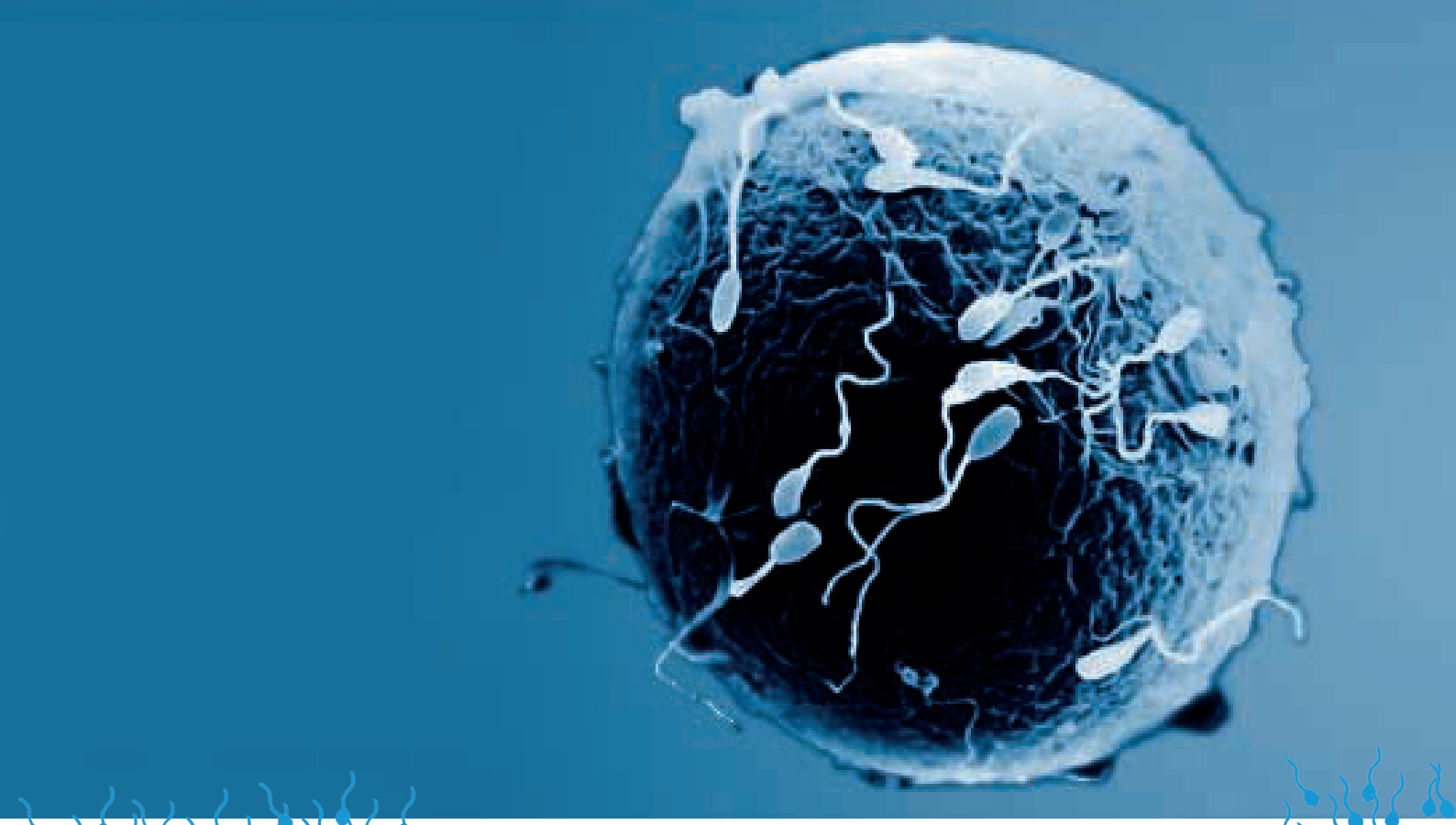


The international AI news from Minitube

SpermNotes®

www.minitube.com

minitube

Superior CASA technology: the secret behind highly precise measurements with AndroVision®

The new generation of CASA Systems uses high tech equipment to gain a maximum of precision in a minimum of time. Minitube provides the AndroVision® system with a high speed camera equipped with a high resolution USB 3.0 interface and the latest IMX sensor generation. This sensor is four times faster compared to standard camera sensors of the same resolution and works with a frame rate of up to 75 fps.

It provides AndroVision® with a very large analysis area per field and therefore allows to evaluate up to 1000 sperm per field. This also increases the precision: a high sample size increases the predictive power. The CASA analysis of a semen sample with AndroVision® is thus extremely quick, reliable and precise as you can read in the following article about AndroVision® in animal semen laboratories on page 5.

The AndroVision® USB 3.0 camera also stands out because of the small pixel size of 3.45 x 3.45 µm combined with a high resolution of 2048 x 2048 pixels. This feature makes the sperm recognition highly precise: a great advantage for the morphological sperm analysis, because it allows the morphological characteristics such as proximal or distal plasma droplets, to be displayed and therefore analyzed with very high precision. A topic that will be further explored in the article on page 8 about the precision of the AutoMorph analysis.

The technology behind AndroVision® also enables high throughput and fast custom analysis; This also contributes to the outstanding precision, because the residence time of the sperm in the measuring chamber is short.

These features predestine AndroVision® for semen analysis in the professional semen production laboratory like no other system.

Imprint

Minitüb GmbH
Hauptstr. 41
84184 Tiefenbach
Germany
Phone: +49 8709 9229 0
E-mail: minitube@minitube.de
Managing Director: Dr. Christian Simmet
Registration: HRB 4129 Register Court Landshut
VAT-ID-Nr.: DE 182191255

minitube

AndroVision®: one system, numerous possibilities

AndroVision® is a highly precise CASA system for standardized, interactive semen analysis. AndroVision® not only provides classical analyses of motility, concentration and morphology, but also various fluorescence based assessments of sperm functionality. The basic system with PC and accessories is complemented by optional software modules.

