The importance of the male and the semen quality in swine reproduction

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The use of artificial insemination in swine is widespread worldwide, having a major impact on genetic improvement through the use of top genetic boars. So, the production efficiency of the breeding herds is highly dependent on the reproductive capacity (fertility) of these males.

Thus, poor quality boars will affect the reproductive performance of a great number of sows. Using traditional insemination, a boar can produce 600-800 litters per year, but if post-cervical insemination is used, a boar will produce about 2,000 litters per year and if this technique is combined with the new technology of single insemination at fixed time this amount can reach the amazing number of 4500-5000 litters per year. This gives us an idea about how important the role of the boar and the semen quality in swine reproduction is at present.

The aim of this article is to explain why some boars with 'good semen quality' have very low reproductive results, fertility and/or prolificacy, when used at farm level.

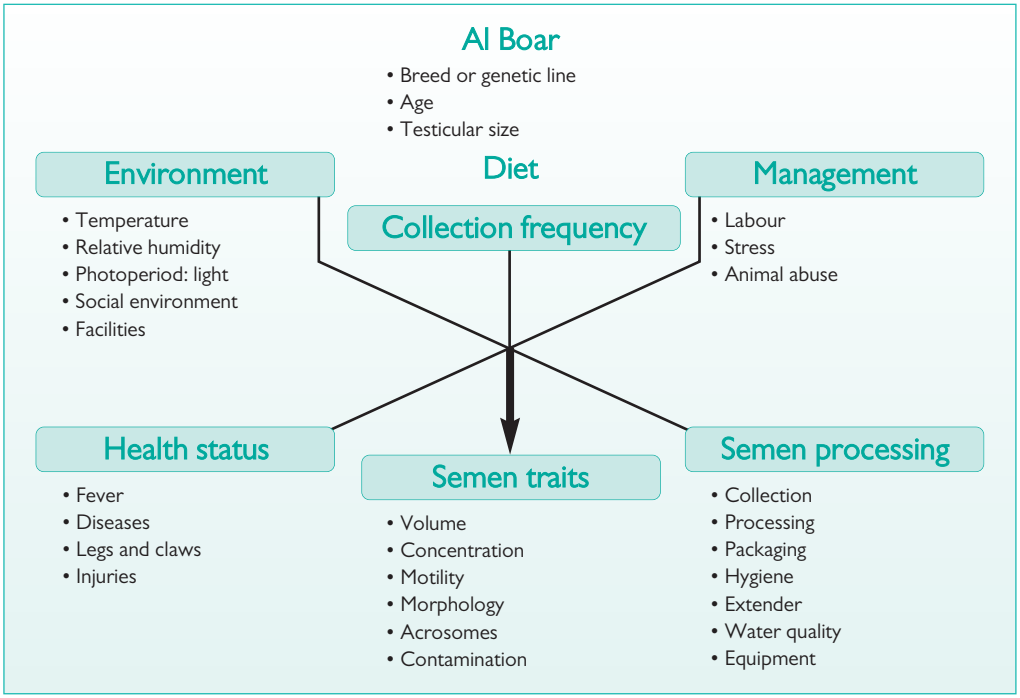

Fig. 1. Factors affecting boar semen quality.

Semen quality

Although semen quality is very important to obtain good reproductive results, the numerous factors that may affect the quality of the ejaculates (Fig. 1) make the correlation between these factors and the presence of one or more abnormalities very difficult.

Fig. 2. Sperm Chromatin Dispersion Test (Eva Green).

There are many articles which confirm that morphological aberrations in sperm have a negative effect on both farrowing rate and litter size. Feitsma et al., 2005 showed that an increase in abnormal forms from 10 to 20% decreases farrowing rate by 0.6%. In all their trials, farrowing rate decreased as the percentage of abnormal cells increased. Every 10% increase in abnormal sperm cells decreased litter size by 0.1 piglets.

Boars selected as breeding animals, and hence as semen donors in the boar studs, must show good to excellent semen quality. However, this does not ensure that they have a good or excellent in vivo fertility.

