Semen evaluation – how can we predict field fertility?

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Over the last decade sow productivity has increased significantly. This growth is the consequence of several different factors like improved management, genetics and feed, but improved reproductive performance has been a key driver. Litter size has increased worldwide due to strong selection for this trait, but also due to a large shift from natural mating to the use of AI.

Artificial insemination is almost the exclusive breeding method in Western Europe (95-99%), the USA (90%) and Mexico (90%). A similar trend is seen in South America with high percentages of AI in Chile (99%) and Brazil (66%).

The use of AI increases breeding efficiency, because more sows can be bred with one single ejaculate, but when farrowing rates reduce or litter size declines below the expected value, often semen quality is blamed.

However, data indicate that when commercially approved semen (for instance >70% motility and <20% abnormal cells) is used the effect of semen on litter size is very small. This shows that good semen quality control at the initial AI dose production side is essential.

Microscopic evaluation

The traditional way to evaluate semen uses a microscopic evaluation of each sample. First a drop of fresh undiluted semen is evaluated under the microscope to score massal motility (wave motion of the semen).

Subsequently, diluted semen (1:10 or 1:25) is looked at and the percentage of motile sperm cells is estimated.

This technique is used by many AI centers or on-farm AI practitioners all over the world and is an economic method of semen evaluation. But, scientists show that this technique is very sensitive for subjective interpretation by individual technicians. This means that the evaluation of the same sample has a high chance of being evaluated differently by two technicians.

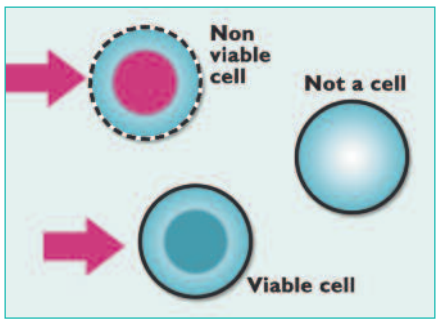
The use of AI has increased breeding efficiency allowing for more piglets to be born from the same ejaculate.

Also the technique has a limitation as a technician can only classify semen with intervals of 10%. For instance an average sample receives 70%, while a slightly better sample will receive 80%.

CASA systems

In the late 70s and early 80s an image analysing computer system was developed to avoid this technician bias in semen analyses. The first evaluations were performed on bull, rabbit, ram and stallion semen with good relations to the microscopic evaluations.

Fig. 2. Principle of staining with specific reactive agents for flow cytometry.



Technology has evolved since then and current computer assisted semen analysis (CASA) systems are fully automated systems that visualise and digitise successive images of sperm samples.

They are able to process and analyse data randomly and convert this into accurate and precise information about the semen sample and also about individual sperm cells in that sample.

Standard analyses include concentration and motility characteristics of the sample and some CASA systems can even perform morphology analyses. Because there is no longer an effect of the technician that performs the analyses the use of CASA allows AI centers to standardise their semen evaluations between different locations.

Farm fertility

If were able to 'measure' the fertility potential of a semen sample this would be of great value for both AI centres and farmers. It would allow for pre-selection of fertile ejaculates and/or boars, which would improve on-farm farrowing rate and litter sizes. This would be of high economic importance for the producer.

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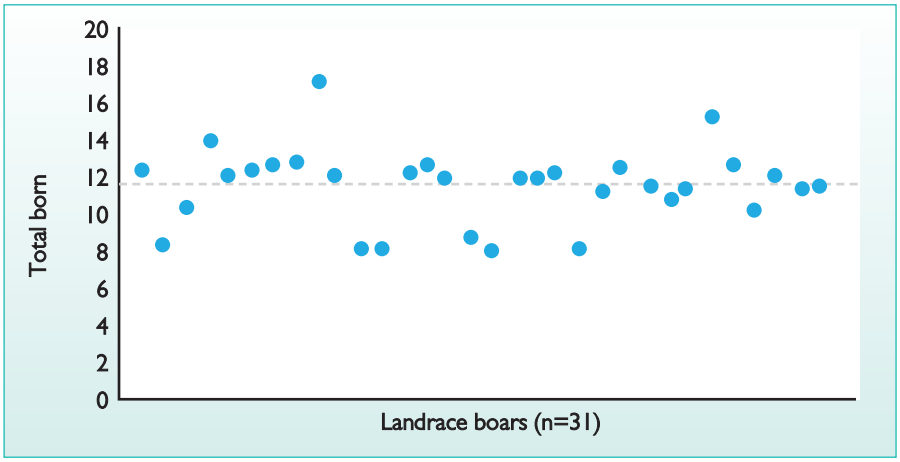

Fig. 2. A large variation exists in litter size between boars. All semen samples were qualified as commercially fit (>75% motility and <15% abnormal cells) and semen was not pooled (Adapted from Foxcroft el al. 2010).

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However, even though the current characteristics of motility and morphology give us a good idea of semen quality, the relation with on-farm fertility in swine is not always that clear. It seems that these characteristics alone do not completely explain the physiological status and thus the fertilising potential, of the semen.