AutoMorph is a module for the automated recognition of proximal and distal plasma droplets as well as bent tails of porcine and bovine semen. Plasma droplets are symptoms of a defective maturation of sperm which can be attributed to various causes such as stress or disease. AutoMorph is integrated in the analysis of motility and concentration. The semen sample should be diluted in clear extender.

The Module **Dose Calculation** automatically calculates the number of doses which can be prepared from an ejaculate and the volume of extender to add to the ejaculate.

The Module **Lab Software Link** allows the link of AndroVision® to external software and external devices, like scales, photometer, scan stage. IDA, IDEE and PRISM can be integrated with AndroVision® to a complete lab setup. The **Quality Control Module** analyses samples post thaw and in holding and also allows the link to a native ejaculate analysis. It offers the possibility to analyze samples during production: Samples of one ejaculate can be analyzed more than once and can be compared with the native ejaculate values. This function allows also checking the quality of already processed ejaculates. To analyze a holding sample or thawed sample the donor is selected out of a list showing processed ejaculates for which no control analysis was performed so far. Multiple analyses within the production chain allow for tracking the ejaculate quality during several steps in production.

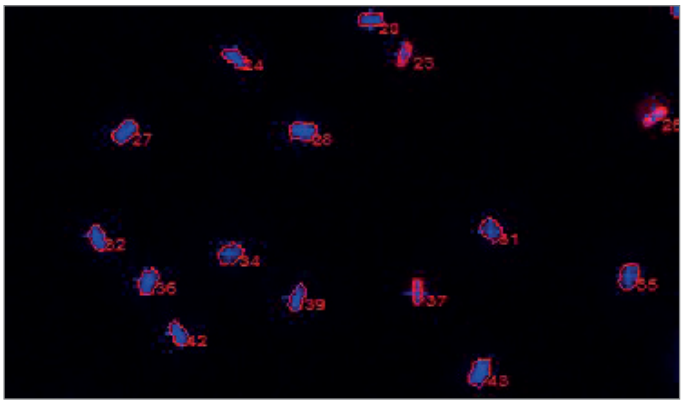
Ready-made semen doses which were brought in from outside or belong to older stock, and need to be quality checked are easily analyzed with the quality control module. A user interface for adding the donor to the data base is provided.

Morphology and Morphometry

The Interactive system for analysis of sperm morphology and morphometry identifies sperm of stained and unstained samples and measures length and width of the sperm head, head shape and mid-piece asymmetry of each single sperm cell (acc. to Krueger). Results can be classified into a large range of morphologic abnormalities.

Viability – why analyze?

The plasma membrane encases completely the sperm. One of its main functions is that of a protection and the selection of molecules to pass from the outside to the inside. A defect of the plasma membrane can easily lead to the death of the sperm. The viability analysis is mainly used for the quality control of holding samples of fresh semen or thawed samples of frozen semen.



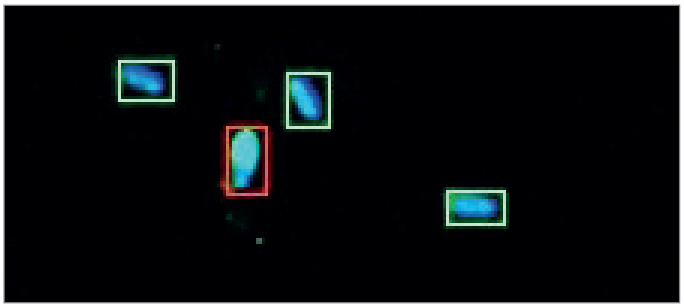
Automatic detection of sperm with damaged plasma membrane (marked red/violet) by means of a double fluorescence staining: H33342/PI

A viability test can help to detect fertility problems of individual donor animals or to detect ejaculates of poor quality before the semen delivery, rather than to be faced later with fertility deficiency. The **Viability Module** offers an automatic count of the percentage of membrane intact sperm, based on a double fluorescence assay.

For the assay of the plasma membrane integrity, a double fluorescence stain with Hoechst 33342/PI or SYBR14/PI is used. The stain Hoechst 33342 permeates cell membranes and binds specifically to the DNA. All sperm are marked blue. The PI stain (Propidium Iodide) only permeates damaged membranes. It overrides the blue Hoechst stain. Sperm with damaged membranes are marked red/violet. On this basis, AndroVision® determines the percentage of sperm with damaged and intact membranes. When the dye SYBR14/PI is used, first all sperm are marked green. In addition, sperm with damaged membranes are marked red by the permeating PI dye.

Acrosome Integrity – why analyze?

The acrosome reaction is a key step for a successful fertilization. It enables the sperm to penetrate the ovum. Prerequisite: an intact plasma and acrosome membrane. Various stressors during semen processing can



Automatic detection of sperm with defective acrosome (outlined in red) by means of double fluorescence staining: H33342/FITC-PNA

AndroVision® - One system, numerous possibilities

cause damage to the acrosome membrane or can provoke a premature acrosome reaction. A successful fertilization is then no longer possible. The **Acrosome Integrity Module** automatically counts the percentage of sperm with damaged acrosome, based on a double stain fluorescence assay.

For the assay of the acrosome integrity, a double fluorescence staining with H33342/FITC-PNA is used. All sperm are marked blue (H33342). Damaged acrosomes of these cells are marked green (FITC-PNA). On this basis, AndroVision® determines the percentage of sperm with damaged and intact acrosomes.

