EFFECTS OF EXPOSURE TO X-RAY AT AIRPORT SECURITY CHECKPOINTS ON MEMBRANE INTEGRITY OF CHILLED CANINE SEMEN

Simona Sakalauskaitė[1], Jakov Šengaut[3], Neringa Sutkevičienė[2], Henrikas Žilinskas[2], Arūnas Rutkauskas[2], Vita Riškevičienė[1]

[1]Department of Veterinary Pathobiology
[2]Large Animal Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės 18, LT-47181, Kaunas, Lithuania; e-mail: simona.sakalauskaite@lsmuni.lt
[3]Private Small Animal Clinic 'Jakovo veterinarijos centras', Gerosios Vilties 1, LT-03147, Vilnius, Lithuania

Abstract. This study was conducted to investigate effects of X-irradiation on motility and viability of canine spermatozoids in chilled semen samples over the storage period of 5 days. Diluted semen samples from 10 different dogs were divided into 2 aliquots, and 1 group of aliquots was exposed to X-irradiation by transporting it through airport security X-ray machine (HI-SCAN 100100 T). Evaluation of total and progressive spermatozoids motility was done by Sperm Class Analyzer (SCA) (Spain, 2011). To detect spermatozoa with a biochemically active plasma membrane, a hypo-osmotic swelling test was used. Assessment of spermatozoa with a structurally intact plasma membrane was done using SYBR-14/PI (Molecular Probes) fluorescent staining. Our study showed that the total and progressive motility ($P > 0.05$) and the percentage of canine spermatozoa with a functional ($P \leq 0.05$, $P \leq 0.01$) and intact ($P > 0.05$) membrane over the storage period of 5 days were lower in the samples exposed to X-irradiation compared with the control group.

Keywords: canine semen, X-ray, plasma membrane integrity

Introduction. Insemination with chilled semen is commonly used in dog breeding. In the recent years, it has become usual to transport chilled canine semen internationally to prevent inbreeding and to improve genetic variety (Kmenta et al., 2011). Nowadays, it is common to use air transportation for biological material, including semen (Hendricks et al., 2010). Canine semen, as every other cargo, is exposed to X-irradiation at airport security checkpoints. It is well known that exposure to X-irradiation can cause double and single DNA strands breaks. In one study, the motility of frozen domestic feline spermatozoa decreased after exposure to X-ray. These results show that exposure to X-irradiation at airport security checkpoints may have a negative impact on semen and other important biological material's quality parameters (Gloor et al., 2006). However, there is no information whether exposure to X-ray at airports has any negative effects on motility and viability of spermatozoa of chilled canine semen.

Although there are several quality parameters of canine semen, one of the most important is integrity of the spermatozoal membrane. The plasma membrane is essential for sperm survival in a female reproductive tract, capacitation and fertilisation (Mocé & Graham, 2008; Santos et al., 2011). These processes depend on the integrity of the spermatozoal plasma membrane, which can be assessed by the hypo-osmotic swelling test (HOST) (Goericke–Pesch & Failing, 2013) and fluorescent staining.

The HOST method is based on the principle that under hypo-osmotic conditions biochemically active spermatozoa with a functionally intact membrane absorb water and swell increasing in volume until equilibrium is reached (Jeyendrean et al., 1984; Cabrita et al., 1999; Amorim et al., 2009). This test is used to assess sperm membrane functional integrity (Kargei et al., 2014). Spermatozoal plasma membrane structural integrity is assessed using amphipathic probes with the acyl group, which can penetrate intact membranes (Donoghue et al., 1995).

The aim of our study was to investigate effects of exposure to X-ray at airport checkpoints on motility and viability of chilled canine spermatozoa over the storage period of 5 days.

Methods and materials. Semen samples were collected from 10 different healthy dogs. The dogs were 2 to 5 years old. The semen was collected by digital manipulation into plastic tubes separating 3 fractions of the ejaculates. Only the sperm rich fraction was used in this study. Each ejaculate was divided into 2 equal aliquots. Both aliquots were diluted with CaniPlus Chill 10 extender (Minitüb, Germany). One group of aliquots was transported through airport security X-ray machine (HI-SCAN 100100 T) one time.

Volume of ejaculate, concentration and morphology of spermatozoa and subjective motility of fresh canine semen were assessed using conventional semen evaluation methods (Januškauskas, 2010). Only the samples with normal sperm concentration and low spermatozoa morphological abnormalities were used in the study.

The extended semen samples were kept at 4°C and examined every day over the storage period of 5 days. The total and the progressive motility parameters of the extended semen samples were determined using the computer-assisted sperm analysis (CASA) system Sperm Class Analyzer (SCA V.5.1.) (Microptic. S. L., Spain). The motility of spermatozoa was examined at 37°C under phase-contrast microscope Olympus BH2 with a pre-warmed 37°C stage (Olympus Optical Co., Ltd., Japan) using a 100X magnification. A 5-µL aliquot was placed on a pre-warmed 37°C microscope slide, covered with a

coverslip (18 × 18 mm) and examined through the SCA. The proportions of the total and the progressive motile spermatozoa were recorded. Three fields of each sample were recorded, and after recording each field was manually edited for misidentified cells.

