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Sperm DNA fragmentation in boars is delayed or abolished by using sperm extenders

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Abstract

The semen quality of seven young adult boars was assessed for percentages of sperm motility, normal acrosomes, abnormal sperm, cells positive to sHOST (short Hipoosmotic Swelling Test), HPNA cells (sHOST Positive with Normal Acrosome cells) and the percentage of sperm heads, which exhibited DNA fragmentation using the Sperm Chromatin Dispersion test (SCD). These parameters were analysed in sperm samples both undiluted and diluted using a commercial extender and stored at 15 °C for 21 days. Results showed that semen quality decreases faster in the undiluted semen samples from day 0 to day 7 compared to diluted semen samples that remained with a high quality up to day 11. The undiluted semen exhibited a low DNA fragmentation index (DFI) during the first days and then a significant increase from day 7 up to day 21. This increase in the DFI coincided with the lowest levels of the other semen quality parameters. On the contrary, the samples diluted in the commercial extender showed very low levels of DNA fragmentation in all boars during the preservation period. When the evolution of DNA fragmentation was analysed in the undiluted samples, differences were found among boars. These differences were not shown in the samples diluted in the extender where the basal DFI remained stable during the 21 days. The main conclusion of this study was that some sperm extenders delay or partially prevent sperm DNA fragmentation.

Keywords: Sperm DNA fragmentation; DNA damage; Sperm chromatin; Sperm Chromatin Dispersion test; Boar spermatozoa

1. Introduction

Damaged DNA in the single sperm cell that fertilizes a female oocyte can have a dramatic negative impact on foetal development and health of the offspring. Thus, sperm DNA fragmentation tests provide a reliable analysis of the DNA fragmentation index (DFI) that may help to identify individuals who are at risk of failing to initiate a healthy pregnancy [1]. Information about sperm DNA integrity may help in the clinical diagnosis, management and treatment of male infertility and may be of prognostic value in assessing the outcome of an assisted conception treatment [1,2]. Although most information about DNA fragmentation has been obtained from humans, in animals, especially in mammals, it may be assumed that similar factors are the effectors of sperm DNA fragmentation. However, they have been less studied because of the absence of adapted technologies to visualize such effects. In the particular case of boar sperm samples DFI has been

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analysed using four different strategies, SCSA (sperm chromatin structure assay; [3], comet-assay [4,5], TUNEL assay [6] and recently a modified SCD test (Sperm Chromatic Dispersion test; [7,8]). These studies point to the fact that DNA fragmentation is positively correlated with a decrease in fertilization success.

In humans, the causes of high DNA fragmentation are usually related with chemical or radiation exposure, heat exposure, several types of infection, testicular cancer, age, and of course, oxidative stress through an increase of the free radical levels in the semen [9]. In addition, it is very important to be aware that DFI can change with time during the life of a male. Therefore, a good tracking of the sperm quality during different periods of life could assure the selection of the best samples, which could be preserved until its utilization. In boars, especially those used for reproductive purposes, a high incidence of those causes, which may produce sperm DNA fragmentation in humans are not expected, although they can not be disregarded. This is mostly because donor males are young and have been selected for reproductive strategies. However, no strict biological policies, such as the use of extenders and storage at 15 °C to fertilize receptive females, are a common practice. Even within this scenario, it is completely unknown how the quality of the DNA, in terms of DNA fragmentation, is affected in sperm samples, which have been stored in diluents. The aim of the present investigation was to analyse the stability of DNA in boar sperm samples under different conditions of preservation, in an attempt to throw some light on the dynamics of DFI.

2. Materials and methods

Seven adult boars aged 18–24 months were used to perform this experiment. These animals were selected from a series of 52 individuals, which were studied for DFI and standard semen parameters. The criterion to select the seven individuals to be included in the analysis was animals with DFI lower than 5% and high quality in standard semen parameters. Thus, semen quality was assessed for percentage of sperm motility, percentage of normal acrosomes, percentage of abnormal sperm, percentage of cells positive to sHOST (short Hipoosmotic Swelling Test), percentage of HPNA cells (sperm cells sHOST positive with a normal acrosome) and percentage of DNA fragmentation.

Boars were fed with a commercial porcine ration. Water was available *ad libitum*. Semen collection was performed with the gloved hand method, discarding gel fraction and seminal plasma.

DFI was independently assessed in two different laboratories and the mean was included in the analysis. From every ejaculate two samples were taken: one sample of 20 ml was kept in a plastic tube without extender and the other sample of 2 ml was extended in 18 ml of ACROMAX® (GVP, SL, Madrid, Spain). After an equilibration period of 3 h at room temperature, both samples of each ejaculate were preserved at 15 °C for 21 days. During the preservation period, samples were taken on days 1, 3, 4, 5, 7, 8, 10, 15 and 21 to check for the same parameters of semen quality as stated above.

Sperm motility was checked manually using negative phase contrast microscopy with 20× objectives. Semen samples were placed on a warm slide at 37 °C. Acrosomal status was analysed in semen samples fixed in a 2% glutaraldehyde solution [10]. Observations were performed under phase contrast using 100× objectives. One hundred sperm cells were evaluated as intact or damaged acrosome according to the criteria previously established [10]. Sperm cells with an intact acrosome were considered those with a normal apical ridge. Sperm cells with damaged acrosome were those that showed damaged or missing apical ridges or loose acrosomal caps. In the same slide, the percentage of abnormal sperm was evaluated by counting 100 sperm cells and calculating the percentage of proximal and distal droplets, coiled tails and other sperm abnormalities.

The percentage of sHOST positive cells was calculated by placing a 0.1 ml (undiluted) or 0.35 ml (diluted) semen sample into 1 ml of hypoosmotic solution (75 mOsm/kg) in a water bath at 37 °C for 5 min. After incubation a sample of 0.35 ml was removed and fixed in 0.5 ml of 2% glutaraldehyde solution and evaluated in a phase contrast microscope using 100× objectives and counting in 100 sperm cells those with any degree of a coiled tail (sHOST positive cells) and those with a straight tail (negative sHOST cells) [11]. In the same preparation, the HPNA value was calculated by counting in 100 sperm cells positive sHOST cells with a normal acrosome [12]. This fraction represents the population of sperm cells with the most resistant sperm membrane.

To determine DFI in boar sperm cells, the Sperm-Sus-Halomax® kit (ChromaCell SL, Madrid, Spain) was used. Twenty-five microliters of diluted sperm were added to a vial with low melting agarose and mixed. Provided pre-treated slides were placed onto a metallic plate, which had been previously cooled at 4 °C. A drop of the cell suspension was spread onto the treated face of the slide and covered with a glass coverslip for 5 min