


The international AI news from Minitube

# SpermNotes®



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# Modern reproduction techniques in swine breeding

The most important factor for success in animal husbandry and breeding seems to be the degree of efficiency. In animal breeding, not only optimised care and feeding but also improved genetics help to reach high efficiency. As international trade enables breeders to get animals and semen from different parts of the world, genetic improvement has never been easier. In order to take full advantage of these possibilities, modern breeding techniques and equipment have to be utilised. An overview of these techniques with a focus on the latest innovations and topics is given in this issue of the Minitube SpermNotes.

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# AndroVision® in boar semen laboratories

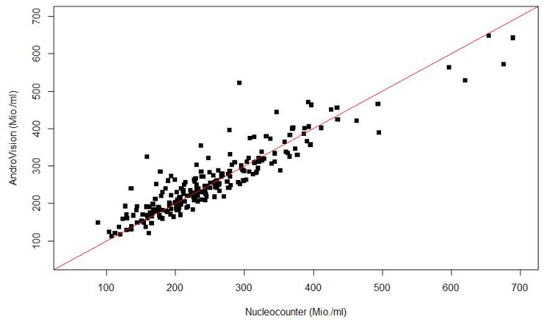
Rudolf Großfeld, Ph.D., Minitube

The correct and precise measurement of the qualitative and quantitative ejaculate parameters is prerequisite for the production of boar semen doses with correct number of viable sperm. The analysis of the quantitative semen parameters volume and concentration has been automated in professional semen laboratories by using scales, photometers and other tools. With computer assisted sperm analysis (CASA) systems like AndroVision®, the evaluation of semen motility has shifted from subjective estimation on the microscope, to an objective measurement. With the option to detect morphologically abnormal sperm with plasma droplets or bent tails, the use of CASA systems is today even more efficient.

Besides just measuring the core parameters of boar ejaculates (semen motility, concentration, abnormalities), a CASA system offers many more advantages in a boar semen laboratory. With the reduction of the human influence, boar semen production gets precise and efficient. This is due to the higher degree of standardisation by using automatic dilution pipettes, scan stages and disposable counting chambers.

## 1. Reliability of concentration measurements

The error prone semen concentration measurement can be made significantly more reliable with AndroVision®. We could show that the semen concentration measurement of AndroVision® correlates very well with a reference method (NucleoCounter, SP 100). The mathematical coefficient for concordance of measurement methods in this measurement series of 220 boar ejaculates is 0.9164 which represents an almost complete agreement. This shows that AndroVision® provides reliable semen concentration measurement.




In order to offer laboratory technicians information on their individual pipetting capabilities and to prevent mistakes, AndroVision® features a control function. The system automatically evaluates the sperm count per measurement field in the counting chamber, calculates the coefficient of variation and issues a warning message, if this value is too high. This can happen for example, if the chamber is not completely filled or if air bubbles are within the measurement fields due to pipetting errors. With this information, the technician can take action and therefore prevent incorrect measurement results.

## 2. Tools to increase speed

Further tools for rationalising the measurement of boar ejaculates are a barcode reader, a touchscreen and the direct data-transfer of measurement results to the laboratory management software. If the boar



# AndroVision® in boar semen laboratories

ID is printed as a barcode on the ejaculate container, AndroVision® can use a barcode reader to identify the semen donor in the software. This tool can be applied to raw ejaculates and to retain samples. It not only prevents errors in data recording, but reduces the number of clicks in the software significantly. After the scan of the barcode, AndroVision® opens a new analysis screen, then only the counting chamber has to be filled and by pressing one button, the measurement is started and completed. A scan-stage will automate and standardize this process further and a touch screen allows the direct input of user commands on the screen. So a mouse and keyboard are not needed to run the system. All this speeds up the work with AndroVision® and speeds up semen processing.

## 3. Data transfer and remote access

An important part of a standardised semen production which prevents mistakes and speeds up the process, is the direct and automated transfer of all measurement results from AndroVision® to the laboratory management software. This data transfer is very flexible. The data can be pushed via a RS232 connection or they can be pulled via internet protocol (IP) directly from the AndroVision® database. The latter method offers a variety of additional features. The AndroVision® database is based on structured query language (SQL), a well-defined standard for querying databases. Virtually any software that is capable of handling SQL queries can obtain semen analysis data from AndroVision®. This query can be performed remotely within an intranet. So it is possible, that a person has online access to analysis data only seconds after the analysis of an ejaculate is finished.

