

The importance of semen analysis in a boar semen laboratory

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The growth of AI in recent years has been spectacular. Nowadays, the estimate is that of over 85 million sows in the world, more than 40% are serviced using AI.

Evidently, this reproductive method has advantages over natural mating: reduction in the total number of boars per farm, use of higher quality genetics, exploitation of group or batch management, equal or greater farrowing rates than those obtained with natural mating, ease of management by reducing time and effort per service, better control on semen quality and better hygiene control.

Cornerstone of quality

From the invention of the microscope in Holland in the 17th Century, the early works of Spallanzani in Italy, to the first artificial inseminations done by Ivanov with a bamboo made straw, semen analysis has become the cornerstone of semen quality and, therefore, of farm performance.

Today, proper semen analysis is used with several objectives:

- To control the ejaculate quality.
- Quality control of the end product.

IMV's AccuCell photometer.



The AccuRead photometer.

- Boar selection (culling decision) based on their semen quality.

The microscope was the first tool to evaluate semen quality to find its place in a semen processing laboratory. The minimum requirement for an efficient andrology analysis asked for a warm plate and phase contrast.

As time went on, a good motility could be perceived and the notion of live and dead cells determined. Morphological analysis of semen as an indicator that any wrong shape is influencing the fertility power of semen was always an essential part of the microscopical evaluation.

Modern sophistication

Today, sophisticated microscopes with fluorescence and differential staining procedures give a more precise status of the sperm morphology and motility. Some basic information like membrane integrity and acrosome status can be assessed through fluorescent microscopy.

Inherited from clinical haematology, the Thoma Zeiss chamber was used for concentration assessment (number of spermatozoa per millilitre).

At that time the length of the protocol, the never ending counting under an objective

and the laboratory technician effect did not discourage the users to declare this technique as the gold standard.

In the early 1980s, more sophisticated systems appeared on the market – spectrophotometers calculating an absorbance value.

Nowadays, equipment that indicates the concentration, the number of doses produced and the level of extender to be added are available on the market. Semen analysis that combines microscope and photometry is used worldwide as the most frequent tool for semen evaluation.

CASA systems

Using photometers with LED stable wavelength analysis without a filter, proved to be affordable equipment with very high repeatability, reliability and accuracy.

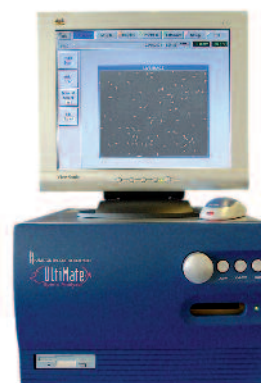
The very simple consumable makes this technique very popular and affordable in a boar semen laboratory, but is not comparable with CASA systems and quality control flow cytometry.

The evaluation of semen quality with a microscope is done by individuals and is therefore very subjective. Naturally the idea emerged to make a 'machine' that will analyse samples and give a more objective evaluation automatically.

The original idea to put together a micro-

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UltiMate CASA system for swine semen.



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scope with a warm stage and phase contrast under the eye of a camera and all under the control of software appeared on the market in the 1980s in the USA for stallion semen. These were the first computer assisted semen analysers (CASAs).

With the availability of faster and better computers automated morphology or DNA assessment can be used routinely in production.

Today, CASA systems in a boar stud are used for two main purposes – quality control and in-line assessment of ejaculate. This gives fast, reliable, and objective evaluation and reduces the technician effect on the result of the semen analysis.

A second very important impact is the correlation between boar quality assessed by CASA and field fertility factors indicating the range of quality of semen.

The correlation is important as a control of semen production, fertility rate and litter size indicator.

Fertility trials

Two fertility trials were undertaken to evaluate the relationship between boar semen quality and fertility (conception rate and litter size) after on-farm artificial insemination.

The change in sperm velocity during the first two hours of incubation and the magni-



The EasyCyte Mini.

tude of the velocity parameters after two hours were identified as the most consistent indicators of fertility. These results demonstrate that fertility information can be derived from the CASA analysis of boar semen provided it is combined with a period of incubation in capacitating conditions.

The relationship between various semen evaluation tests and fertility in fertile and subfertile artificial insemination boars was examined.

Those tests presenting the highest correlation with non-return rate were CASA assessed total motility and subjective sperm motility after seven days of storage.

The main purpose of sperm evaluation is to predict its fertilising ability. However, basic sperm test results show a low correlation with fertilising ability.

The purpose of the study was to determine whether there is an association between induced acrosome reaction (IAR) and the incidence of subfertility of normal boar sperm.

The percentage of progesterone induced acrosome reaction was significantly lower in subfertile boars (5.75%) as compared with fertile boars (10%).

These results suggest that assessment of IAR in vitro may be a useful parameter to identify subfertility in boars.

Today, the use of flow cytometry to assess semen quality is gaining acceptance. This technology goes one step further in semen evaluation, by actually looking into the physiological profile of the sperm cell.

By individually analysing and counting microscopic particles (such as cells), flow cytometers allow simultaneous multiparametric analysis of the physical and/or chemical characteristics of up to thousands of particles per second.

The newest generation of the flow cytometer is user friendly and uses a very small amount of semen and reagent. These systems are compact in size and work fast. They also generate a very small amount of

waste compared to the previous generation of flow cytometry.

The equipment is available along with sets of tests specifically developed for semen analysis in specific species.

Available tests automatically measure the viability of the sperm cells, the potential of the mitochondria to generate energy, the level of calcium inside the cell (one early event related to capacitation), the level of DNA fragmentation, and the level of bacterial contamination of a semen sample.

This type of equipment will allow the boar stud to have a lot better information on the quality of the semen, therefore providing the industry with an extra tool to:

- Improve the quality control and added value of semen produced by centres and supplied to producers.

- Screen and select the boars on their semen quality merits.

The use of different tests brings a precise answer not only to semen quality but also to predict the effect of fertility:

- The chromatin is the complex of DNA and protein that make up chromosomes. If a large percentage of a sperm (greater than 30%) have damaged chromatin, the chances of pregnancies are significantly reduced and, furthermore, the risk of embryonic losses is increased.

The portion of sperm with damaged chromatin may be determined with a Sperm Chromatin Structure Assay (SCSA).

- In an unpublished study, using mitochondrial assay on 300 ejaculates a threshold was determined that on fresh boar semen more than 70% polarised mitochondria decreases significantly the fertility power of semen.

- In the same trial all semen with lower than 80% of intact membrane had a significant loss of fecundity power.

Conclusion

The ability to screen the structural and functional integrity of the spermatozoa in vitro has increased over the past decades, but our capacity to estimate the fertility of a semen sample or of the boar, especially in selected semen processing laboratories has not.

The estimation of fertility is constrained by several factors (for example type of cell, analysis strength, sperm deposition strategies, recordings of fertility), including the fact that the ejaculate is composed of a diverse sperm population.

Determination through semen analysis of how large such a sperm population is, with competence for fertilisation and an in-built ability to display these attributes under physiological signalling, would allow for a better estimation of fertility.

The unique opportunity to have a number of discriminating assays done

by flow cytometry gives us a useful skeleton to more closely determine the boar fertility index.

To build up tools that would detect viral antibodies, bacterial determination or improve the assays for physiological parameters of sperm would significantly increase this analysis and determination. ■

References are available from the authors on request.

The EasyCyte Plus.

