiment 1, a second trial was conducted with the aim to investigate the extender effect on samples subjected to a compensatory incubation period of 6 hours at +25°C.

Take home message:

- Androstar Plus protects boar semen down to +10°C
- Boar semen in BTS must not be exposed to +10°C
- Compensatory incubation period at +25°C before cooling is helpful
- Shipping can be done at +25°C
- Androstar Plus eliminates the need for a cold room at the boar stud

Fig. 1: Progressive motility of boar semen extended in Androstar Plus before over night cooling to 10°C, n=6

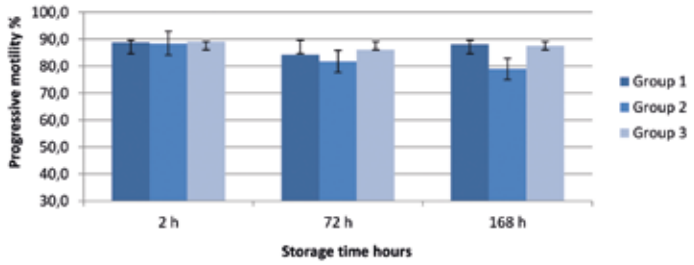
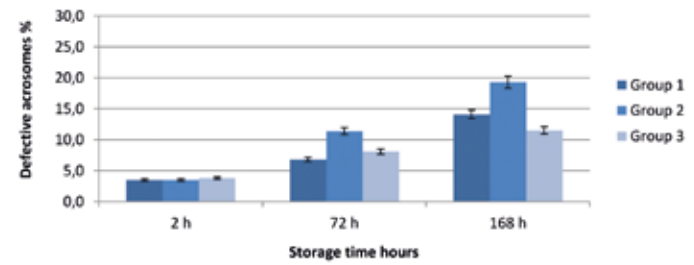
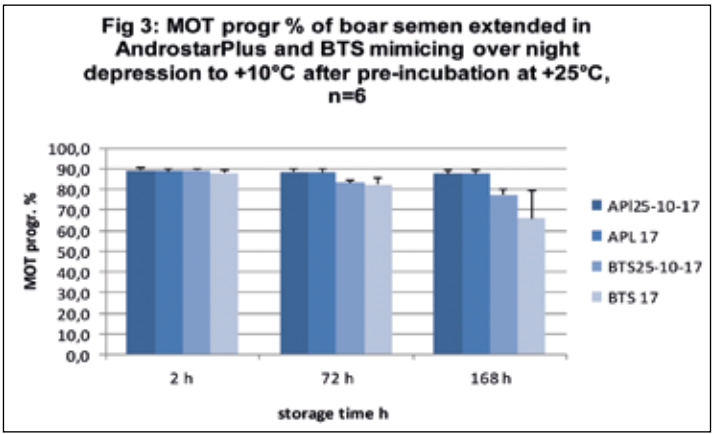


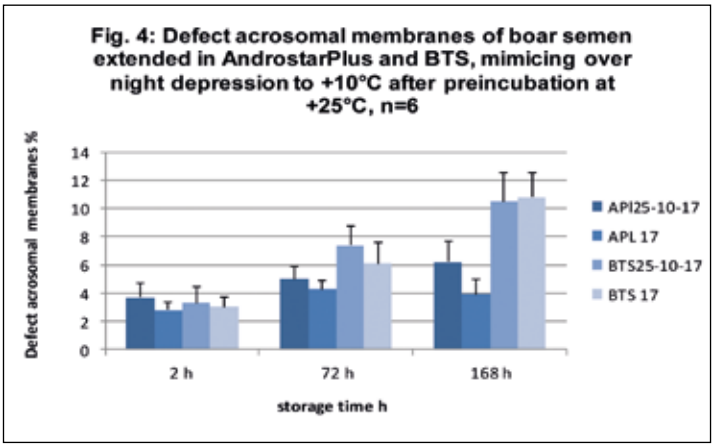
Fig. 2: Defective acrosomes of boar semen extended in Androstar Plus before over night cooling to 10°C, n=6



In experiment 2 (Fig. 3 and 4) split samples were prepared with Androstar Plus and BTS using for group 1 and 2 of each extender the same semen preparation procedure as in experiment 1 for group 1 and 3.