Good quality boar semen is essential to obtain satisfactory fertility rates. Standard assessments currently used to evaluate boar semen quality include sperm motility, viability, morphology and determination of concentration. When used individually, these standards tests have limited utility in determining the fertilising potential of an ejaculate, that is to say, their correlation with the field reproductive results is not too high.

However, these tests do have the ability to identify ejaculates of overtly poor quality. The evaluation of classical seminal parameters, under commercial conditions, allows the identification of ejaculates with poor fertility potential, but does not have high efficiency in predicting field fertility.

Effective prediction of relative boar fertility is essential and will allow the removal of less productive or sub-fertile boars from the commercial studs.

Fertilising capability

Semen quality is not the same as fertilisation capacity. This means that the use of males with excellent semen quality does not guarantee obtaining good results on fertility and/or prolificacy.

Fertilisation is a complex process involving a huge number of events:

- Transit to the place of fertilisation.
- Sperm-oviduct junction.
- Sperm capacity.
- Sperm-Zona pellucida junction.
- Acrosome reaction.
- Penetration of Zona pellucida.
- Fusion of the membranes of the sperm and oocyte.
- Penetration of the oocyte.
- Chromatin decondensation.
- Embryonic development.

Knowing all of this, we are able to identify the characteristics of a fertile ejaculate:

- Progressive motility.
- Appropriate morphology.
- Sufficient energy production.
- Capacity for hyper-activated motility.
- Structural and functional integrity of the membranes.
- Integrity of fertilisation associated enzymes.

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- Penetration ability.
- Intact and functional genome.

Therefore, all of these characteristics should be assessed before processing the ejaculate. The problem is that the routine examination of AI boar semen quality only includes, at most, the first two factors, motility and morphology, and if both are correct we assume that the rest is too.

Unfortunately, in many cases, this is not the case and when we use this semen at farm level poor reproductive results are obtained.

Even more, some cases of male infertility could be misdiagnosed as idiopathic because certain types of sperm abnormalities occur at the molecular level, many times in the absence of morphological manifestation detectable by light microscopy.

In 1994 Saacke et al. classified the sperm characteristics into compensable (problems with motility and morphology) and non-compensable traits (problems related to fertilisation and embryo development) based on whether reproductive performance improves when the number of cells is increased.

So far, the traditional use of insemination with a high number of sperms in each semen dose (usually more than three billion total sperm per dose), the widespread use of pooling semen (3-6 ejaculates) and the large number of services per heat (three and even more) have masked and compensated the negative effect of some boars which show reduced fertility when a lower number of sperm are used in the insemination.

In recent years this situation has radically changed: a reduced number of sperms per semen dose (2.0-2.5 billion with traditional insemination, 1.0-1.5 billion with post-cervical insemination or no more than 0.15 billion with deep intrauterine insemination); less services per heat (protocols with two inseminations per heat or just one with the new technology of single insemination at fixed time). This new situation meant that boars used without problems with traditional insemination have showed reduced reproductive performance when used in post-cervical insemination.

Using all of these new techniques and technologies, semen quality is the key to get excellent reproductive performances and the use of perfectly assessed semen is critical.

Semen assessment

Do field semen parameters always reflect fertility? The answer to this question is yes, but to a limited extent. Why? Because appearances are deceiving.

It is not difficult to find ejaculates classified as excellent after routine assessment which have very low reproductive performance especially when used at low concentration.

Sperms that seem to be perfect by light microscopy can have important non-compensable defects, mainly at chromatin or DNA level, which make the fertilisation or the normal development of the embryo impossible, resulting in a sub-fertile boar.

So, identifying these sub-fertile boars is essential to be successful when post-cervical or single fixed time insemination is used.

Without a doubt, the best way to identify these sub-fertile boars would be the single sire evaluation with the consideration that a boar that appears sub-fertile at a particular sperm concentration may improve if the number of sperms per dose is increased.

However, this system is slow and delayed and can be influenced by many factors that affect the female. In addition, almost all males show variations in semen quality throughout the year which would require having to repeat the test several times.

In recent years some biomarkers have been developed to identify molecular anomalies of defective sperms.

