

The international AI news from Minitüb

Sperm Notes

Evaluation of semen quality: morphology

Page 2

Automatic filling systems for boar semen

Page 4

**Androstar® Plus - Extender medium for long term
preservation of boar semen**

Page 4

How breeder Giles found his "clear" solution

Page 6

New posters made by Minitüb

Page 7

Minitüb at the EuroTier

Page 8

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Evaluation of semen quality: morphology

Semen evaluation uses macroscopic, microscopic and, if necessary, physiochemical methods to diagnose the quality of an ejaculate (see figure 1).

Figure 1: Standard evaluation of animal semen

macroscopic	microscopic	physiochemical
<ul style="list-style-type: none"> • volume • aspect • smell 	<ul style="list-style-type: none"> • concentration • motility • agglutination • contamination • morphology 	<ul style="list-style-type: none"> • pH-value • osmolarity

All these mentioned criteria are important to decide, if an ejaculate is usable for preservation and insemination - or not. Particularly the microscopic examination of sperm cells has gained in importance in recent years, due to new technological developments: CASA (Computer Assisted Semen Analysis) systems today allow both an objective and time saving method to evaluate the quality of semen. Besides the analysis of motility and concentration, in particular the morphology of sperm cells is taken into account to determine the fertility of the ejaculate of a boar. The reason is, that motile sperm cells, too, can be morphologically abnormal, and this defect makes them not available to fertilize an ovum.

Morphological defects of sperm cells can be found on the head's cap (acrosome integrity), the head itself or the middle piece. Various types of coiled tails as well as cytoplasm drops are frequently found abnormalities. Figure 3 shows some samples of abnormal sperm cells.

Sperm morphology, similar to sperm motility, needs to meet minimum requirements which have to be fulfilled for an ejaculate being used in AI. The Umbrella Association of German Pig Production (ZDS), for instance, defines the requirements for boar ejaculates as shown in figure 2.

When those figures are exceeded, this indicates a low fertility of the semen dose. A survey of Waberski et al. (1994) showed, that an increased number of morphologically abnormal sperm cells correlates negatively with pregnancy rates. Furthermore, an increased percentage of sperm cells with plasma drops corresponded negatively with the litter size.

What options are offered today to evaluate sperm quality? A full analysis of sperm morphology is only possible with a phase contrast microscope with 1000x magnification and oil immersi-

on. This analysis is quite time-consuming and needs to be done by trained staff. Only few laboratories have such equipment or so much spare time.

„basic morphology“

Therefore, there are basic however valid methods, allowing a superficial but practical evaluation of sperm morphology. With the CASA system Sperm Vision™, this evaluation can be done while the motility is analysed. This is enabled by the tool "basic morphology", where primarily bent tails and protoplasm drops are identified by the user.

„automatic morphology“

This analysis can now also be done automatically: Minitüb is the first company worldwide to present a tool for Sperm Vision™ for automatic detection of sperm morphology. Bent tails as

Figure 2: Minimum requirements for boar semen

Total morphological abnormalities (%)	≤ 25
Sperm with head abnormalities (%)	≤ 5
Sperm with acrosome abnormalities (%)	≤ 10
Sperm with plasma droplets (%)	≤ 15
Sperm with coiled tails (%)	≤ 15
Other morphological abnormalities (%)	≤ 15