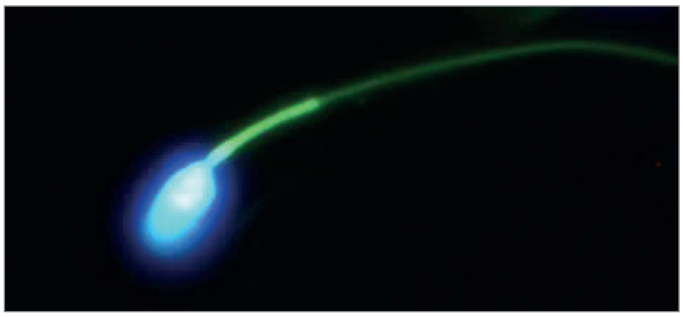
Mitochondrial Activity – why analyze?

The analysis of the mitochondrial activity is a test for the assessment of the energy metabolism of the sperm.

The mitochondrial activity is among other things necessary for:

- Maintenance of the motility
- Capacitation ability of the sperm
- Maintenance of the basic cell functions

The **Mitochondrial Activity Module** offers automated count of percentage of sperm with active Mitochondria, based on a double stain fluorescence assay.



Sperm with high mitochondrial activity

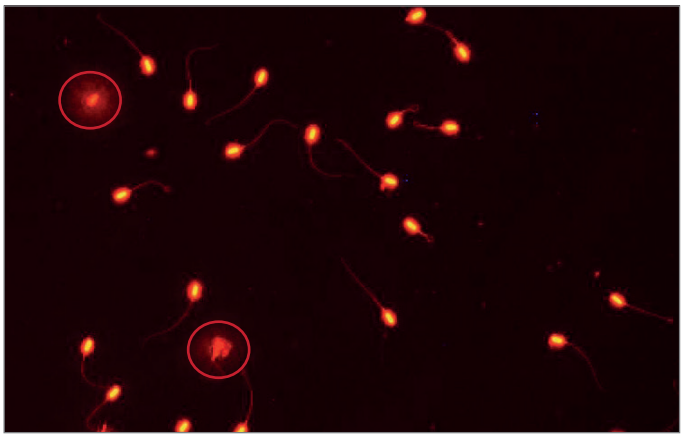
For the evaluation of the mitochondrial activity, a double fluorescence staining with H33342/Rhodamin123 is used. All sperm are marked blue (H33342). In addition, the mid-piece of the sperm with active mitochondria is marked green (Rhodamin123). On this basis, AndroVision® determines the percentage of sperm with high mitochondrial activity.

DNA Integrity – why analyze?

The success of insemination and embryo development is highly dependent on the integrity of the DNA in the sperm.

Consequently, the DNA structure can be used to indicate the fertility potential, or to explain sub-fertility, of a certain breeding animal. DNA integrity testing therefore offers a new approach to the clarification of lower fertility rates.

The **Module DNA Integrity** offers the automated count of the percentage of sperm with fragmented DNA based on a special dispersion technique combined with fluorescent staining.



Sperm with fragmented DNA exhibiting halo-effect

This technique detects sperms cells with defective DNA: If the DNA of a sperm cell is fragmented, an aura of light, or halo, will form around the head of the sperm cell. In contrast, all sperm cells that do not exhibit a halo have intact DNA. The halo effect is visible when the sample has been stained with propidium iodide (PI) and exposed to fluorescent light. The percentage of sperm cells with fragmented DNA in a given ejaculate or sample can thus be determined.

Download the leaflet

https://www.minitube.com/storage/web/source/en/pdf/androvision-more-than-casa/pdf_12500-0000_Leaflet-AndroVision_en_191204.pdf

AndroVision® in animal semen laboratories

Rudolf Großfeld, Ph.D., Minitube

The correct and precise measurement of the qualitative and quantitative ejaculate parameters are the prerequisite for the production of semen doses with a 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 in studs has been shifted from subjective estimation on the microscope, to an objective measurement with the CASA. With the option to detect morphologically abnormal sperm with plasma droplets or bent tails in porcine ejaculates, the use of CASA systems is today more efficient.

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

1. Reliability of concentration measurements

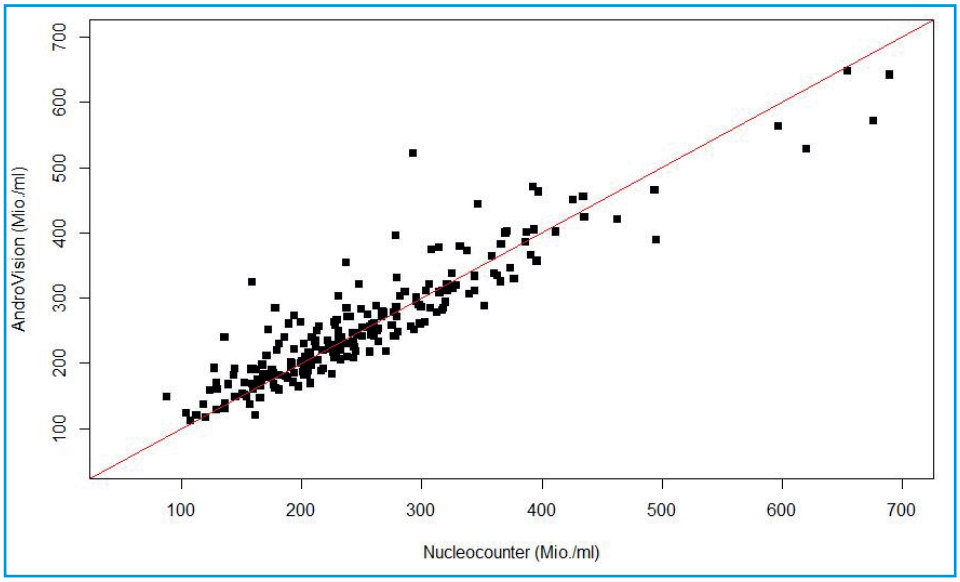
The error prone semen concentration measurement can be made significantly more reliable with AndroVision®. We could show that the semen concentration measurement correlates very well with a reference method (NucleoCounter, SP 100) as the following graph shows.