Fig 3: MOT progr % of boar semen extended in AndrostarPlus and BTS mimicing over night depression to +10°C after pre-incubation at +25°C, n=6

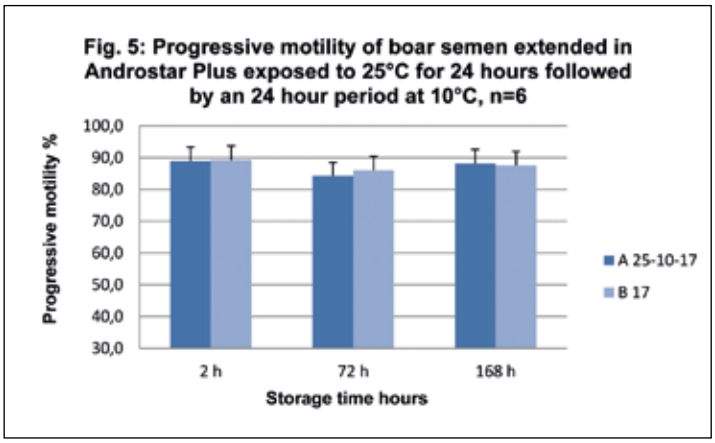


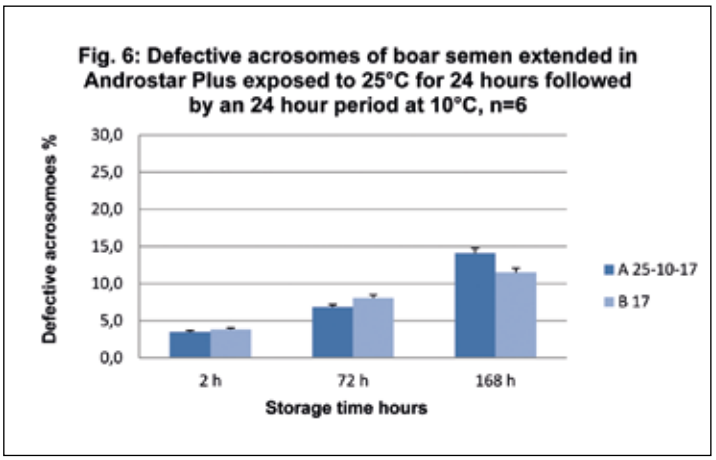


Progressive motility and percentage of intact acrosomes was significantly higher in the Androstar Plus samples compared to BTS. In the case of Androstar Plus no difference was seen between group 1 cooled after a compensatory incubation period at +25°C to +10°C and stored overnight at +10°C and the control group maintained at +17°C throughout the complete storage period.

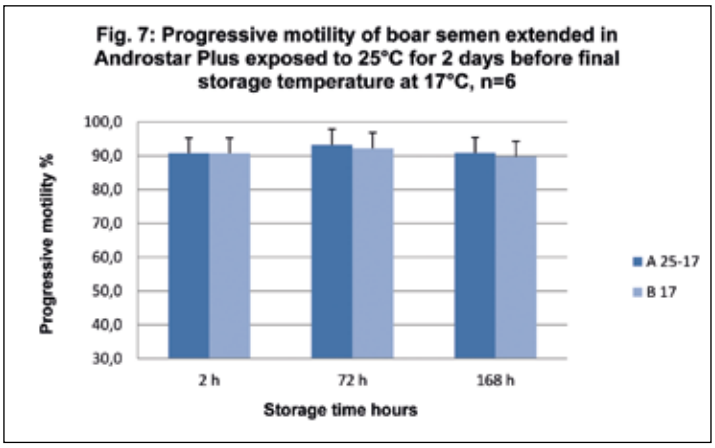
Boar semen shipping in hot climates

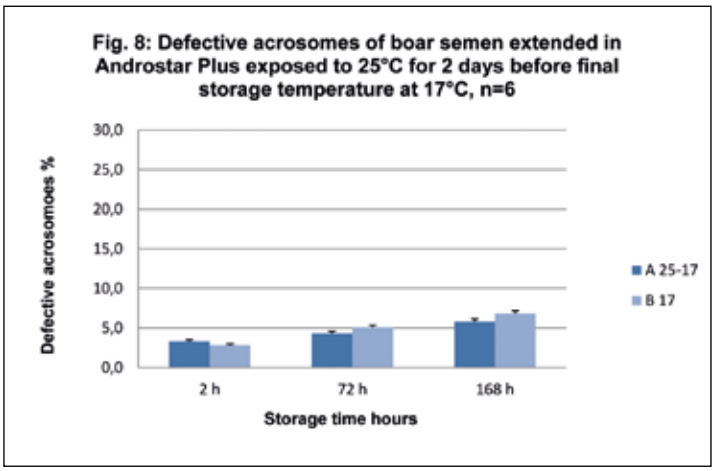
As the compensatory incubation period of 6 hours has given so good results, further experiments were conducted to investigate the effects of an extended exposure to +25°C on boar semen as it happens sometimes during shipping. In experiment 3 we repeated the procedure of experiment 2 but held the semen for 24 hours at +25°C.





Finally in experiment 4, a two day semen storage at +25°C was performed before lowering the temperature to +17°C.





As shown in Fig. 5 to 8, Androstar Plus does also an excellent job when shipping conditions require a semen exposure to +25°C for even 2 complete days.

Conclusions

The final conclusion from this set of experiments is that Androstar Plus with the CSP cold protection agent in combination with a compensatory incubation period can completely prevent the detrimental effect of cooling the semen down to +10°C as it can occur during shipping in cold climates.

