

NEW TESTS TO CONTROL SEMEN QUALITY IN BULLS

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Summary

Until recently, the microscopical control of sperm motility was the most important measure to determine the quality of bull sperm. The introduction of more sophisticated laboratory tests will help to get a better correlation between laboratory findings and fertility in the field. Testing of motility with standardized computer programmes give a big number of new parameters for the characterization of sperm movements. On the other hand, the flowcytometer technology introduced new methods for characterization of live and dead sperm, the determination of intact acrosomes and the detection of the sperm chromatin structure. Correlation of these new tests with field fertility results (non-return rates) give in total the chance to produce more doses of semen of the highly desired bulls.

Key words: semen, quality control, bulls

For many years the control of semen quality was mainly based on the microscopical control of mass movement or single cell motility by the use of light-microscopy and the human eye. This gave sufficient results and a certain correlation to fertility which helped to exclude the worst ejaculates from processing and use in artificial insemination (AI).

The number of sperm cells per dose in routine work amounts to as many as 15-20 millions. Forward motility has to be at least 70% in raw ejaculates and 50% after thawing of frozen semen doses. These parameters include an excess of security in processing the ejaculates taking into account that there are differences in sperm cell per dose due to lack of standardization in filling of the straws. For example, experimental work of GRIGA (6) showed a possible deviation from the medium 15 mio. sperm cells per dose of up to 25%. In such a case, it is not advisable to extend to a higher level (lower number of sperm cells per dose). On the contrary, high-demand bulls can be extended down to 8-10 mio. sperm cells without loss of fertility if semen quality is determined exactly and the best ejaculates are used only.

The development of the computer-assisted sperm motility analysis (CASA) and a variety of fluorescence dyes offer new possibilities, to achieve more and more objective information about the functionality of fresh and frozen/thawed sperm (9).

CASA-systems

Amann and Katz (1) were the first to discover the benefit of CASA-systems to detect environmental influences on the quality of semen production and sperm function. Besides the percentage of total motility, progressive and local motile

sperm with CASA it is possible to measure different parameter of velocity (for example velocity straight line, VSL, velocity curvilinear, VCL) or the beat cross frequency, BCF. Instead of the strong automatization process, good results mainly rely on precise handling of the sample, the machine and the software.

Flow cytometry

Flow cytometry is a fully automated and highly standardized method of measurement. By this method a big number of fluorescence-marked cells can be measured objectively and quick and investigated in the physiological status. The advantage of this method is the possibility to analyse thousands of cells within seconds. The sensitivity compared to a microscopical staining is better and different cell characteristics can be determined simultaneously (5). This method has a vast growing importance in semen quality control and, possibly, in the prediction of fertility in the future. Combining different fluorescence dyes offers even more possibilities.

The acrosomal status is best measured by FITC-PNA/propidiumiodine (PI)-stain. Fluorescein-isothiocyanate (FITC) which is bound to lectin is qualified nicely as a selective fluorescence marker of the acrosome. The lectin is derived from *Arachis hypogaea* (peanut agglutinin, PNA). This lectin binds selectively to the outer acrosome membrane and emits green light due to laser stimulation (3). The plasma membrane of vital sperm cells, which is covering the outer acrosome membrane, is not permeable for FITC-PNA.

Propidiumiodine is non-permeable for membranes. A stain of the sperm core can only be achieved in non-intact sperm cells (4). Non-intact sperm cells are red. FITC-PNA/PI-stain is best for frozen/thawed sperm cells diluted by egg-yolk-containing extenders (8).

Sperm chromatin structure assay (SCSA)

This method was developed to detect chromatin defects in sperm cells. The principle is a higher sensitivity of abnormal structured chromatin to physically induce denaturation in comparison with physiological chromatin (2).

Rhodamin-123-staining

To measure the mitochondrial activity rhodamin-123 is used as a stain. It passes the mitochondrial membrane to the inner side of the mitochondria and accumulates (2). This requires vital cells with functioning mitochondria.

Reactive oxygen species (ROS)-test

Another flowcytometrical test is used to detect the susceptibility of sperm cells to lipid peroxidation. It plays a major role for the functionality of the cells. The evidence of ROS was done by using dihydrorhodamine-123 (DHR). DHR is membrane permeable and start fluorescing by oxidation of the cell internal ROS (7).

C11-Bodipy/PI-staining

This method can be used for the detection of lipid peroxidation. Bodipy is a fatty acid which enters into most cell membranes and – by oxidation – shifts the emitted fluorescence light from red to green (7).

All these tests give us a huge amount of valuable data to detect semen quality in routine work much better than years ago. The extension of the ejaculates can be adapted to these findings and will lead to more semen doses of high-demand bulls with bigger security to ensure high fertility.

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