## 4. Comprehensive quality control

AndroVision® is also a very valuable tool for the quality control (QC) of retain samples. The QC is very important for a boar stud. Of approximately 20-25% of the produced boar ejaculates retain samples should be kept and evaluated after two or three days of storage, or on the use-by-date of the semen tube which can be after seven days. In AndroVision® the analysis data of the native ejaculate and the QC are connected in the database. This allows the analysis of the stability of motility over several days, and in turn allows for the selection of boars with higher semen motility after several days of storage and therefore a higher prospective fertility.

QC of the produced semen tubes is an important self-monitoring tool for a boar stud. Besides checking semen motility, also the final sperm content in the semen tube should be monitored. A proof that those two parameters fulfil minimum requirements is very useful in customer service of a boar stud, as AndroVision® allows issuing ejaculate certificates which can be provided to end-users of the semen doses. It is very easy to retrieve the semen quality history of individual boars in the AndroVision® reporting module. This information makes selection decisions on boars easier.

## 5. Advanced analysis options in AndroVision®

The advanced analysis options in AndroVision® add a further toolset. The acrosome evaluation of QC samples enables the boar stud to closely monitor the temperature management of semen, as acrosomes are sensitive to temperature fluctuations. Also, monitoring the acrosome status of a subset of produced semen samples is a tool to detect possible mycotoxin contents in the boar feed at a very early stage.[1]

The DNA module of AndroVision® is useful for checking the DNA integrity in sperm of young boars, i.e. as an entry test. This test in young boars can prevent low fertility results after AI, in case the young animal has an impaired DNA integrity. The DNA integrity test is also useful for aging boars and boars that show a low fertility albeit normal semen quality.

With the fluorescence options of AndroVision®, relevant additional sperm functional parameters can be examined in a boar stud without the need to invest in a costly flow cytometer.


## 6. Review functions

AndroVision® includes an extensive review function of all obtained data. All analysis data, pictures and videos are stored and can be reviewed at any time. The level of detail extends to individual sperm data. These data are also available via SQL query over a network connection.

In summary, AndroVision® is a management tool for a boar stud with capabilities that go far beyond the basic semen analysis. Extracting data from AndroVision® for further use is very flexible and very easy. The QC sample analysis and fluorescent options are valuable tools to monitor the performance of individual boars and of the whole stud.

*References: [1]Tsakmakidis, I.A.; Lymberopoulos, A.G.; Alexopoulos, C.; Boscos, C.M.; Kyriakis, S.C. In vitro effect of zearalenone and α-zearalenol on boar sperm characteristics and acrosome reaction. Reprod. Dom. Anim. 2006, 41, 394–401.*

# Effect of management on semen quality during long-term storage in boar studs

M. Schulze, C. Kuster, J. Schäfer, M. Jung, R. Großfeld

The processing of ejaculates is a fundamental step for the fertilizing capacity of boar spermatozoa. The aim of the present study was to identify factors that affect quality of boar semen doses. The production process during 1 day of semen processing in 26 European boar studs was monitored. In each boar stud, nine to 19 randomly selected ejaculates from 372 Pietrain boars were analyzed for sperm motility, acrosome and plasma membrane integrity, mitochondrial activity and thermo-resistance (TRT). Each ejaculate was monitored for production time and temperature for each step in semen processing using the special programmed software SEQU (version 1.7, Minitüb, Tiefenbach, Germany). The dilution of ejaculates with a short-term extender was completed in one step in 10 AI centers (n = 135 ejaculates), in two steps in 11 AI centers (n = 158 ejaculates) and in three steps in five AI centers (n = 79 ejaculates). Results indicated there was a greater semen quality with one-step isothermal dilution compared with the multi-step dilution of AI semen doses (total motility TRT d7: 71.1 ± 19.2%, 64.6 ± 20.0%, 47.1 ± 27.1%; one-step compared with two-step compared with the three-step dilution; $P < .05$). There was a marked advantage when using the one-step isothermal dilution regarding time management, preservation suitability, stability and stress resistance. One-step dilution caused significant lower holding times of raw ejaculates and reduced the possible risk of making mistakes due to a lower number of processing steps. These results lead to refined recommendations for boar semen processing.