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® allows 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 rationalizing the measurement of ejaculates are a barcode reader, a touchscreen and the direct data-transfer of measurement results to the laboratory management software. If the male 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 ScanStage 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.



AndroVision® in animal semen laboratories

3. Data transfer and remote access

An important part of a standardized 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 and post-thaw samples. The QC is very important for a stud. 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, after cooling or freezing and thawing. This allows the selection of males with higher semen motility after processing and storage and therefore a higher prospective fertility.

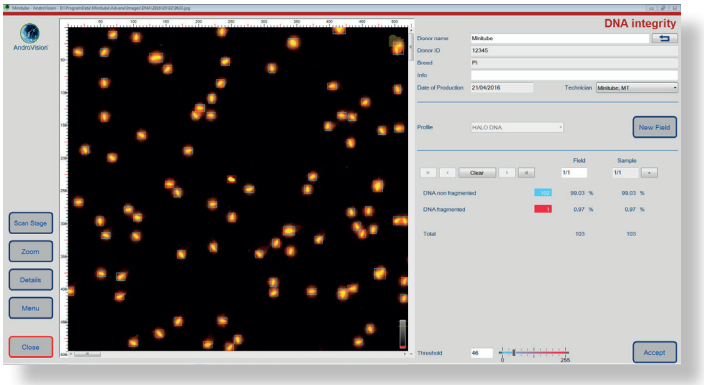
QC of the produced semen doses is an important self-monitoring tool for the studs. Besides checking semen motility, also the final sperm content in the semen dose should be monitored. A proof that those two parameters fulfill minimum requirements is very useful in customer service of a stud, as AndroVision® 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 donors in the AndroVision® reporting module. This information makes selection decisions on donors easier.

5. Advanced analysis options in AndroVision®

The advanced analysis options in AndroVision® add a further toolset. The acrosome integrity evaluation of QC samples enables the 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 feed at a very early stage[1] .

AndroVision® in animal semen laboratories

The DNA module of AndroVision® is useful for checking the DNA integrity in sperm of young males, i.e. as an entry test. This test in young males 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 males and males that show a low fertility albeit normal semen quality. With the fluorescence options of AndroVision®, relevant sperm functional parameters can be examined in a 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 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.

[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.

User Reference:

For the production of over 4 million semen tubes sold annually, the demands for top quality standards are equally high from all sides. With the introduction of computer-assisted semen analysis for ejaculate assessment, the GFS has taken a major step forward in the further development of semen laboratory testing methods, which has in turn enabled a number of other important innovations.

After intensive testing of several different CASA systems, the GFS opted for the AndroVision® system from Minitube. Following numerous comparative tests of GFS semen at the reference laboratories TiHo Hannover and IFN Schönow, the conversion to the AndroVision® CASA system in the GFS sperm laboratories was completed during the course of 2018.

As a result of the unbiased sperm screening, a larger random sampling, and the exact parallel measurement of sperm concentration and motility together with the proportion of sperm with malformations, it is possible to determine an exact number of quality sperm in the ejaculate.

GFS customers are thereby assured of receiving an equal number of fertile sperm in every insemination unit in accordance with the new and more precise BRS quality standard. More offspring from the best tested boars translates into higher profitability for the end customer.

Accuracy of AndroVision® AutoMorph measurement

Rudolf Großfeld, Ph.D., Minitube

State-of-the-art CASA systems, like AndroVision®, can measure semen motility and concentration very precisely. This is important for semen production in professional laboratories, where these values are the basis for the calculation of the number of semen doses that can be produced from a given ejaculate. Beside the use for dose calculation, semen motility serves as a quality criteria allowing an assumption about the estimated fertility of the semen. Therefore, every laboratory usually uses minimum requirements for total and/or progressive sperm motility to decide, whether an ejaculate is approved or rejected for further production.

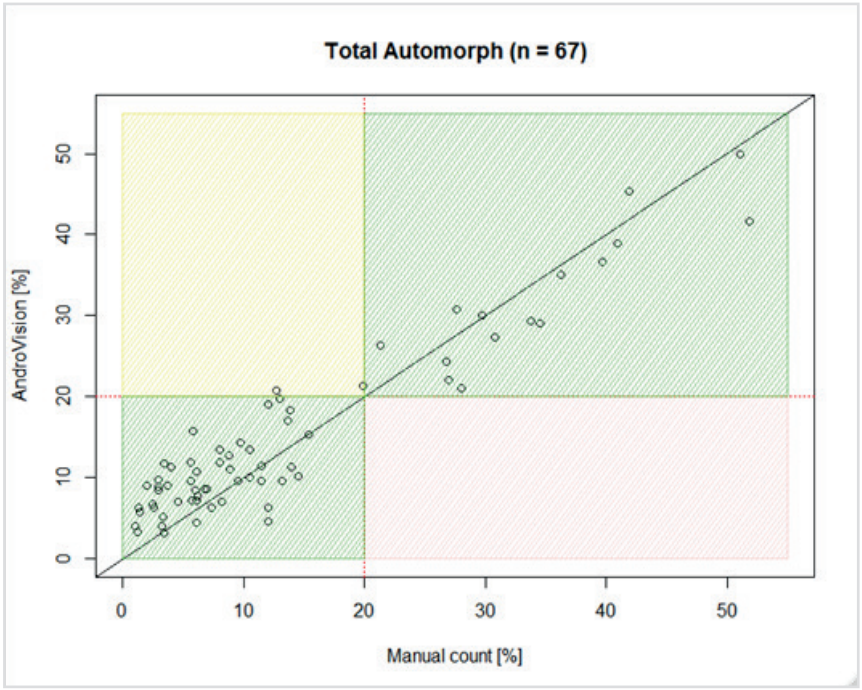
But sperm motility is not the only semen quality criteria that is used in semen production. Sperm morphology is as important for high fertility[1] after insemination of the produced dose. A high quality sperm dose needs both, high sperm motility and intact sperm morphology.

