Sperm under the microscope

How to interpret boar sperm morphology when inspecting semen samples in the AI laboratory

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AI

An accurate prediction of the fertilising ability of sperm in boar semen is of great economic importance to breeding herds practising artificial insemination (AI). At the moment, unfortunately, the classical methods for evaluating sperm are poor at predicting fertility because they detect only the lowest-quality samples. But there is still a good case for carrying out routine evaluations of semen after collection while new and better techniques are being developed. Measurements of the concentration of sperm cells, their progressive motility, the percentage of viable cells and their structure or morphology build up to a valuable analysis that allows samples with markedly poor quality to be detected and discarded.

PHOTO 1: Seen through a phase-contrast microscope, this boar semen has a high percentage of spermatozoa with abnormal morphology: cyotplasmic droplets (marked as stars), folded tail (arrow), coiled tail (plus sign). White dots mark the sperm cells with normal morphology. (*All photos courtesy of University of Murcia)*

PHOTO 2: Using an eosin-nigrosin staining technique, viable sperm appear white while the pink one has damaged membranes. All are of normal morphology.

PHOTO 3: Examples of altered sperm morphology: (a) Cytoplasmic droplets and folded tail; (b) macrocephalic sperm with 2 fused tails; (c) bicephalic perm (2 heads); (d) round head; (e) normal and folded tail. Images 'a-d' from contrast-phase microscope, image 'e' from scanning electronic microscope.

Of these various checks, it may be helpful in this report to concentrate on the ways in which morphology is assessed. Their purpose is to determine whether the sperm cells have developed correctly in the testicles and have matured fully in the area of the boar's scrotum known as the epididymus. Anomalies that have occurred during spermatogenesis (the production of new spermatozoa) or in the maturation process can reduce the semen's fertilising capacity.

One sign of immaturity is when a tiny droplet of cytoplasm can be seen attached to the cell, because these fragments disappear in the mature sperm. The presence of a high number of

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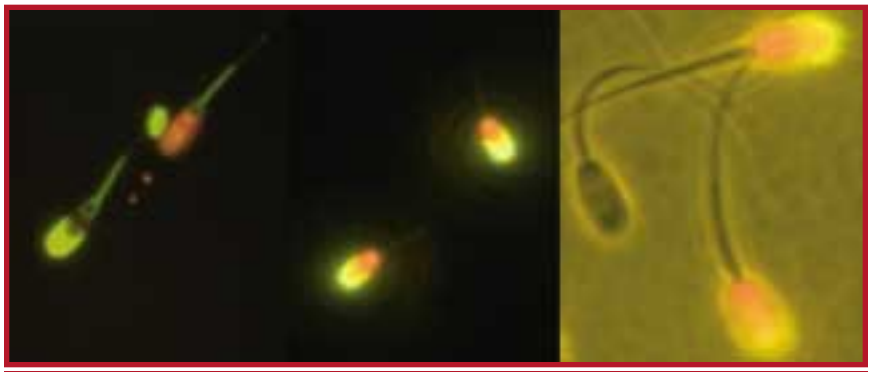

PHOTO 4: Double flourescent staining (PNA-FITC and IP). Green area represents acrosome altered. Orange area = membrane altered. Black sperm corresponds with intact membrane (acrosomal and cytoplasmic).

such droplets could indicate a lack of synchrony between sperm production and the frequency of semen collection from the boar. Where the tails of the spermatozoa appear folded or coiled, it may signify that their development in the epididymus was impaired.

Most importantly, we need to check on the acrosome — the cap around the

PHOTO 5: Boar sperm with a distal cytoplasmic droplet (marked with a star), viewed by scanning electronic microscope.

head of the cell which contains enzymes necessary to egg fertilisation. Acrosome status can be classified as intact, altered or lost. But the only functional categories that are interesting are intact and altered, because intact acrosomes are demanded to fertilise eggs.

How to evaluate sperm morphology and acrosome status: A rough evaluation can be performed easily at the same time as sperm concentration in measured with a haemocytometer chamber, using a simple bright-light microscope. The sperm samples must be fixed with formal saline or buffered glutaraldehyde solution. More precise examination needs the semen to be fixed and inspected under a phase-contrast microscope (1000x magnification) so general morphology and acrosome status are visible in greater detail: see examples in **Photos 1 + 3**.

For a simple yet precise evaluation, a semen sample is diluted 1:1 with staining solution (5% eosin, 10% nigrosin in a citrate solution pH = 7.4) and smeared. The air-fixed, stained spermatozoa are observed under a bright-light microscope at 400x or 1000x magnification. Their viability and morphology can be analysed at the same time. Those appearing red-pink in colour have a damaged membrane whereas white sperm are viable, as in **Photo 2**.

Also it is possible to use a fluorescence technique (by applying lectines, for example) to stain the acrosome and combine this with a staining agent such as propidium iodide to show viability, as in **Photo 4**. However, the cost of the equipment is high. Finally, at the research level, some interesting sperm images (**Photos 5 + 6**) have been obtained using a scanning electronic microscope.

Cut-off values for discarding an ejaculate: In the microscope observation, spermatozoa are categorised according to their morphology into those with normal appearance, cells with attached cytoplasmic droplets, folded tail, coiled tail and others (including a double tail and heads that are abnormal for size and structure). Most of the anomalies seen in practice relate to the presence of cytoplasmic droplets and folded tails. Coiled tails and others represent only a low percentage of total anomalies.

Sperm from fertile boars generally has fewer than 10% of anomalies. Any samples with more than 20-25% must be discarded. Usually acrosome alteration is lower than 10%. It must not be over 15%.

In a recent unpublished study we found significant cut-off values of 93% for normal acrosomes and 9% for total anomalies. But we do not think it is possible to suggest a general value because many factors relate to fertility, ranging from the number of sperm per dose to the insemination schedule and the length of time semen is stored.

How morphology and acrosome status relate to fertility: Many anomalies have been linked to cases of infertility. Certainly it is true that poorer fertility rates and litter sizes are observed when the number of morphological anomalies increases.

Cytoplasm droplets can be assumed to mean a lower fertilising potential since they indicate the sperm are immature. Tail alterations (folded or coiled) usually make the spermatozoa less motile by comprising the movement of the flagellum.

Acrosome damage is more difficult to quantify, even though we know that this cap must be intact in order for fertilisation to take place. Several authors

PHOTO 6: This scanning electronic microscope image shows boar sperm with coiled tail (arrowed). Acrosome seems to be intact (marked with star).

have described how high numbers of altered acrosomes are associated with fertilising problems. However, only a low degree of correlation has been found by research, between the percentage of normal acrosomes in a semen sample and the fertility resulting from its use in AI.