These biomarkers include fluorescent markers of acrosomal status, fluorochromes detecting altered sperm, chromatin or DNA integrity, vital dyes revealing sperm mitochondrial activity, probes detecting apoptotic events and antibodies detecting proteins that are either up or down-regulated in defective spermatozoa.

Within this last group we find some proteins or ligands that are uniquely associated with the defective spermatozoa carrying gross or subtle morphological defects, but critical, not detectable in conventional light microscopic analysis.

Peter Sutovsky and his group are working with a small protein called Ubiquitin which is associated with the surface of defective spermatozoa of some species including swine and the results are very promising.

There are several tests indicated to detect chromatin or DNA integrity: TUNEL Assay, COMET Assay, Sperm Chromatin Structure Assay (SCSA) and Sperm Chromatin Dispersion Test (SCDT) (Fig. 2).

Boar	Morphologically normal sperm (%)*	Sperm with chromatin instability (%)**	Farrowing rate (%)***	Piglets born alive
1	76.50^{ac}	0.16^{a}	88.92^{a}	11.21
2	74.29^{a}	0.92^{d}	83.57^{ab}	9.30
3	71.50^{ac}	0.60^{bd}	82.05^{ab}	10.61
4	64.00^{ad}	2.09^{bcd}	74.00^{bcd}	12.25
5	62.53^{cd}	4.67^{e}	65.91^{ce}	12.00
6	48.50^{ad}	0.55^{b}	75.88^{be}	10.56
7	47.20^{bd}	3.78^{ce}	59.30^{c}	10.60

^{abcde}Means with different letters in the same column are significantly different at *p<0.01 **p<0.05 and ***p<0.05 or p<0.001.

Table 1. The relationship between sperm quality traits and fertility of porcine semen (Adapted from Tsakmakidis).

Interesting correlation

An interesting study of Tsakmakidis et al., 2010 shows a strong correlation between fertility with live normal sperm and chromatin stability after the combination of both sperm traits. Seven boars were used in this study over a period of six months (Table 1).

Although the data shows a weak relation between farrowing rate and litter size, an inverse correlation was observed between fertility and morphological abnormalities. However, both parameters were affected by sperm DNA damage (SCSA).

If we pay attention to this table, we can see boar number 6 with less than 50% of live normal sperms but with good chromatin stability.

Conversely, boar 4 and 5 have more than 60% of live normal sperms but both show high percentage of chromatin instability and, consequently, less than 75% of fertility.

Due to the difficulty of assessing sperm chromatin integrity, it is almost impossible to involve this in the routine seminal tests. However, it could be applied periodically to be sure about the fertilisation capacity of the AI boars.

The sperm head is 90% composed of DNA and its shape is based on the structure of the DNA, so any change in chromatin structure will be reflected by a change in the sperm shape.

On this basis, K. L. Willenburg et al, 2012 studied this shape by Fourier Harmonic Analysis finding significant differences in the sperm head shape between boars with good and poor fertility rate, concluding that this analysis could be very useful for the boar centres in identifying boars with unacceptable fertility.

On the other hand, the impact of some chromosomal abnormalities on the reproductive performance of carrier animals has been widely reported in pigs where the most frequently reported chromosomal rearrangements are the reciprocal translocations which lead to a reduction in litter size. Nowadays, some genetic programs offer a karyotype analysis in all AI boars. The homologous in vitro fertilisation test is also a very good tool to identify sub-fertile boars.

Sperm concentration

Finally, with this new situation of reduced number of sperms per semen dose, the sperm concentration has become a critical point. We have to be totally sure about the sperm concentration of the ejaculate.

Many AI centres use photometers, colorimeters or haemocytometers for routine analysis of sperm concentration but, in the last years, CASA (Computer Assisted Semen Analysis) equipment is becoming more common on AI stations for routine analysis of semen (concentration, motility and morphology). However, all of these are not totally accurate and repeatable.

Newer instruments for measuring sperm concentration include the NucleoCounter SP100 which is a fluorescent counting chamber based method, but with higher repeatability and accuracy than the previous systems. Future developments will reduce the cost of all these analyses enabling AI centres to use them periodically to identify sub-fertile boars and, so, allowing work with low sperm concentration with full warranty. ■

References are available from the author on request