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DNA integrity in sexed bull sperm assessed by neutral Comet assay and sperm chromatin structure assay

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Abstract

During the production of sex-sorted spermatozoa from bull semen, the cells are exposed to a number of potential hazards including: dilution, centrifugation, incubation, exposure to DNA stains and laser light. These factors may affect the survival capacity and fertilization potential of the sperm. The objective of this study was to determine whether sex-sorted bull spermatozoa have more DNA damage than sperm from conventional processed bull semen. Two methods were used to determine DNA integrity: the neutral Comet assay (NCA) and the sperm chromatin structure assay (SCSA). The NCA showed that the conventional samples had a higher tail moment (TM) ($P < 0.017$) than the sorted samples and that there was no difference between the samples in tail length (TL) ($P = 0.36$). The SCSA showed that the DNA fragmentation index (DFI) was higher for conventional than the sorted samples ($P = 0.011$), but the standard deviation of DFI (SD-DFI) was higher for the sorted samples ($P < 0.001$). We conclude that the NCA and SCSA can be used in assessing DNA integrity in bovine sperm and that cell sorting by flow cytometry improves the integrity of the sperm cell population. Additionally the results from the SCSA indicated that the sex-sorted sperm had less homogenous sperm chromatin. In the future assessment of sperm DNA

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integrity may be used to select bulls for sperm sex sorting and optimizing sperm sex sorting procedures.

Keywords: Flow cytometry; Single cell gel electrophoresis; Sex sorting; Spermatozoa; Bovine; DNA

1. Introduction

Sex-sorted bovine spermatozoa have been commercially available since the beginning of this millennium. The pre-selection of sex is of great interest in the dairy and beef cattle industry, making production of offspring with a desired sex an advantage in commercial production [1]. In 1987, Johnson et al. [2] were able to separate animal X and Y chromosome bearing spermatozoa using flow cytometry into two enriched populations based on the difference of total cellular DNA (US Patent #5135759). The method measures DNA content of individual sperm cells via fluorescence of the DNA-binding dye, Hoechst 33342, while the sperm are processed and sorted through a flow cytometer. The procedure has proven effective in the X and Y sorting of bovine spermatozoa, with a purity of about 90% [3].

During the production of frozen sex-sorted sperm, the sperm cells are exposed to a number of potential hazards. The factors include dilution, incubation, exposure to DNA stains, elevated pressure and laser light, centrifugation and freezing. These factors may affect the survival capacity and fertilization potential of the sperm. It has previously been shown that the DNA of the sperm can be damaged by external factors such as type of semen extender, dilution, prolonged incubation time [4] centrifugation and freezing-thawing [5]. The external factors can contribute to an increase in the number of damaged and dying sperm in the sample, which may produce reactive oxygen species (ROS) [6]. It has been shown that ROS can result in oxidative damage to the membranes and may also attack DNA and cause strand breaks and base damage. As maturing sperm discard the majority of their cytoplasm during the final stages of spermatogenesis, they lose some of their defense-enzymes, which protect cells from oxidative damage and the exchange of histone to protamine condenses the chromatin and thereby limits the proteins of the DNA repair machinery to gain access to the DNA [7]. Spermatozoa rely entirely on the very tight compact packing of their DNA around protamines, which reduces the exposure of the DNA to free radicals, as well as the antioxidative capacity in the seminal plasma [8]. Damage induced to the DNA of the mature ejaculated sperm during semen processing and handling will therefore accumulate.

The fertilization potential of flow-sorted bovine sperm has been demonstrated in IVF systems. In one study it was found that the blastocyst rate of sorted frozen semen was significantly lower compared to the blastocyst rate when using unsorted frozen semen [9]. In a more recent study using ejaculates from three different bulls, it was shown that neither sorting nor staining affected the embryonic development up to the blastocyst stage. However, a variation among bulls for sorted semen for both cleavage and blastocyst rate was observed [10]. In vivo, only a few studies have compared the fertility of sex-sorted