AndroVision® is capable of evaluating sperm morphology in parallel to semen motility and concentration. This function, called AutoMorph, will count sperm cells with proximal and distal droplets and sperm cells with bent tails.

Compared with the so far, more or less, rough estimation of plasma droplets and bent tails from the computer screen by the technician, the automatic count of sperm abnormalities is a major breakthrough. With this function, the evaluation of the proximal and distal plasma droplets and the bent tails shifts from subjective estimation to objective measurement. Up to the availability of the automatic AutoMorph measurement, the lab technician did have to evaluate the number of sperm cells with plasma droplets and bent tails on the screen, often under time pressure during production. Under such circumstances, it is unlikely a lab technician can precisely determine the percentage of abnormal sperm cells at any time. Consequently, approval or rejection of an ejaculate for further production relies on a less fact-based decision. This method therefore leaves a risk, that either ejaculates with too high percentages of abnormal sperm are approved for production or that ejaculates which are OK, get discarded.

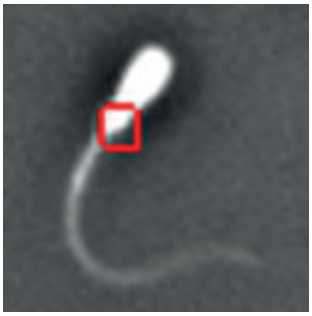
AndroVision® with the AutoMorph function takes a lot of responsibility from the lab technician while simultaneously increasing the level of measurement standardization.

However, it has to be assured that AndroVision® AutoMorph produces reliable measurement results. This can be tested in a process called method validation. AndroVision® AutoMorph was validated on a set of videos that have been recorded from semen samples with varying percentages of sperm with plasma droplets and/or bent tails. These videos were individually and manually evaluated on a PC screen and the number of malformed sperm were systematically counted and recorded by experienced lab technicians. Subsequently the same videos were analyzed with AndroVision® AutoMorph for the number of malformed sperm as well. The following graph shows the result of such a comparison.

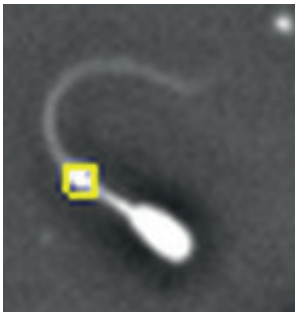


For this evaluation, 67 videos of sperm samples were evaluated for plasma droplets (proximal, distal) and bent tails in two ways, manually and with AndroVision® AutoMorph. The diagonal line in the graph is the line of perfect agreement. Points on this line resulted in equal results of percentage of abnormal sperm, counted either manually on the screen or by AndroVision® AutoMorph. The closer the data points are distributed around the diagonal line, the better the method is in agreement of the manual count and AndroVision® AutoMorph.

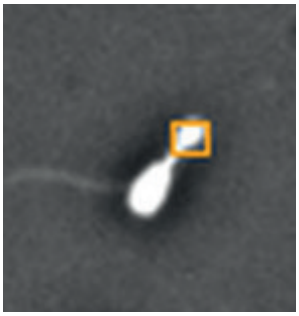
There are also mathematical coefficients to describe the method agreement. One is the Concordant Correlation Coefficient (CCC)[2].



Proximal Plasma Droplet



Distal Plasma Droplet



Bent Tail

Accuracy of AndroVision® AutoMorph measurement

The CCC for the above test series is 0.92. The closer this value is to 1, the better is the method agreement. In the statistical literature[3], a CCC of 0.92 is described as "nearly complete agreement".

Another way of evaluating such tests for method agreement is to check whether a given ejaculate would have been correctly approved or rejected depending on the number of sperm with plasma droplets and/or bent tails. The manual count is the reference method in this case. The above graph features such an evaluation by assigning four sectors in the graph, around a threshold of maximal 20% abnormal sperm with plasma droplets (proximal and/or distal) and bent tails. The cut-off approval was set to 20% droplets/bent tails. This is lower than the max. 25% morphological abnormalities allowed by the BRS[4] criteria (German Livestock Association). As AndroVision® AutoMorph does not yet evaluate all possible abnormalities (e.g. head abnormalities), the threshold was set more strict. Data points in the lower left and upper right sector (both green) would have been correctly approved or discarded. Data points in the upper left sector (yellow) would have been falsely rejected, as the AndroVision® AutoMorph system revealed a higher percentage of abnormal sperm, than were counted manually on the screen. The data points in the lower right sector (red) would have been falsely approved and an inadequate ejaculate would have been produced. This can happen, if the CASA system does not pick up all malformations in a semen sample, compared to the manual count.

In the present method validation, 97% (n=65) from all (n=67) semen samples were evaluated correctly and either approved or rejected correctly. 3% (n=2) samples were falsely rejected and no ejaculate was falsely approved.

Putting all this together, AndroVision® AutoMorph can reliably decide, whether a semen sample can be used for semen production depending on its percentage of proximal and distal plasma droplets and bent tails. This evaluation is performed in parallel and in online analysis speed to the standard semen motility and concentration analysis. All the measurement results provided by AndroVision® allow for a reliable quality control of semen samples and with this, a high quality semen dose.

