Fragmentation of sperm DNA in boars

by P. García-Casado, Dpto. Reproducción Animal, INIA, C. López-Fernández, A. Gosalvez and J. Gosalvez, Unidad Genética, Facultad Biología, UAM, and R. Sala Echave and B. Pérez-Llano, Gestión Veterinaria Porcina SL., C. Villalba, Madrid, España.

High standards of semen quality in boars exploited for artificial insemination are of economic relevance due to its association with fertility and offspring results. On average a boar produces approximately 1,800 semen doses per year and with a mean fertility of 85% would yield 510 litters per year. Using three semen doses per inseminated sow, with an average of 10 piglets per litter, a total of 5,100 piglets per boar per year would be obtained.

Therefore, the control of quality in semen used for artificial insemination (AI) must be as precise as possible. The usual parameters for assessing boar semen samples for AI include sperm motility, sperm morphology, acrosomal status and plasma membrane quality, whose normal values are already known for pigs, although in some cases they do not fully explain the loss of fertility observed in some boars.

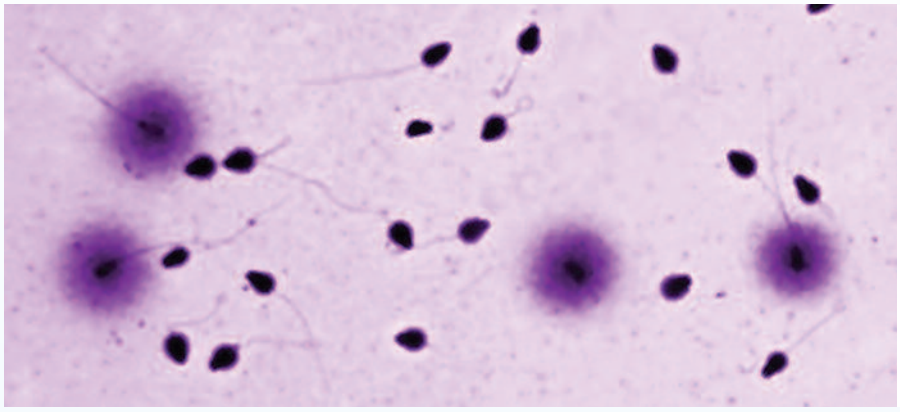
Other sperm characteristics, such as the status of the DNA structure in the sperm cell, of relevance in human sterility, are not considered important in the boar, in spite of its economic relevance.

During spermatozoa maturation from testis to caput epididimus, many structural and biochemical processes take place to achieve a highly chromatin package.

One of these processes is the replacement of histone by protamins as the DNA condensation takes place to achieve a compacted DNA molecule free of nicking.

When this process is not fully finished or when the sperm cell goes to apoptosis, DNA degrades, decreasing the fertile capacities of the animal.

In fact, a relationship between the level of sperm DNA fragmentation and fertility has been reported in humans and in other mammalian species.



Visualisation of sperm DNA fragmentation using the SCD test. Those sperm cells showing a large halo of chromatin dispersion contain a highly fragmented DNA molecule.

From a theoretical viewpoint, a fragmented DNA molecule cannot achieve fertilisation or if this occurs, probably an increase of embryo loss through damaged DNA would occur. However, little is known about sperm DNA fragmentation in boars and even the normal threshold values for sperm DNA fragmentation and its influence on fertility have not yet been well established.

This study, and other studies in the future, aim to establish sperm DNA fragmentation values which could be considered normal in a random population of boars used for AI purposes. In the first experiment 218 boars from different Spanish farms were included in the analysis. The boars were aged between one and three years and were Landrace, Large White, Pietrain and Duroc.

Semen was collected manually, diluted in Acromax extender (Gestión Veterinaria Porcina SL, Madrid, Spain) and preserved at 15°C.

The analysis of motility (M), normal acrosomes (NA), abnormal sperm morphology (ASM), proximal citoplasmic droplets (PD), distal citoplasmic droplets (DD) and coiled tails (CT) and sperm DNA fragmentation index (DFI) were performed after 24 hours of sperm storage at 15°C.

DFI was evaluated using the sperm chromatin dispersion (SCD) test. All measures were performed using a Sperm-Sus-Halomax commercial kit (Chromacell SL, Madrid, Spain; distributed by Gestión Veterinaria Porcina SL).