Semen extended with BTS shows a clear quality decrease concerning motility and acrosome damage when exposed to +10°C, even after a compensatory incubation period.

Holding boar semen extended with Androstar Plus at +25°C for up to 48 hours gives also similar results to the standard +17°C temperature management, even if combined with a +10°C overnight cooling.

Hence, Androstar Plus opens the window from +10°C to +25°C for shipping of boar semen if the protective effect of CSP is combined with a compensatory incubation period at +25°C of at least 6 hours.

The widely used practice to cool semen as quickly as possible to the final storage temperature of +17°C is detrimental for semen quality. Androstar Plus offers a wide range of shipping and short term storage temperature, thus eliminating the need for a cold room at the boar studs and providing a safer product to the farmer.

Challenge: semen transport at high and low ambient temperatures

High ambient temperatures and strong solar irradiation in summer, as well as very low temperatures in winter are a challenge for maintaining the optimal temperature range during transport and storage of boar semen. Semen should be cooled slowly and continuously after dilution and should be stored at a stable temperature of approx. +17°C until insemination.

If the temperature of the diluted boar semen exceeds +40°C, the proteins coagulate in the sperm and the cells die. But also temperature variations in the lower range already reduce the fertilizing ability. Storage in the physiological range (+30°C to +35°C) does not damage the sperm cells but reduces the shelf life significantly.

High ambient temperatures

In summer the temperature inside vehicles left unprotected in the sun can easily reach up to +90°C. A styrofoam box is the minimal required protection for semen transport; better are air conditioned boxes or air conditioned transport vehicles, if the temperature is adjusted correctly. In the optimal case the boar semen is transferred directly from the refrigerated transport box respectively from the air conditioned vehicle of the supplier to the pre-cooled temperature controlled semen storage unit or the air conditioned box of the receiver.

However in practice the optimum cannot always be implemented and the semen quickly gets damaged by the high temperatures. Where this risk exists, the insemination centre can take precautionary measures by using boar semen extenders with a membrane protection factor. These extenders reduce the sensitivity of sperm cells towards temperature variations and to too high or too low temperatures.

The ultraviolet irradiation of the sun causes the formation of oxygen radicals in the extended boar semen. These oxygen radicals alter the lipids of the sperm membrane, which in turn reduces the fertilizing ability, because the sperms cannot be identified by the oocyte and thus the insemination fails. Furthermore the UV irradiation damages the DNA of the sperm cells and this prevents embryo growth or causes an embryonic premature death of the inseminated oocytes. For this reason contact with sun or other UV irradiation must definitely be avoided. A protection against accidental contact with sunlight is provided by the UV-protect boar semen tube, which filters the UV irradiation.

Low ambient temperatures

In winter, respectively in cold countries the risk of a cold shock is most dangerous for the sperm cells. If the temperature of the boar semen goes below a certain limit, the sperm cells suffer an alteration of the membrane fluidity (flexibility) and certain elements of the semen cells get damaged irreversibly. Due to the use of the cooling protection factor in top quality boar semen extenders, semen cells are protected to low temperature down to +5°C.

The use of insulating packaging, e.g. with styrofoam boxes is particular important. Additional thermo elements help to maintain the transported boar semen at a stable temperature. Note: Many air conditioned boxes only provide a cooling function! When transferring the semen to the receiver, cold shock must also be avoided. The direct transfer from one air conditioned box to the other is also optimal here. If boar semen must be transported between buildings in an AI centre, it is also recommended to use pre-warmed air conditioned boxes. By using extenders with cooling protection factor, the insemination centre can also in winter reduce the potential risk of very low ambient temperatures.

Very high and very low ambient temperatures are a challenge for the transport and the storage of boar semen. However it is relatively easy to face this challenge by using the appropriate equipment, such as air conditioned boxes and high quality boar semen extenders.

Transport of boar semen tubes: examples of good practice

Very high ambient temperatures: SABOR Artificial Breeding Centre, Clare SA, Australia

Foto with friendly support of Graham Reu, SABOR Artificial Breeding Centre, Clare SA, Australia

The breeding centre SABOR supplies sows farms in the surroundings with two delivery vans equipped with a fully air conditioned storage room, where a temperature range of +16°C to +18°C is maintained. Thick-walled pre-cooled styrofoam boxes are used. SABOR exclusively uses Androstar Plus semen extenders. In that way even deliveries of up to two days can be protected.

Very low ambient temperatures: Finnpig OY, Seinäjoki, Finnland

Finnpig OY cooperates with an external logistics company, which takes over the produced semen tubes early in the evening. The boar semen is already packed for the individual customers in air conditioned boxes with minimum 5 cm wall thickness. If a delivery only comprises a few tubes a heat bag is added. These heat bags are produced by Finnpig itself, in order to protect the boar semen from decrease of temperature during the transport. The logistics company delivers the boar semen overnight in an air conditioned van to distributer centres in the whole country. Also Finnpig works with the Androstar Plus extender, in order to optimally protect the boar semen from low temperatures.