Effects of airport screening X-irradiation on bovine sperm chromatin integrity and embryo development

K.E.M. Hendricks [a], L.M. Penfold [b,*], D.P. Evenson [c], M.T. Kaproth [d,e], P.J. Hansen [a]

[a] Department of Animal Sciences, University of Florida, Gainesville, Florida, USA
[b] White Oak Conservation Center, Yulee, Florida, USA
[c] SCSA Diagnostics, Brookings, South Dakota, USA
[d] Genex Cooperative, Ithaca, New York, USA
[e] Department of Animal Science, Cornell University, Ithaca, New York, USA

Abstract

Biological samples, including cryopreserved sperm, are routinely X-rayed during air shipment. The goal was to investigate the impact of X-irradiation used for checked and carry-on luggage on bovine sperm chromatin integrity and postfertilization in vitro embryonic development. Frozen domestic bull sperm (*Bos taurus*) (n = 9 bulls) stored in a dry shipper (-160 °C) was screened by X-irradiation 0, 1, 2, and 3 times as either carry-on or checked luggage. Duplicate straws were thawed, and sperm were assessed for chromatin damage using the sperm chromatin structure assay (SCSA) and by postfertilization in vitro developmental competence of mature oocytes. Multiple exposure to X-rays did not significantly affect sperm chromatin integrity assessed by SCSA. There were lower proportions of oocytes cleaved (P = 0.07; $21.6 \pm 3.1\%$ vs. $29.4 \pm 3.1\%$, $24.9 \pm 3.1\%$, and $25.7 \pm 3.3\%$ for 3 vs. 0, 1, and 2 times, respectively; least-squares means $\pm$ SEM) and that developed to blastocysts (P = 0.06; $9.0 \pm 1.7\%$ vs. $13.8 \pm 1.7\%$, $11.5 \pm 1.7\%$, and $12.6 \pm 1.9\%$, respectively) when fertilization was performed with sperm X-rayed 3 times using checked luggage irradiation; developmental competence (percentage cleaved embryos becoming blastocysts) was unaffected. There were no deleterious effects of other X-irradiation treatments on embryo development. We inferred that screening by X-irradiation may reduce the ability of sperm to activate oocyte cleavage after multiple exposures at the checked luggage dose. However, there was no evidence that competence of embryos to become blastocysts was reduced by X-irradiation ($45.4 \pm 5.7\%$, $40.4 \pm 5.7\%$, $46.4 \pm 6.1\%$, and $41.8 \pm 5.7\%$ for 0, 1, 2, and 3 doses, respectively), but potential long-term epigenetic effects are unknown.
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1. Introduction

Air transportation of biological material such as sperm, ova, and embryos has become common with the advent of assisted reproductive technologies. These biological materials are exposed to X-irradiation at ports of entry and exit. It is not known whether the levels of X-irradiation employed at airport security checkpoints adversely affect bovine sperm chromatin or affect sperm potential fertilizing ability and embryo development rates, although in a previous study, DNA in domestic cat spermatozoa was negatively affected by exposure to multiple doses of X-rays through airport screening devices [1]. It is noteworthy that X-irradiation has been shown to cause DNA strand breaks and

chromosomal aberrations in somatic cells [2,3], germ cells [4–6], ejaculated sperm [7,8], and embryos [9,10]. Furthermore, DNA damage in the male gamete has been attributed to low fertilization rates [11–13], reduced embryo development [14,15], and increased embryo and fetal losses [16,17]. Sperm DNA damage has also been associated with an increased incidence of cancer in offspring produced from such damaged sperm [18,19]. The goal of this study was to investigate whether exposure of frozen bovine sperm to X-rays generated by airport security X-ray machines for carry-on luggage and for checked luggage would induce changes in sperm chromatin and cause a reduction in rates of in vitro fertilization and embryo development.