Studies show that when commercially approved semen is used (semen that meets the set quality criteria) there still exists a large difference in litter size between boars.

This cannot be explained by the motility or morphology parameters and leads to a large difference in pigs produced per boar, so if we are able to better predict field fertility it may result in a high economic gain.

Flow cytometry

Flow cytometry is used to evaluate semen quality in more detail. Flow cytometry allows for an assessment of different characteristics of the sperm cell.

To analyse semen samples with flow cytometry a small volume of the sample is incubated with reactive agents that stain specific parts of the sperm cell.

Subsequently, this is passed through the cytometer, which counts the amount of stained cells.

Current flow cytometer tests can be divided in tests that evaluate sperm physiology and tests that detect (in)fertility biomarkers.

The first ones evaluate multiple physiological characteristics of sperm cells: concentration, sperm viability, acrosomal integrity, mitochondrial membrane potential, DNA fragmentation, membrane disorders and can even measure oxidation (detrimental for sperm) and calcium levels inside the sperm cell (this is an indication for over maturation of the sperm cell) at a speed of 850 cells/second.

The second type of tests can detect certain biomarkers for (in)fertility like specific proteins on the sperm membrane, which allow for the (pre)selection of high fertile ejaculates and/or males.

Predicting fertility

Does the use of cytometry really help us to predict the fertility of a semen sample?

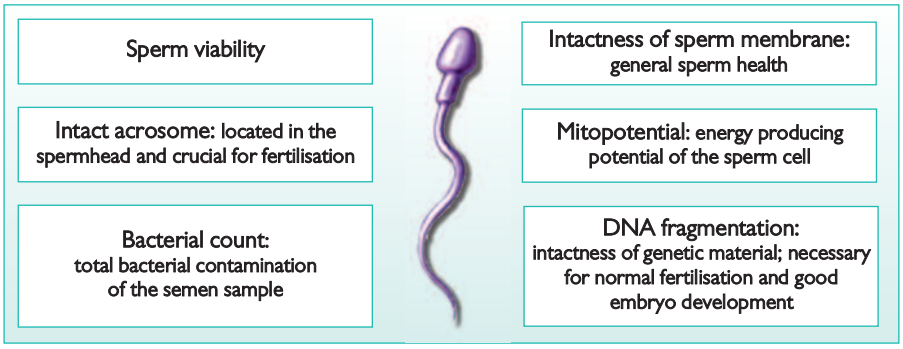
Research shows that it does. The sperm physiology characteristics are directly related to on-farm farrowing rate and litter size.

Studies from the R&D department of IMV Technologies show that high fertility boars have a higher percentage of sperm cells with intact DNA and an intact acrosome.

The disadvantage of conventional cytometers is that they are very large, quite expensive, difficult to operate and that they use large quantities of reactive agents and need a relatively large part of the semen sample for analyses.

IMV Technologies developed a bench top cytometer – the Easycyte – that works with a capillary system, which has the advantage that it needs only a very tiny portion of the semen sample to perform the analyses and, as a consequence, the amount of reactive agents can also be largely reduced. 100 samples can be tested in less than an hour.

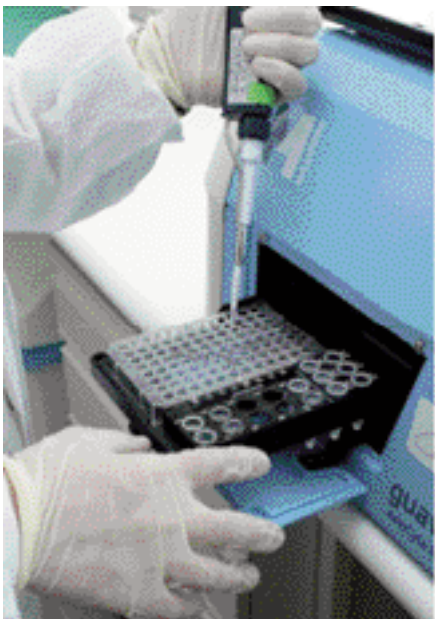
Fig. 3. Different sperm fertility tests with flow cytometry.



Conclusions

Semen quality analyses is important to maintain breeding efficiency high and more sophisticated techniques make it even possible to pre-select ejaculates for fertility, which even further increases AI efficiency.

While the traditional method of semen evaluation by microscope may be sufficiently accurate for on-farm AI evaluation of semen, the large influence of the technician results in a variation between analyses that is not recommendable for AI centres.



Easycyte, IMV's bench top flow cytometer.

Computer assisted semen analyses systems (CASA) evaluate semen quality automatically and reduce the variation between analyses significantly.

This allows (larger) AI centres to reduce variation between analyses and between different locations, which improves their efficiency. The evaluation of motility and morphology alone are not sufficient to predict fertility or to (pre)select fertile ejaculates.

The physiological status of a semen sample can be investigated in more detail by flow cytometry. These physiological characteristics are well related with reproductive performance like farrowing rate and litter size.

A better and more accurate prediction of the fertility potential of a semen sample will increase breeding efficiency and thus farm productivity. ■