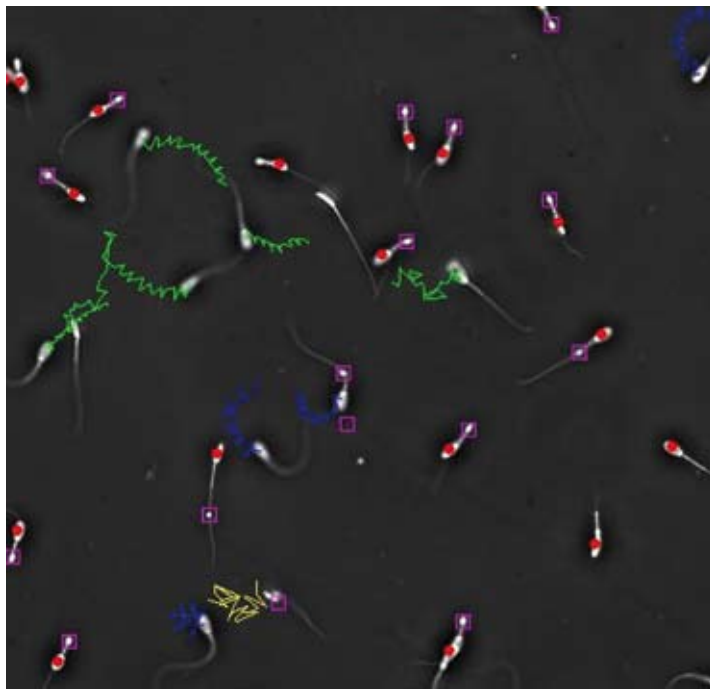
Source: ZDS, 2006

well as (proximal and distal) cytoplasm drops are now detected automatically in production line speed. The examination is done simultaneously with the measurement of the motility and concentration. The results are automatically included in the ejaculate calculation - a time-saving process resulting in an increase of quality of the produced semen doses. As usual, the videos and pictures can be stored for documentation or monitoring of a boar. Thus, the ejaculate quality can be continuously controlled for every production boar.

„extended morphology“

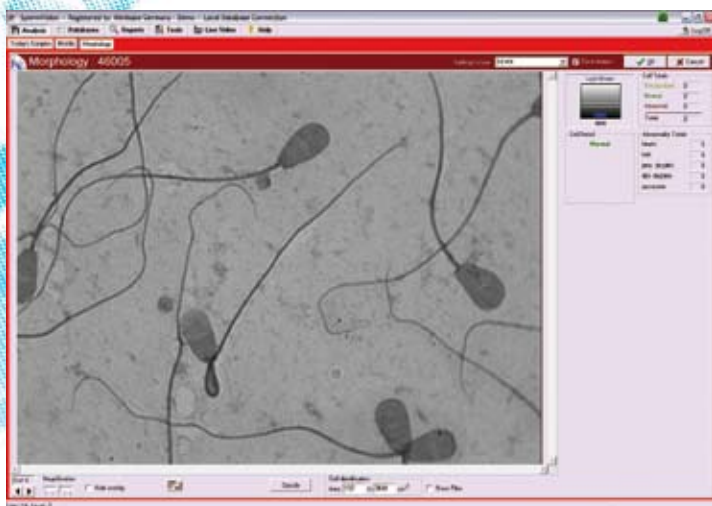
With „extended morphology“, Sperm Vision™ offers an additional module to simplify a full morphology analysis,

Evaluation of semen quality: morphology



allowing the user to save the results. To carry out this full analysis correctly, approx. 5µl of diluted boar semen (in proportion 1:10) is pipetted on a slide and then covered by a cover slip. In case the probe can not be evaluated immediately (>30 min), the sample should be cured with a Formalin stain (e.g. Ref. 15405/0100). This does not only keep the sample stable, but also immobilizes the sperm cells so that they can be evaluated easier and more precisely.

For each analysis, 100 cells should be counted and the proportion of abnormal sperm be determined. Sperm Vision™ stores all taken pictures, so that recording and evaluation of the semen can take place at different times. In a data base, the history of the sperm quality of the animals can be followed



up and monitored. A reminder function makes sure that an evaluation is done regularly. A basic evaluation of sperm morphology should be done with each ejaculate, a full analysis at least once a month for each boar.

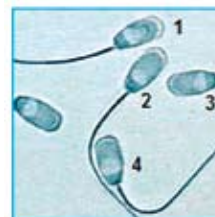
The analysis of all quality criteria does not only serve for breeding and monitoring purposes. It also has a deep impact on the profitability of boar studs: only through precise knowledge of the overall quality, the ideal number of semen doses, which can be produced from one ejaculate, can be determined. ■

Figure 3: Samples of abnormal sperm cells

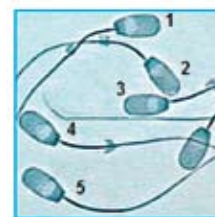
Bromphenol blue stain:
 1 = detached head, stained (dead)
 2 = normal sperm, unstained (alive) with distal cytoplasmic droplet