**Conclusions**

- Production time and temperature management have an impact on semen quality.
- One-step dilution reduces the time until final dilution.
- One-step dilution processes are less susceptible to production errors.
- Science-based quality control mechanisms facilitate progress in semen production.

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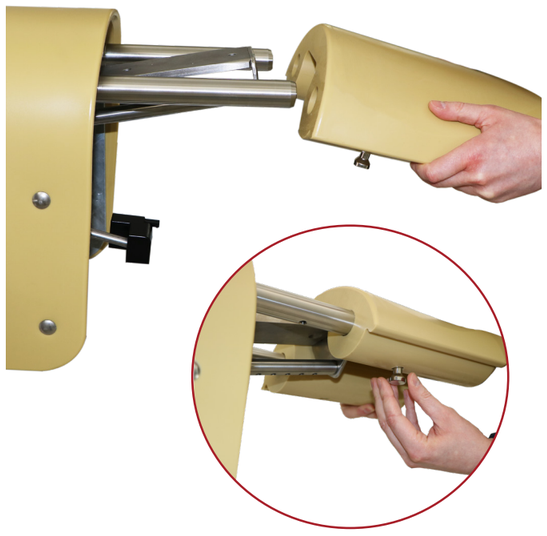
# BoarMatic: Hygienic and efficient boar semen collection

The aim of boar semen collection is to obtain an ejaculate with the highest possible concentration and sperm quality. Also, the semen collection process must be efficient and as time saving as possible.

With the BoarMatic, Minitube has developed an efficient, improved technique of boar semen collection. The BoarMatic boar dummy is provided with an integrated mechanism for automatic semen collection. In combination with the Blue Bag, boar semen can be collected efficiently under optimal hygienic conditions.

The mounting part of the BoarMatic dummy is now easily exchangeable. It snaps in place by itself and is secured with a spring-type screw. The exchangeable mounting part can now be replaced several times during collection hours, in order to maintain good hygiene and a low bacteria load on the dummy. When the mounting part gets dirty and contaminated, it can now be replaced within seconds. Mounting parts and dummy are hosed down at the end of the operation, dried and eventually disinfected. The removable mounting part simplifies the cleaning procedure, as the inner part of the dummy can be accessed much easier without the mounting part in place. The dummy design with exchangeable mounting part ensures maximum hygiene conditions during collection.



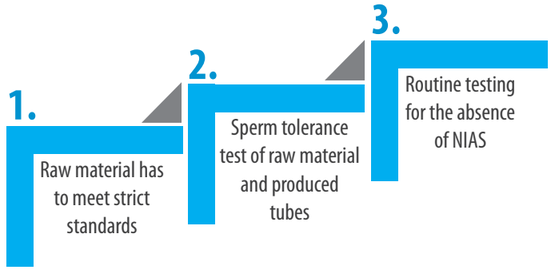


# Quality standard of Minitube's boar semen tubes

Dr. Monika Esch, Minitube

Pig production in the 21st century relies heavily on artificial insemination to ensure efficient breeding programs, fast genetic progress and high biosecurity standards. The benefits of A.I. are based upon the highly increased availability of superior genetics through the number of semen doses produced from one ejaculate, the storage time, and the improved logistics enabling shipment of semen conveniently to any place these genetics are needed.

Materials used to manufacture boar semen tubes must be tested and proven to be biologically inactive and sperm friendly. Minitube as a specialised and certified producer of boar semen tubes has a rigorous QC system in place, consisting of a three level concept for the quality assurance and testing of raw materials and final products.




## 1. Raw material standards

Each batch of the raw material Low Density Polyethylene (LDPE) assigned to the production of boar semen tubes at Minitube's manufacturing plant is accompanied by a batch specific certificate.

Pure LDPE is by definition free from any toxic ingredients like Bisphenol A, heavy metals, phatalates or adipates.

The raw material used in the production of boar semen tubes meets a broad scope of EU and other standards, regulations and directives. A detailed list is available (Minitube Technical Report on Quality Standard of Minitube's Boar Semen Tubes, www.minitube.com).