Basically, the methodology is simple to apply and includes some steps that are easy to perform in laboratories with basic equipment for routine semen analysis. After the SCD treatment, sperm cells with fragmented DNA develop a big halo of chromatin dispersion around a compact core. This morphology can be easily discriminated from those presenting absence of halo of chromatin dispersion, which correspond to those presenting unfragmented DNA.

For sperm DNA fragmentation assessment under bright field microscopy, a simple staining of SCD treated samples using a 10% Wright solution allows evaluation of sperm DNA fragmentation using 20 or 40x magnification. The DFI was calculated as a percent-

Continued on page 9

Table 1. Descriptive statistics of the semen quality parameters of a sample of 218 boar ejaculates.

	Mean	SD	Minimum	Maximum	Median
Motility (%)	68.8	16.1	10	90	70
Normal acrosomes (%)	74.6	13.1	12	100	76
Proximal citoplasmic droplets (%)	8.5	12.8	0	72	4
Distal citoplasmic droplets (%)	12.7	12.5	0	60	8
Coiled tails (%)	12.1	15.1	0	84	6
Sperm DNA fragmentation index (%)	3.7	6.9	0	47.9	1.67

	Motility	Normal acrosomes	Proximal citoplasmic droplets	Distal citoplasmic droplets	Coiled tails
Sperm DNA fragmentation index	-0.07	-0.11	0.44	0.03	0.13
Level of signficance	0.2	0.09	0.0001	0.7	0.05

Table 2. Pearson correlation coefficients between DNA fragmentation index and other semen quality parameters in a sample of 218 boar ejaculates.

Continued from page 7
age using a total of 500 cells. Data obtained from all the standard parameters of semen quality were compared with those of sperm DNA fragmentation using a Pearson correlation coefficient.

Mean values of semen quality and DFI in the 218 boar semen samples are shown in Table 1. The range of DFI values are similar to that observed in boars using alternative techniques based on flow cytometry or comet assay. Taking into account the values used as limits to select ejaculates for use in AI (≥ 70% M, ≥ 70% NA, < 20% ASM), it has been found that between 70-90% of the samples analysed are within these limits.

In fact, if we observe the medians in Table 1, these values correspond with a semen sample of acceptable quality for AI use.

As regards DFI, 86.2% of the analysed sample presented DFI ≤ 5%, 6.4% showed DFI >10% and 3.7% exhibited DFI >20% (Fig. 1). According to some previous data from human semen samples, a negative effect on fertility has been associated with DFI values reaching 15%, additionally the individuals are reported as sterile when DFI is 30% or higher.

When assisted reproductive techniques are used, a significant decrease in the fertilisation rate has been found with DFI values higher than 10%. If we take the values of 20% DFI as a limit, similar to that used with the percentage of abnormal sperm morphology in order to discard the ejaculates for AI, we should reject 3.7% of those considered 'good quality'.

Otherwise, we would risk losing 2.7 ejaculates/boar/year, 67 semen doses/boar/year, 19 litters/boar/year and finally 190 piglets/boar/year.

However, if we take into account that the normal DFI in the whole population does not exceed 5% DFI, higher values could be considered out of the norm, 13.8% of the samples analysed, and the possible undetected and detrimental economical effect would be greater.

In the present experiment no correlation between DFI and M, NA, DD or CT (Table 2) has been found. However, a positive and significant correlation was detected between DFI and PD. This association could be due to different causes related to the maturation process of the sperm cell or to the existence of oxidative stress associated with enzymatic activities on the cytoplasm droplets which has not been fully excluded from the sperm head.

On one hand, a lack of complete maturation in the sperm cell correlates with the incidence of proximal droplets and also with the presence of nicks in the DNA molecule that has not yet completed its process of condensation into the sperm nuclei.

On the other hand, the enzymes present in a cytoplasm droplet, placed in the neck near the sperm head, could affect the cell structures placed around it, such as the nuclear DNA.

In conclusion, in a sample of 218 random boar ejaculates, where 70-90% of them are of acceptable quality to use in AI, an incidence of 3.7% of ejaculates with a DFI higher than 20% has been observed.

This fact, together with the lack of correlation between some parameters, such as motility, acrosomal status, distal droplets, coiled tails and DFI, means that the convenience of adding sperm DNA fragmentation as a new parameter to the routine assessment of every ejaculate to be used in artificial insemination currently needs to be viewed with interest. ■

Fig. 1. Frequency distribution of data of sperm DNA fragmentation index in a sample of 218 boar ejaculates.