Papanicolaou stain:
 1 - 4 = sperm with abnormal (swollen/loosening) acrosomes



Papanicolaou stain:
 1 - 4 = sperm with distal cytoplasmic droplets
 5 = normal sperm



Bromphenol blue stain: All sperm unstained (live)
 1 = bent tail
 2 = primary abnormality of the head
 3 = normal sperm
 4 = normal sperm with proximal cytoplasmic droplet



Automatic filling systems for boar semen



	TwinPacker®	MiniBSP®	SPS 21®
Tubes per hour	550	up to 900	1400
Capacity of tube magazine	40 in 10 magazines	210 with integrated magazine or 2100 with external magazine	2100 on external magazine
Minimum labour needed	1 operator	0,75 operator	1 operator (for max. output)
Compressor	internal	external	external
Labelling	included printer, barcode ready	included printer, barcode ready	included printer, barcode ready
Tube sealing technique	heat-sealing	ultrasound-sealing	ultrasound-sealing
Control	touch screen	touch screen	touch screen
Serial interface	yes	yes	yes
Power supply	230/110 V, 50/60 Hz	230/110 V, 50/60 Hz	230/110 V, 50/60 Hz
Automatic ejaculate change	no	yes	yes
Options		<ul style="list-style-type: none"> • filling station for extender • external tube magazine • hole puncher for tube hanger • automatic ejaculate sorting with conveyor belt 	<ul style="list-style-type: none"> • filling station for extender • automatic ejaculate sorting with conveyor belt
Available languages	de, en, es, ru, pl	de, en, es, ru, it, nl	de, en
Ref.	13214/0000	13210/0005	13209/0000

Androstar® Plus - Extender medium for long term preservation of boar semen

The fertility of semen cells must be maintained during the whole storage period. This is the crucial task of modern semen preservation media. Besides motility of the semen cell, functionality of the sperm membrane must be preserved, since it is a major requirement for the establishment of a semen reservoir in the lower oviduct and therefore for the progress and success of the fertilisation process.

Newly developed extenders aim to protect essential attributes of semen cells over an extended storage period with sometimes unstable and less favourable storage conditions. These essential attributes are in particular motility, integrity of the acrosome as well as the functionality of the entire plasma membrane. Androstar® Plus, extender for boar semen, was designed by Minitüb to fulfil these requirements: it allows a semen storage of up to 7 days at a temperature of +10°C to +20°C. Besides, Androstar® Plus is a fully synthetic extender, being free of any animal or herbal proteins.



Androstar® Plus - Extender medium for long term preservation of boar semen

Androstar® Plus protects membranes, acrosomes and cytoplasm by absorbing stress which sperm cells are exposed to. Stress occurs especially ...

- during semen processing in the laboratory while the ejaculate is manipulated and diluted. A fast dilution or decanting as well as the use of pumps imposes mechanical stress upon the cells. Especially boar semen is extremely sensible to cold-shock. Thus, it is of crucial importance that both semen and extender have the same temperature at the time of dilution. A difference of more than +/- 1 °C may result in a decrease of quality of the semen dose.
- during storage and transport: semen doses are quite often exposed to lower or higher temperatures than the optimum quality and over a longer period. Or, they are cooled down too quickly from dilution (38°C) to storage temperature (17°C). Besides, it regularly occurs, that during transport or on the farm the optimal temperature of 17°C is not kept consistently.

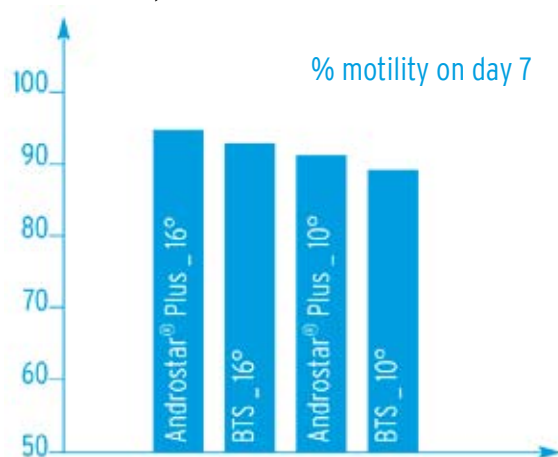
It has to be kept in mind, that even the best preservation medium cannot compensate for mistakes occurring in semen processing and even less, for quality of the semen itself.