## 2. Sperm tolerance

Quality assurance tests are regularly performed at the Unit of Reproductive Medicine of the University of Veterinary Medicine Hannover Foundation, which is a DAkkS accredited GLP laboratory in accordance with the international ISO/IEC 17025 norm. Any new raw material has to pass the tests before it is released to enter the boar semen tube production chain.

For these tests, boar semen of at least 3 different boars is extended with Androstar Plus, divided into split samples and incubated in the boar semen tubes to be tested and in glass vials as a control. Semen is evaluated over a semen storage period of at least 6 days. All split samples are stored in a semen storage unit at 17° C. The following tests are performed:

- Motility: total and progressive (CASA) after 24 and 144 h
- Acrosome integrity (wet mount, phase contrast microscopy 1000x) after 24 and 144 h
- Membrane integrity (flow cytometry, FITC/PNA-PI) after 24 and 144 h
- Thermoresistance test after 144 h storage at 17°C (incubation at +38°C for 120 min), then analysis of progressive motility (CASA)
- DNA Integrity / SCSA (flow cytometry; acridine orange)
- Mitochondria membrane potential (flow cytometry; propidium iodide / JC-1) after 24 h and 144 h
- Membrane fluidity (flow cytometry, Yo Pro 1/ Merocyanin (M540)) after 24 and 144 h

## 3. Tests for the absence of non-intentionally added substances

NIAS are substances which are non-intentionally added to the raw material during production or transport and are potentially harmful. Though, substances like heavy metals, endocrine disruptors and plasticizers like Bisphenol A, phthalates and adipates must not be present in the material as per its formulation and certification, they can cause adverse biological effects in case they are added non-intentionally. Most importantly in the context of boar semen preservation, these substances can cause damage to the sperm during storage and hamper fertility and productivity of the sows inseminated.

Therefore in the third level of the Minitube quality assurance program, the absence of NIAS is controlled with routine testing of the raw materials for endocrine disruptors and plasticizers like Bisphenol A, phthalates and adipates as well as heavy metals.

It is a matter of principle for Minitube as a manufacturer of semen packaging material to take full responsibility of only using substances and raw materials which are not harmful to animals or sperm. This is only possible when the full production line is in control of the manufacturer, as is the case with Minitube's production of boar semen tubes in its own specialised production facility.

*Tube production facility at Minitube*

# Androstar® Premium – Long term extender with superior semen protection capabilities

Rudolf Großfeld, Ph.D., Minitube

Minitube's Androstar® Premium is a boar semen extender with outstanding preservation properties designed for long term storage. Androstar® Premium is completely free of animal protein. The extender supports diluted boar semen at standard storage temperatures of +17°C and also at lower temperatures of even +5°C only.

The ability of Androstar® Premium to protect boar semen when temperature fluctuations occur during semen transport in cold environment is especially beneficial in winter time. In addition, a storage temperature of +5°C prevents bacteria, which may contaminate the semen, from further growth. Low temperature storage is therefore an option, when resistant bacteria are present in a boar stud and semen cannot be stored at +17°C without risking bacterial growth. In such a situation, as an emergency action, boar semen can be produced with Androstar® Premium and stored at +5°C until use.

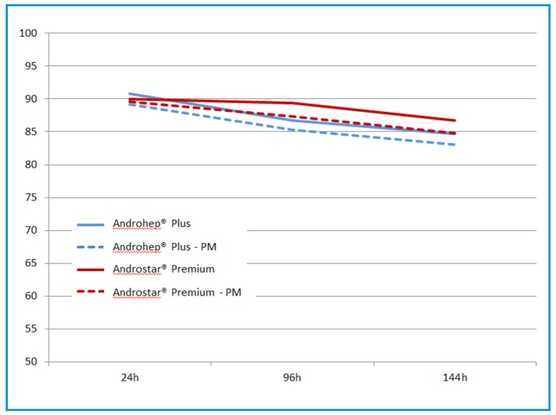
Androstar® Premium provides several further advantages. As the semen extender does not contain any animal protein, foam building during semen handling does virtually not occur. The freedom from animal protein also makes exporting and importing diluted semen to many countries much easier or even possible at all. Also occasional immune reactions in certain sow genetics after AI do not occur.