[1] Kummer, AB et al. Multivariate analyses for determining the association of field porcine fertility with sperm motion traits analysed by computer-assisted semen analysis and with sperm morphology. Reprod. Dom. Anim. 48, 747-54 (2013).

[2] Lin, L. I. A concordance correlation coefficient to evaluate reproducibility. Biometrics 45, 255–68 (1989).

[3] Koch, R. & Spörl, E. Statistische Verfahren zum Vergleich zweier Messmethoden und zur Kalibrierung: Konkordanz-, Korrelations- und Regressionsanalyse am Beispiel der Augeninnendruckmessung. Klin. Monbl. Augenheilkd. 224, 52–57 (2007).

[4] Bundesverband Rind und Schwein – Anforderungen an Besamungseber hinsichtlich ihrer Eignung zum Einsatz in der KB, https://www.rind-schwein.de/services/files/gesetzevo/gb_201005.pdf, accessed 20.8.2019

User Reference:

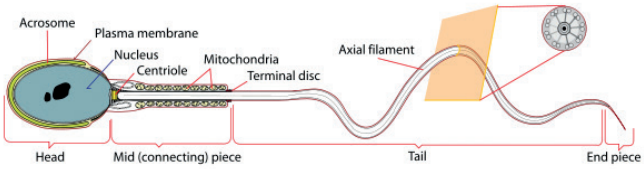
The Cooperative Central Aurora Alimentos is one of Brazil's largest industrial complexes and global reference in meat processing technology, with 11 affiliated cooperatives, plus 100 thousand associate members and over 28 thousand employees. The Cooperative has a herd with more than 200,000 sows in their swine production base. Currently, it already has a Gene Transfer Center (GTC I) with capacity for 372 animals and average production of 60,000 doses/month. The newly inaugurated GTC II has a total built area of 4,266.09 m², capacity for 45,000 doses/month and housing of 300 boars in a barn installation for the best conditions of animal welfare.

Minitube is Aurora's partner in the operation of the two GTCs, where various equipment and technologies are used to collect and process the ejaculates, such as BoarMatic collection dummies, AndroVision® Automorph CASA System, IDEE Management software, IDENT software for electronic animal identification, SmartDispenser dilution system with extender vats, MiniBSP packaging machines and water purification system (reverse osmosis). All consumables used in the processes are also provided by Minitube and the technical staff provides ongoing support to the GTCs through visits, training and remote connections.

The importance of membrane and acrosome integrity in connection with semen freezing

Dominika Becherer, Minitube

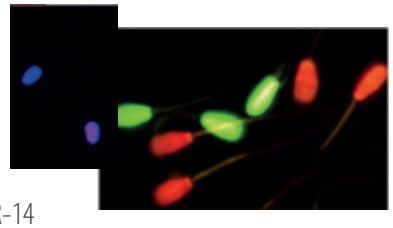
Although sperm cryopreservation is widely used, it is well-known that freezing and thawing processes damage sperm because of changes in temperature, induction of osmotic stress and formation of ice crystals. It is noteworthy that these alterations affect the plasma membrane, acrosomes, mitochondria and DNA integrity and reduce sperm motility and survival[1]. Intact plasma membranes and acrosomes are essential for sperm capacitation, acrosome reaction, and ultimately fertilization of the oocyte. Several studies showed the correlation of these functional cell parameters to the fertility[2]. When data from multiple sperm assays are used, higher correlations with the fertilizing potential of a semen sample is achieved[3]. Additionally the value of sperm functional traits as predictor of bull fertility was shown. If the cell membrane gets permeable, it comes to a loss of cytoplasm and ends in cell death.



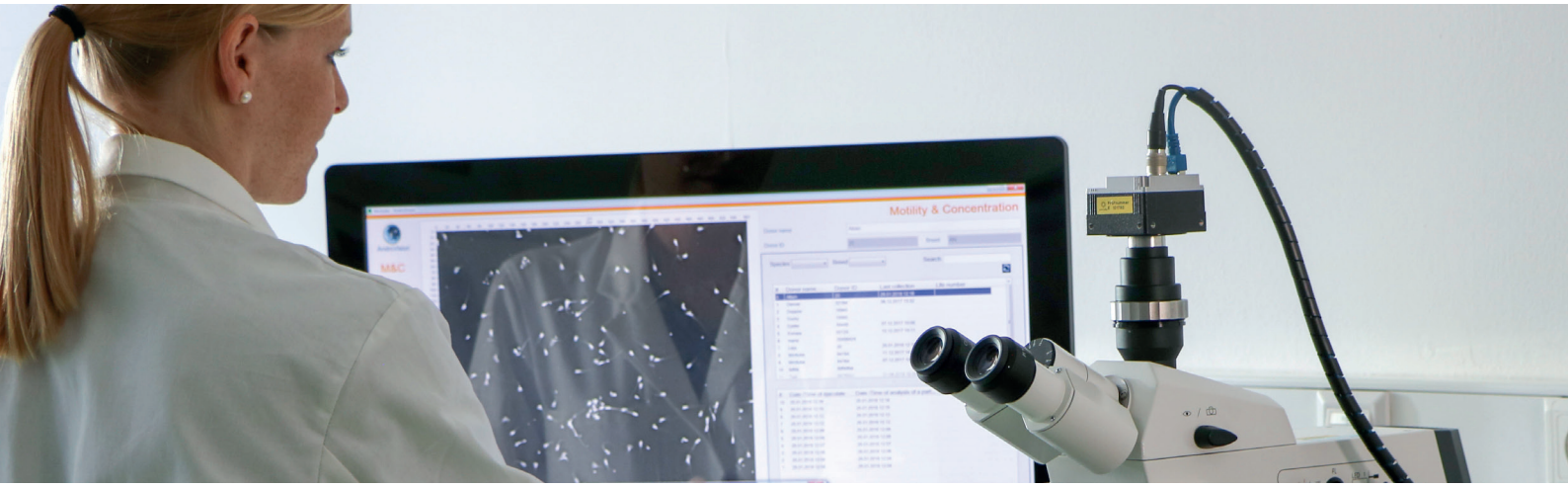
Beside the possibility to predict the male fertility more accurately by using these analysis data, detailed quality checks of the ejaculate during the process in the semen production lab can be helpful to improve the different steps of processing the semen. Dilution rates, equilibration times, freezing rates and semen thawing management are critical steps in semen production and are reasonsable for the loss of semen quality during the process. Functional sperm parameters like membrane or acrosome integrity, evaluated at the different processing steps, are convenient markers to figure out the processing steps with a high loss of semen quality and to improve them. For example, Minitube showed in a field trial on a commercial bull stud, how important a controlled and standardized freezing process is to maintain optimum quality of semen[4].