Helping a good ejaculate to keep its quality and fertilization capacity is where a preservation medium such as Androstar® Plus makes an important contribution. This is due to its special composition: the interaction of pH-value, ionic strength, type of ions, osmotic pressure, anti-oxidants and a special membrane protection factor compensate for the stress caused by bad temperature management or extended storage periods.

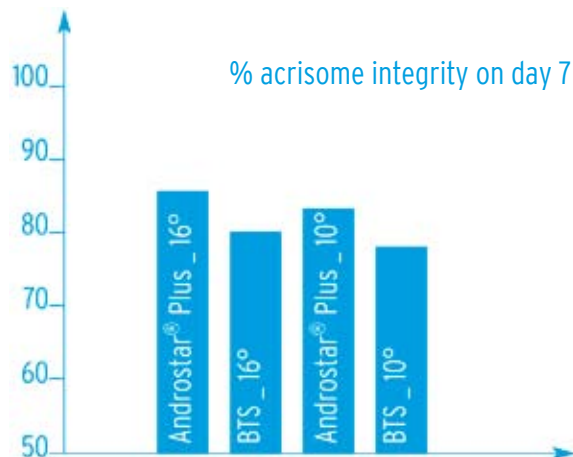
In a scientific survey, it was the aim to analyse the effect of storage time and different temperature regimes on cell motility (percentage of progressively motile cells, Sperm Vision®) and on the percentage of damaged acrosomes (swollen apical ridge and detached acrosome). The study compared Androstar® Plus and BTS, which served as a control in this assay and which is a widely used extender for short term semen conservation. The results are shown in figure 1.

Another large insemination trial (> 350 sows both in the experimental and the control group) has been closed just recently, with Androstar® Plus being compared to a conventional long term extender (as a control medium). The unambiguous results of this essay will be published in November at the ESDAR (European Society for Domestic Animal Reproduction) Conference.

Figure 1: Motility and acrosome integrity of boar spermatozoa incubated in the two extenders BTS and Androstar® Plus for up to 7 days at two different temperatures (+16°C and +10°C)



Motility: For semen stored in BTS at +16°C, motility decreased significantly from day 0 to day 5/7 ($P < 0,05$), while semen stored in Androstar® Plus showed only a small - but not significant - decrease of the motility from day 0 to day 7. Semen samples extended in Androstar® Plus and stored at +10°C showed significantly higher motility from day 3 to day 7 in comparison to BTS semen samples stored at that temperature.



Acrosome integrity: The data of Androstar® Plus semen samples stored at +16°C show significantly better values at the end of the storage period than BTS semen samples stored at the same temperature. Semen stored with Androstar® Plus at a temperature of 10°C showed only little reduction in acrosome integrity from day 5 on, while the BTS samples present a much higher increase of damaged acrosomes. It needs to be highlighted, that the amount of acrosome damage does not differ significantly for Androstar® Plus semen samples stored at +10°C and +16°C over the same storage period.

How breeder Giles found his "clear" solution

Giles is a modern farmer. Only recently he has built a new barn for 400 sows. And now he runs a rearing unit, taking his weaners to 30 kg. Just a few minutes ago his courier Johannes Brown, from the boar centre Boarvillage has delivered the boar semen doses he had ordered.

Brown gets into the car with running motor and engages gear; the car jerks and stops! "Damn, not again! Now it is completely dead, I have to call the breakdown lorry to tow my car away. "Wait! What about the semen doses in the luggage compartment?" asks farmer Giles. "They will be spoiled! Today it is only 13°C. Shall I put them into my semen fridge?" "Don't worry", Brown replies. "We always extend our boar semen with Androstar® Plus. In case of an emergency the semen doesn't lose fertility until a temperature drop to 10°C. Even, if the breakdown lorry took 7 days to arrive." "Wow!" thinks farmer Giles and throws an interested glance through the window into the cargo bay. It contains some insemination equipment that the courier is going to deliver to his customers. One of the products especially catches his eye: a new catheter.