The complex synthetic formula of Androstar® Premium provides outstanding preservation and protection capabilities as comparisons with Androhep® Plus semen extender have proved. In a split sample trial, both extenders preserved a very high level of sperm quality. Progressive sperm motility did not differ significantly between Androstar® Premium and Androhep® Plus over 6 days of storage at +17°C (see Graph 1).

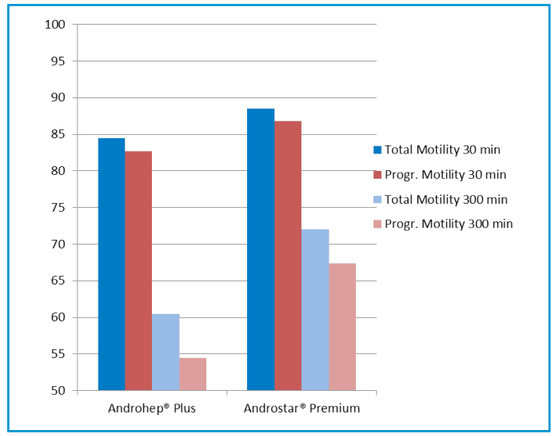
After 9 days of storage at +17°C, the semen samples were submitted to a thermo-resistance test for 5 hours at 38°C, which is today considered the maximum possible sperm stress test. The progressive sperm motility after the stress test was still 67.3% in Androstar® Premium semen, and 54.5% in Androhep® Plus (Graph 2).

The sperm membrane and acrosome integrity also remained at high values after 9 days of storage (Androstar® Premium: 83.2 %; Androhep® Plus: 85.9% intact sperm and acrosome membranes, Graph 3).

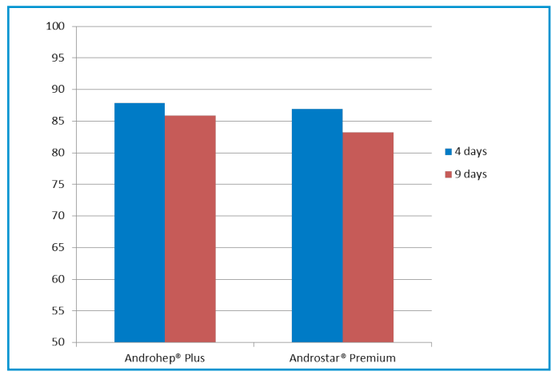
These numbers show that Androstar® Premium is a first class synthetic long term boar semen extender that protects diluted boar semen under normal and stressful conditions. The conservation capabilities compare or are superior to Androhep® Plus.




*Graph 1: Total and progressive (PM) sperm motility during storage at +17°C for up to 6 days (n=6)*




*Graph 2: After 9 days of storage: Motility after thermo-resistance test of 30 min and 300 min at 38°C (n=6)*




*Graph 3: Plasma membrane and acrosome integrity after 4 and 9 days storage (n=6)*



# Successful re-certification: Audit confirms GMP compliance of Minitube's extender production facility

With an intensive audit performed by an external GMP (Good Manufacturing Practice) auditor, Minitube's extender production facility has recently been re-confirmed to fulfill all norms of GMP production.

GMP is "Good Manufacturing Practice" and means that the production methods have to meet strict regulations and guidelines of considerably higher standards as compared to DIN ISO norms. Manufacturing procedures must be exactly described and documented at every level of operation. Seamless traceability of all materials and operating processes has to be assured. Quality control as an integral part of GMP production has to be effective, exception free and fully documented.

Minitube's extender production facility has been designed with the know-how of GMP experts and it provides automation of the production procedures to a maximum degree. Raw material flow during manufacturing is handled in a completely closed system for maximum safety and quality. The production software has been especially developed for Minitube to ensure complete control and documentation for weighing and mixing the extender components.

The whole manufacturing site is supplied with dehumidified and HEPA filtered air from a powerful air conditioning system to ensure perfect conditions for manufacturing and staff.

Diverse production lines for powdered and liquid extender provide automatic packaging in different volumes such as 1, 5, 10, 20, 50 and 100 liter extender for the powdered products. A specially designed robot packages bags into boxes fully automatically.