Flow cytometric procedures have been developed which simultaneously evaluate sperm cell viability, acrosomal integrity and mitochondrial function. For this analysis, "viable sperm" are defined as cells that possess an intact plasma membrane. This attribute is evaluated by staining a sperm sample with propidium iodide (PI), a fluorescent probe that binds to DNA. Cells having an intact plasma membrane will prevent PI from entering into the cell and staining the nucleus. However, cells possessing a damaged plasma membrane will permit PI to enter into the cell and bind to the DNA causing the cells to fluoresce red . Acrosomal integrity is measured using fluorescently labeled plant lectins. The peanut agglutinin PNA, a plant lectin derived from the fruits of Arachis hypogaea, has become the lectin of choice in most laboratories to evaluate acrosomal integrity. PNA binds to β-galactose fraction associated with the outer acrosomal membrane of sperm, causing the acrosomal region of cells exhibiting acrosomal damage to fluoresce. In each case, the lectins, themselves, are not fluorescent, but can be labeled with many different fluorescent probes[3].

According to the flow cytometric assays described above, AndroVision® offers the possibility to analyse these important functional sperm cell parameters easily in the semen production lab. With the modules Viability and Acrosome integrity, the membrane and acrosome integrity of the semen dose can be analyzed. Both assays are performed with a counter stain principle:



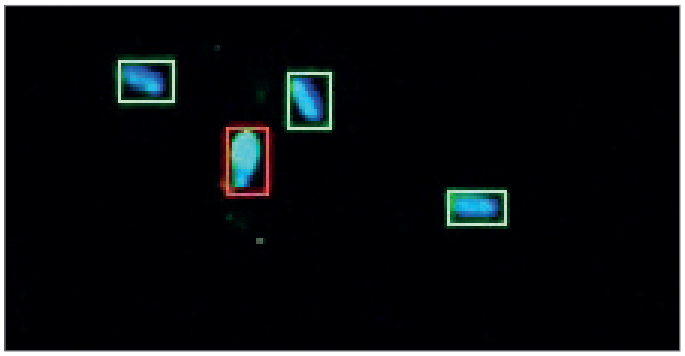
Viability:
Fluorochrome 1: H33342 or SYBR-14
➲ membrane permeable DNA- dye
Fluorochrome 2: Propidium Iodide ➲ non- membrane permeable DNA-dye with high binding affinity ➲ verification of permeabilized, damaged cell membrane



The importance of membrane and acrosome integrity in connection with semen freezing

Acrosome integrity:

Fluorochrome 1: H33342 ➲ membrane permeable DNA dye
Fluorochrome 2: FITC conjugated to PNA (Arachis hypogaea lectine (peanut) ➲ plasma membrane covers outer acrosome membrane and is non-permeable for PNA– PNA binds to outer acrosome membrane only after acrosome reaction (fusion with plasma membrane) – FITC has higher staining capacity than H33342



Automatic detection of sperm with defective acrosome (outlined in red) by means of double fluorescence staining: H33342/FITC-PNA

A detailed protocol is provided with every staining kit. The pictures are analyzed with the referring modules of AndroVision®, giving a percentage of each sperm cell population within a few seconds. Subsequently such information is used for the assessment of the quality of the ejaculate.

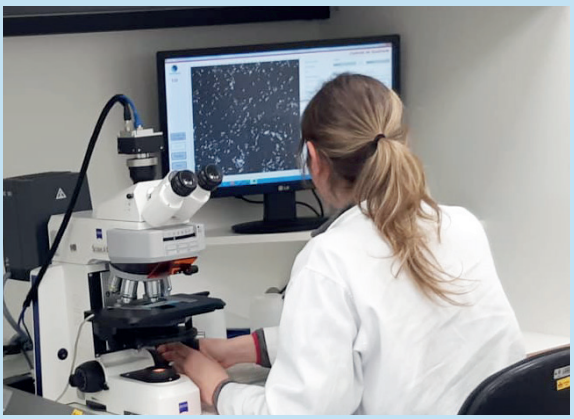
[1] Effects of cryopreservation on sperm viability, synthesis of reactive oxygen species, and DNA damage of bovine sperm
H. Gürler et al. ; Theriogenology 86(2) · February 2016

[2] Changes in motility, morphology, plasma membrane and acrosome integrity during stages of cryopreservation of buck sperm
A. Mushtaq et al., Journal of the South African Veterinary Association | Vol 85, No 1, 2014

[3] Assessment of sperm quality: a flow cytometric approach
James K. Graham, Department of Physiology, Colorado State University, Fort Collins in Animal Reproduction Science Volume 68, Issues 3–4, 3 December 2001, Pages 239-247