Breeder Giles had never seen one like that. Up to now he had always bought his catheters from a retailer of general farming equipment passing by. Normally, he doesn't care for the kind of catheter. It's all right as long as it's cheap! Though they are from China, up to now there had always been something coming out of a sow.

"This is the ClearGlide™ made by Minitüb", Brown calls out to him. What first arouses his interest is the colour: a clean ice blue. Completely different to the catheters he normally uses. And this is also new for him: each catheter is packed in an individual sheath. He had never seen that either. He had already heard about it from colleagues, but he himself had never used one. On the sheath he can read: SafeBlue®. Now farmer Giles is curious. He moves the catheter in the protection sheath. An extra perforation is provided at the front. The catheter can be pushed very smoothly through the plastic sheath. 'This was easy', thinks farmer Giles. 'And what's that all about?' "For hygiene reasons", explains the courier. He had already called the towing service. "You don't remove the protection sheath

till the catheter is in the vagina of the sow. So the tip of the catheter remains clean and a contamination of the sow's cervix that might lead to infections, can be avoided. The catheters have even been sterilised."

"I see", mutters farmer Giles. This is really new to him. Up to now he had only cleaned the vagina with a paper towel, nothing else. Could that be the explanation for some of the repeat breeder sows?



"And how do I apply the lubricant, with the catheter still in the sheath?" asks farmer Giles. "Lubricant? You do not need lubricant for this catheter any more. As soon as the catheter tip gets in contact

with the humidity of the vagina, it generates a sliding film on the surface. The catheter glides very smoothly without much effort until reaching the cervix. 'Smoothly gliding - that sounds good', thinks farmer Giles.

Now he touches the head of the catheter. It is very flexible and especially on the tip the material stretches nearly like rubber. Now he notices why: the shaft of the catheter is recessed and only reaches up to the middle of the tip. Now he pulls on the tip. It can not be removed. With his old catheters it sometimes happens, and then the tip remains in the cervix. "This can not happen with this catheter" Brown explains. "The whole catheter is produced in one piece instead of gluing the catheter tip manually, as do the Chinese producers." "And why is the tip so flexible?" "It adjusts to the cervix in an optimal way, thus locking perfectly into it and avoiding semen backflow." The courier hands him a leaflet about the ClearGlide™. The picture catches his eye. He has never seen one like that either. The title is: Endoscopic picture of ClearGlide™ in the cervix of a sow. "It is easy to distinguish how the catheter seals the cervix to the rear side preventing the semen backflow."

Less semen backflow: Now farmer Giles is completely convinced. It seems like somebody really had a good idea. He buys a package of ClearGlide™. It is not cheap, but superior quality can not be bought at discount prices. In turn, the catheter provides benefits, that can't be found elsewhere. The insemination is performed with little effort and the quality promises better results - such efficiency always pays off. ■



New posters made by Minitüb



„Porcine Male Reproduction“

The poster highlights all the important aspects of semen collection and processing. As there are:

- semen collection
- semen analysis and dose calculation
- anatomy the sperm cell
- minimum requirements on semen quality

Available as laminated poster, size DIN A2, in English.

EUR 3,-

REF. : 22421/2911

„Porcine Female Reproduction“

The poster highlights all the important aspects of artificial insemination of sows. As there are:

- estrus detection
- timing of AI
- AI technique
- possible reasons for reproductive failure

Available as laminated poster, size DIN A2, in English.

EUR 3,-

REF. : 22421/2912



Minitüb at the EuroTier: Hall 13 - Stand E57



Porcine

- ① ClearGlide™
- ① BoarMatic
- ② Extenders
- ③ IDENT/IDEE
- ④ Sperm Vision™ with automatic morphology
- ⑤ Compact Dispenser
- ⑥ SPS 21

Bovine

- ⑦ CombiSystem
- ⑧ Sperm Vision™ with ECM and Viability
- ⑨ MPP Uno
- ⑩ eProCheck®

We also recommend you to visit our 2nd booth where you find products for bovine reproduction management:

Hall 27, Stand E20