Quality control comprises analysis of all raw material batches and of all batches of ready-made products. Raw materials are exclusively pharmacopoeia grade and sourced from GMP manufacturers only. They have to undergo tests of sperm tolerance of the relevant species in an incubation trial and physical-chemical tests, before they are released for production. QC of produced batches comprises CASA sperm motility analysis, flowcytometric analysis for acrosome and plasma membrane integrity and microscopic evaluation of acrosomes in external testing labs. Minitube's fully automatic production lines, GMP approved facility and procedures and extensive, consistent quality control without any gaps represent the benchmark of highest quality standard for semen extenders in the industry.

# Electronic donor identification with iMale

**Safe and easy electronic reading of animal ear tags and direct printing of barcode labels**

The wireless combination of iMale RFID-Reader and iMale Label Printer allows the reading of animal ear tags and the direct printing of barcode labels with the animal ID in the semen collection area.

The printed barcode label can be stuck on the semen collection bag/cup and can then be used to securely identify and process ejaculates in the laboratory i.e. with IDEE/IDA. As soon as the barcode label is read with the barcode reader in the laboratory, a new ejaculate file of the corresponding semen donor is automatically opened in IDEE/IDA and this prevents mistakes in data entry and assures correct ejaculate and semen dose identification.

The iMale RFID-Reader has a reading distance of 30 cm which adds up to the device length of 65 cm. All HDX and FDX-B transponder ear tags can be read. RFID-Reader and Label Printer are paired via Bluetooth. The maximum distance for transferring the data wireless from the iMale Reader to the Label Printer is about 8 meter.

**Your benefits:**

- Fast and easy printing of RFID ear tag number on barcode label
- Wireless data transfer to printer; no chaos with cables
- No extra software needed to print labels
- Safe reading of ear tags due to long reading distance of the reader
- Robust and battery operated devices can both be carried by one person
- iMale RFID-Reader is robust and water proof (IP 67)
- iMale RFID-Reader comes with built-in quick-charger and external power supply

# AI training model "Suzy" – A new approach to artificial insemination training

"Suzy" is an artificial sow for training AI technicians. It provides a most realistic gynaecological tract. Artificial insemination can be practised in a safe and convenient environment, before AI trainees work with real animals.

The artificial sow consists of a plastic body and silicone internals. The silicone gynaecological tract (GT) can be removed to simplify training in the initial stages. "Suzy's" GT features a cervix, similar to the natural one, so that the lock of the AI catheter in the cervix can be practised. A sound will confirm success when the introduced catheter reaches the correct position in the cervix. Furthermore, the prevention of introducing the AI catheter in the urethra can be trained, as "Suzy's" GT has an opening at the appropriate position to mimic the urethra opening. Inseminating "Suzy" with a "real" dose containing water is also possible as the GT has a drain.

The artificial sow provides flexibility around timing and location of AI training courses, and also lowers the risk of injury to animals by unexperienced trainees. Additionally, training units can be repeated as often as necessary. All these benefits help to provide optimal training and ultimately an improved performance of applicants of AI in sows.

## Product features

- Realistic sow model, gynaecological tract and cervix
- Confirmation sound when the AI catheter has been introduced correctly
- Prevention of introduction of AI catheter in urethra
- Training of actual insemination process
- Training model is compact and light-weight (e.g. it fits in the boot of a station wagon or similar car size)

*Demonstration of correct catheter introduction on removed GT in order to prevent mistaken introduction of AI catheter in urethra*

# The SmartDispenser: Automated semen dilution

The SmartDispenser is a combination of pump, balance and control unit. It allows accurate dosage of boar semen extender into each ejaculate in an easy way. The SmartDispenser control unit activates the peristaltic pump until the target weight is reached according to the reading of the balance.

Due to the precise weighing of extender, the SmartDispenser is superior to the use of a peristaltic pump with rotation control, because diverse weaknesses are eliminated:

- No more dosage inaccuracy due to different tubing materials and diameters, different wall thicknesses, restoring forces and ageing of the tubing
- Aspirated air does not influence the system
- Easy calibration on the balance
- Errors caused by different filling levels in the vat can not occur

All those benefits make the SmartDispenser, available in an L and XL-version, a widely used tool in boar stations worldwide.


*Automated semen dilution with SmartDispenser L*