2. Materials and methods

2.1. Sperm treatment and preparation

Semen from bulls (*Bos taurus*) of known fertility (n = 8 Holstein and 1 Jersey) used in a commercial artificial insemination program were cryopreserved in a standard commercial milk-based extender containing 7% (vol/vol) glycerol in 0.5-mL straws. Conception ratings of the nine bulls (mean value for all bulls set to 0) were −3.8, −3.1, −2.3, −2.2, −1.3, −0.9, 0.6, 0.6, and 3.0 (reliabilities varied from 89.9 to 98.8). Cryopreserved straws were transported in liquid nitrogen by ground transportation from the processing site (Genex Cooperative, Ithaca, NY, USA) to the testing location. Overland transportation ensured that sperm were not exposed to irradiation before treatment. Bulls chosen for this trial had not previously been tested in in vitro systems and included five males of known lower fertility that were selected to examine any chromatin differences between bulls of known normal fertility versus lower fertility. Sixteen straws from each of the nine bulls were transferred from liquid nitrogen storage (−196 °C) to nitrogen vapor (dry shipper, −160 °C) and transported through airport security X-ray machines either 0, 1, 2, or 3 times using a standard checked luggage X-ray machine administering 34 to 36 mrem or a carry-on (hand) luggage X-ray machine administering <1 to 10 mrem, quantified by dosimeters (Luxel; Landauer Inc., Glenwood, IL, USA). A control group of straws was similarly maintained in a dry shipper for an equivalent interval but was not exposed to X-irradiation. Thus, four straws from each bull were exposed to each treatment. Duplicate straws were subsequently taken for sperm chromatin structure assay (SCSA) analysis and in vitro fertilization and embryo development studies.

2.2. SCSA analysis

Duplicate straws of frozen bull semen were thawed in a 37 °C water bath. Thawed semen was diluted with TNE (0.01 mol/L Tris[hydroxymethyl]aminomethane [Tris]-HCl, 0.15 mol/L NaCl, and 1 mmol/L EDTA, pH 7.4; Sigma Chemical Co., St. Louis, MO, USA) buffer to a concentration of 1×10^6 to 2×10^6 cells/mL. Details of the SCSA are described elsewhere [20]. Red and green fluorescence of each acridine orange (AO)-stained spermatozoa was measured using a Cytofluorograf II (Ortho Diagnostic Systems, Westwood, MS, USA) flow cytometer unit equipped with a Lexel 100-mW argon-ion laser operated at 35 mW output and 488 nm wavelength and interfaced to a Cytomation Data Handling System (Fort Collins, CO, USA). Increased red fluorescence was indicative of increased DNA fragmentation and quantified using the DFI (DNA fragmentation index); a ratio of red to total (red + green) fluorescence of each individual spermatozoon [20]. Analysis of the resultant DFI histogram composed of 5000 spermatozoa per sample determines the percentage of spermatozoa with increased DNA fragmentation (%DFI). Each of these values indicates the extent of abnormal chromatin structure, with a higher number indicative of increased DNA fragmentation [20].

2.3. Oocyte maturation, in vitro fertilization, and embryo production

Thawed sperm were centrifuged at $300 \times g$ for 20 min on a mini Percoll gradient of 0.5 mL 45% Percoll (Sigma-Aldrich Corporation St Louis, MO) (vol/vol). The sperm pellet was washed in 5 mL Sp-TALP (Sperm Talp media), centrifuged at $300 \times g$ for 5 min, and the remaining pellet was resuspended in Sp-TALP to a concentration of 17×10^6 spermatozoa/mL. In vitro fertilization and embryo production was performed as previously described [21], except that oocytes in a single replicate were inseminated with semen from a single bull. Cumulus-oocyte complexes (COCs) from abattoir ovaries were washed twice in OCM (Oocyte culture media) and placed in groups of 10 in 50-μL drops of OMM (Oocyte maturation media) overlaid with mineral oil and matured for 20 to 22 h at 38.5 °C, 5% CO_2 in humidified air. Groups of matured oocytes (30 to 50) were fertilized with 30 μL (5×10^5) Percoll-purified spermatozoa from a single bull. After 8 to 9 h of coincubation at 38.5 °C, 5% CO_2 in humidified air, presumptive zygotes were denuded of cumulus cells by vortexing in 100 μL hyaluronidase (1000 IU/mL in approximately 0.5 mL HEPES-TALP) and cultured in