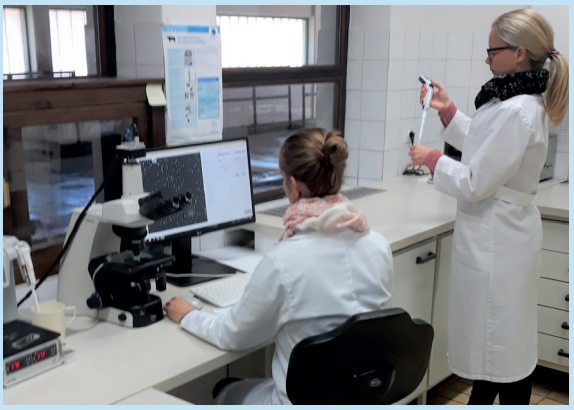
[4] Technical report Minitube TurboFreezer - A standardized freezing process is crucial for efficient production of cryopreserved bull semen

User Voices:



"When it comes to a CASA System, AndroVision® is a great option not only for the excellent technology but also for technical assistance that Minitube offers to us".

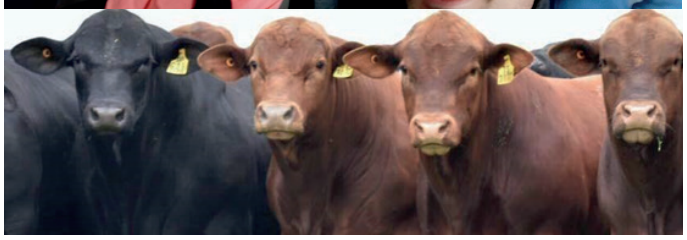
Ana Paula Mellagi – Professor at Swine Sector – Animal Medicine Department – Faculty of Veterinary Medicine – UFRGS - Brazil



„Since our Artificial Insemination center is working with AndroVision®, the quality of our work is better and has improved."

Dr. vet. med. Gregor Plevnik, AI Ptuj, Slovenia

Satisfied customers are the best reference



Minitube's AndroVision® CASA system supports projects in the reproduction of wild animals

The Animal Reproduction Centre A.R.C. in Bratislava, Slovakia, is a family company that provides practical reproductive technology for animal and veterinary services. Specialized in wild animals like mouflons, chamoises and bears, or half domesticated wild animal species in extensive farming conditions like red deer or fallow deer, biologist Jaroslav Pokorádi, Ph.D., and his team rely on twenty years of experience in the field of reproductive issues.

With installing AndroVision®, he got the optimum support for his workflow process extracting any information the he needs for his purposes through precise, standardized and interactive semen analysis.

"I and my team are pleased to see Minitube in the field of biotechnology in the world of animals, which, with its products and reliable instruments, improves the reproductive health of animals. The AndroVision® system is like a good German car, fast, reliable and professional.", Mr. Pokorádi explained.

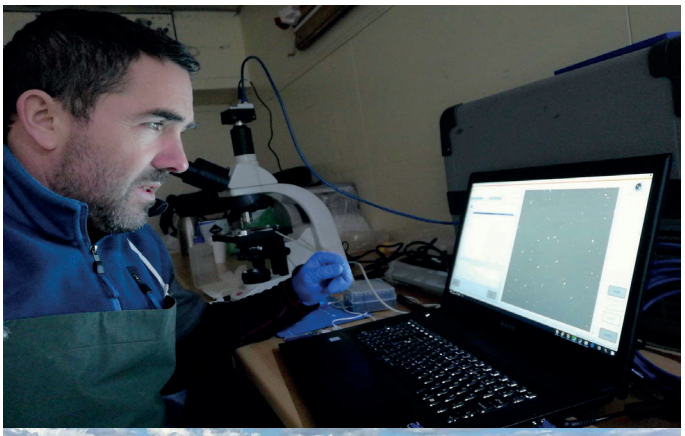
First AndroVision® CASA System in Uruguay for the commercial production of semen

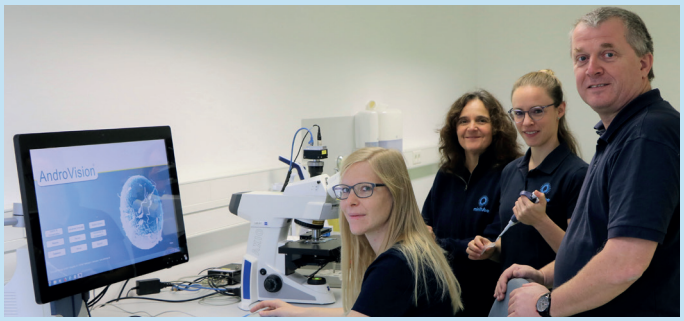
The Bull station "El Coraje" de Bove Itzaina Hnos, purchased one of Minitube's AndroVision® CASA System in Sarandí del Yí - Uruguay, to support their routine semen analysis and quality control.

El Coraje is a family company dedicated to livestock as their main activity and since its beginning always emphasized efficiency, having Brangus breed as a tool to increase productivity and improve economic results.

By installing AndroVision®, El Coraje will be able to reach higher levels of quality which enables them to export semen doses to other countries.

Andrés and Pablo Bove Itzaina, brothers and owners of the bull center comment: „Historical day for El Coraje and for our Reproduction Center. We acquired an AndroVision®, a CASA system with high precision for the standardized and interactive semen analysis, number 1 worldwide and the first to enter Uruguay for commercial production of semen. We made this investment to put aside subjectivity and possible human error and take firmer steps in our production processes. For more and better diffusion of El Courage Genetics!"





Product Training

Minitube provides product training that meets your individual requirements. Our aim is to individually provide all relevant information concerning the system, tips and tricks, as well as help with error analysis and trouble shooting. Our experts will instruct you and your colleagues, either in one of our training centers or on-site at your location.