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Sperm chromatin structure integrity in liquid stored boar semen and its relationships with field fertility

G.B. Boe-Hansen [a,1,*], P. Christensen [a,2], D. Vibjerg [b], M.B.F. Nielsen [b], A.M. Hedeboe [b]

[a] *Section for Veterinary Reproduction and Obstetrics, Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Dyrlaegevej 68, 1870 Frederiksberg, Denmark*
[b] *Danish Meat Association, Axeltorv 3, 1609 Copenhagen V, Denmark*

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Abstract

Extended semen doses from some boars used for AI have been shown to develop high levels of sperm DNA fragmentation during storage. Studies in other animals and humans have shown that if DNA damage is present in a certain percentage of the sperm cells the fertility potential of the semen sample is reduced. The objectives of the present study was to determine the relationship between sperm DNA fragmentation measured using the sperm chromatin structure assay (SCSA) in extended stored semen and field fertility in the boar.

Three ejaculates from each of 145 boars were collected. Preparation of the semen doses included dilution with an EDTA extender and storage for up to 72 h post collection. The semen doses were assessed using flow cytometric methods for the percentage of viable sperm (PI/SYBR-14) and sperm DNA fragmentation (SCSA) at 0, 24, 48, and 72 h. A total of 3276 experimental inseminations in Danish breeding herds were conducted. The results showed that for 11 (7.6%) of the boars at least one of the three samples showed a value of DNA fragmentation index (DFI) above 20% within the storage period. Total number of piglets born (litter size) for Hampshire, Landrace and Danish Large White boars was, respectively, 0.5, 0.7 and 0.9 piglets smaller per litter when DFI values were above 2.1% as opposed to below this value. In conclusion the SCSA technique appears to be able to identify individuals with lower fertility with respect to litter size, and could in the future be implemented by the pig industry after a cost-benefit analysis.

Keywords: Boar; Spermatozoa; Sperm chromatin structure assay; Litter size; Fertility

1. Introduction

Today, artificial insemination (AI) with extended liquid boar semen is extensively used in the pig production. Extended liquid boar semen is often used within the same day of production, but may be stored at a temperature of 15–20 °C for up to 5 days before AI [1]. This semen storage technique has several advantages, including distribution of semen doses with a general preservation of fertility of the semen doses,

* Corresponding author at: Royal Veterinary and Agricultural University, Department of Large Animal Sciences, Section for Veterinary Reproduction and Obstetrics, Dyrlaegevej 68, 1870 Frederiksberg C, Denmark. Tel.: +45 35332986; fax: +45 35332972.
E-mail address: gbh@life.ku.dk (G.B. Boe-Hansen).
[1] Now at School of Veterinary Sciences, University of Queensland, St. Lucia, Queensland, Australia.
[2] Now at SPZ Lab A/S, Fruebjergvej 3, 2100 Copenhagen O, Denmark.

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however, an effect on farrowing rate and litter size may be detected when storage exceeds 48 or 72 h [2,3].

Johnson et al. [1] states that the structural and functional changes of spermatozoa, occurring during liquid storage of extended boar semen, resemble a natural ageing process which may depend on factors including conditions and length of storage. During storage of boar semen, changes in the traditional sperm parameters, including sperm motility, pH, and viability have been found [2,4] and also changes in energy metabolism [5] and sperm DNA integrity have been detected [6]. However, further studies revealing how in vitro storage affect the aging of the boar sperm cell and the subsequent effect on fertility is needed.

Several techniques have been used to evaluate sperm DNA fragmentation, and a number of these have also been applied to boar semen. These include the sperm chromatin structure assay (SCSA) [7], the comet assay [8] and the sperm chromatic dispersion test (SCD) [9]. The SCSA measures the susceptibility of sperm DNA to acid denaturation in situ using the metachromatic dye acridine orange combined with flow cytometry. Some of the advantages of the flow cytometric approach utilized in the SCSA compared to the microscope-based assays are that the number of cells assessed is significant larger and subjective evaluation of the cells is eliminated.

Since mature sperm apparently lack DNA repair mechanisms [10], the integrity of the sperm cell DNA relies on an effective defence against damage. It is speculated that these defence mechanisms include the tight structure of the sperm DNA and the antioxidant capacity of the seminal plasma and environment of the sperm cell [6]. The damaging effects of storage on the integrity of boar sperm DNA has previously been described, both with respect to liquid storage [6,8,11] and frozen storage [12–14]. Dilution conditions [11,15] and time of storage of liquid semen [6] have been shown to affect boar sperm DNA integrity, and these factors may perhaps subsequently result in a decreased fertilization capacity and defective early embryonic development, due to insufficient repair of the DNA damage. Further species specific research is required however studies in the mouse [16] and bovine [17] have shown that defective blastocyst formation may only occur after a certain level of sperm DNA damage induced by irradiation.

A number of studies have indicated the potential of the SCSA in the assessment of boar semen [6,7,18]. However, studies relating the variables obtained from the SCSA, including the DNA fragmentation index (DFI), with fertility are few. In a previous heterospermic boar trial the SCSA correctly predicted the three low fertility boars out of six boars [7]. In other preliminary work, correlation between the sperm DNA integrity and fertility, measured as number of live born piglets, have been found [19,20].

Since the sperm trait DNA integrity is hypothesized to be uncompensable [21], as the above studies also suggest, the effect on fertility in a polytocous species like the pig, may be reflected both on pregnancy and farrowing rates, but also on litter size. However, in order to conclude on the effects of the sperm DNA integrity on fertility variables in the boar, the negative effects of semen handling and storage on boar sperm DNA integrity that has previously been documented needs to be considered in the experimental design. Therefore field fertility studies need both to include factors such as condition and duration of semen storage prior to use and assessment of sperm DNA integrity in the insemination doses as well as detailed insemination results for both pregnancy and litter size at different time points.

The objective of the present study was to determine correlations between SCSA variables and sperm viability and field fertility measured as litter size for boar semen, extended and liquid stored at 18 °C for up to 72 h.

2. Materials and methods

2.1. Sample collection and preparation

During a period of 24 weeks from December 2004 to May 2005, experimental semen doses were collected on Sundays from 160 boars placed in six Danish boar stations. The boars were collected routinely for artificial inseminations and were between 8 and 15 months of age at the time of the experiment. The aim was to analyse semen from three ejaculates from each boar and obtain a distribution of approximately 20% Hampshire, 40% Danish Large White, and 40% Landrace boars. The sperm rich fraction of the ejaculate was collected using the gloved hand technique and was weighed to determine the semen volume. The sperm concentration in the raw sample was determined using the SP-100 NucleoCounter (ChemoMetec A/S, Allerød, DK). The percentage of motile sperm was assessed microscopically using phase contrast at 200× magnification. After the immediate semen assessments, dilution to an approximate concentration of 92×10^6 motile sperm/mL was performed in two steps using an EDTA boar semen extender (Leo Animal Health, Uldum, DK), containing 57.4 g/L glucose anhydrate, 3.5 g/L disodium EDTA, 3.5 g/L sodium citrate, and 1.1 g/L sodium bicarbonate, with a pH between 6.8 and 7.0. To