The biochemical integrity of the spermatozoal membrane was investigated using the hypo-osmotic swelling test (HOST) (Januškauskas, 2010). This test was carried out using 100 mOsm/L hypo-osmotic solution. The extended semen samples were mixed together with 500 µL of hypo-osmotic solution and incubated for 1 hour at 37°C. Two hundred spermatozoa were counted at 400X with the phase-contrast microscope to identify spermatozoa with curled tails. Spermatozoids with curled tails were considered to have functionally intact cell membranes, while spermatozoids with straight tails were considered to have damaged cell membranes.

The sperm membrane integrity was also assessed using LIVE/DEAD® Sperm Viability Kit (Molecular Probes, Germany). The semen samples of 50 µL were mixed with 450 µL phosphate-buffered saline (PBS). Aliquots of 500 µL semen solution were mixed with 100 nM 2.5 µL SYBR 14 and incubated for 10 min at 37°C. Then, 2.5 µL of 12 µM propidium iodide (PI) was added to each suspension and incubated for 10 min at 37°C. Two hundred spermatozoa were counted at 200X magnification using a fluorescence microscope. The spermatozoa which were seen as fluorescent green were regarded as live with intact cell membranes and others seen as fluorescent red or orange as dead with damaged cell membranes.

The statistical analysis was performed using computer software SPSS 22.0 programme (Statistical Package for Social Sciences 22 for Windows). The results were presented as the mean and standard deviation (SD). An independent samples t test was used to evaluate semen exposure to X-ray on sperm motility and viability. The level of significance was set at $P < 0.05$.

The study was conducted in compliance with Lithuanian animal welfare regulations (No. B1-866, 2012; No. XI-2271, 2012) and was approved by the Lithuanian Committee of Veterinary Medicine and Zootechnic Sciences (Protocol No.07/2010).

Results. The mean volume of sperm rich fraction of the fresh semen samples was 1.53 ± 1.1 mL. The mean concentration was 431.1 ± 134.44 × 10^6/mL (minimum 254 × 10^6/mL, maximum 479 × 10^6/mL). The total motility of the fresh canine semen assessed subjectively was 82.25% ± 9.66%. The percentage of the spermatozoa with morphological abnormalities was 17.08% ± 7.45%, of which 12.08% ± 5.41% of those spermatozoa were with tail deviations and 5.0% ± 2.04% with head defects.

The total and the progressive motility were lower over the storage period of 5 days in the semen samples exposed to X-ray compared with the control samples, but no statistical difference was found (Fig. 1.). The percentage of the spermatozoa with a biochemically active plasma membrane evaluated by the HOST was significantly lower 4.44% ($P \leq 0.05$) on the first day in the semen samples exposed to X-ray in comparison with the control group. On the second day, this difference increased to 8.18% ($P \leq 0.01$) and on the third day to 12.38% ($P \leq 0.01$). On the fourth and fifth days of the study, the percentage of the spermatozoa with a biochemically active plasma membrane was lower in the semen samples exposed to X-ray compared with the control samples, but there was no statistical difference between these groups (Fig. 2.).

The percentage of the spermatozoa with a structurally intact membrane assessed by SYBR 14/PI was 84.25% ± 11.60% in the semen samples exposed to X-ray, and it was by 2.44% lower than in the control samples. The percentage of the spermatozoa with an intact membrane decreased by 33.4% in the samples exposed to X-rays, while in the control samples only by 24.88% over the storage period of 5 days, but no statistical difference was found (Fig. 3.).

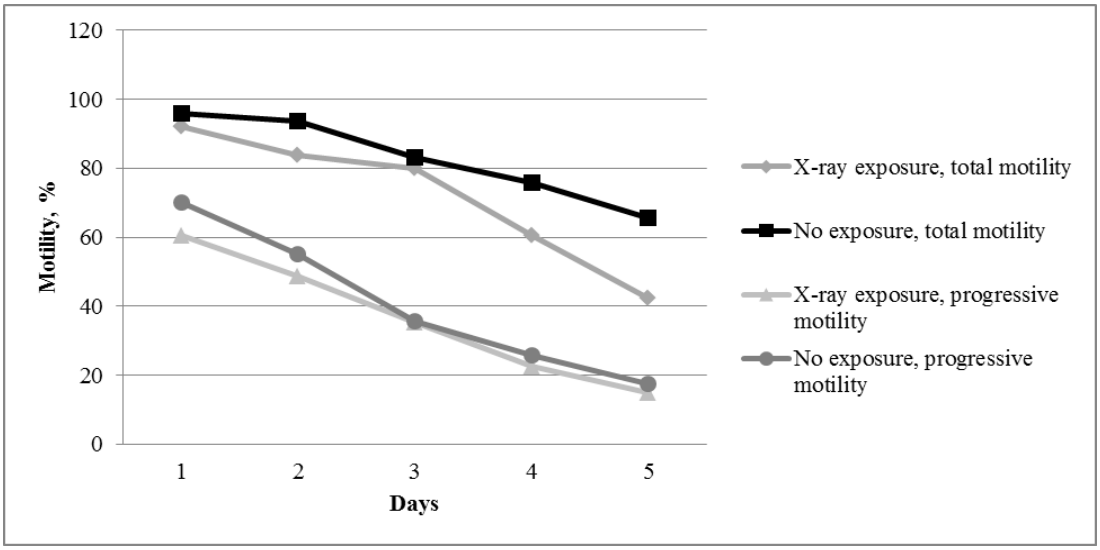

Fig. 1. **Changes of total and progressive motility of canine sperm exposed and not exposed to X-ray over the storage period of 5 days**

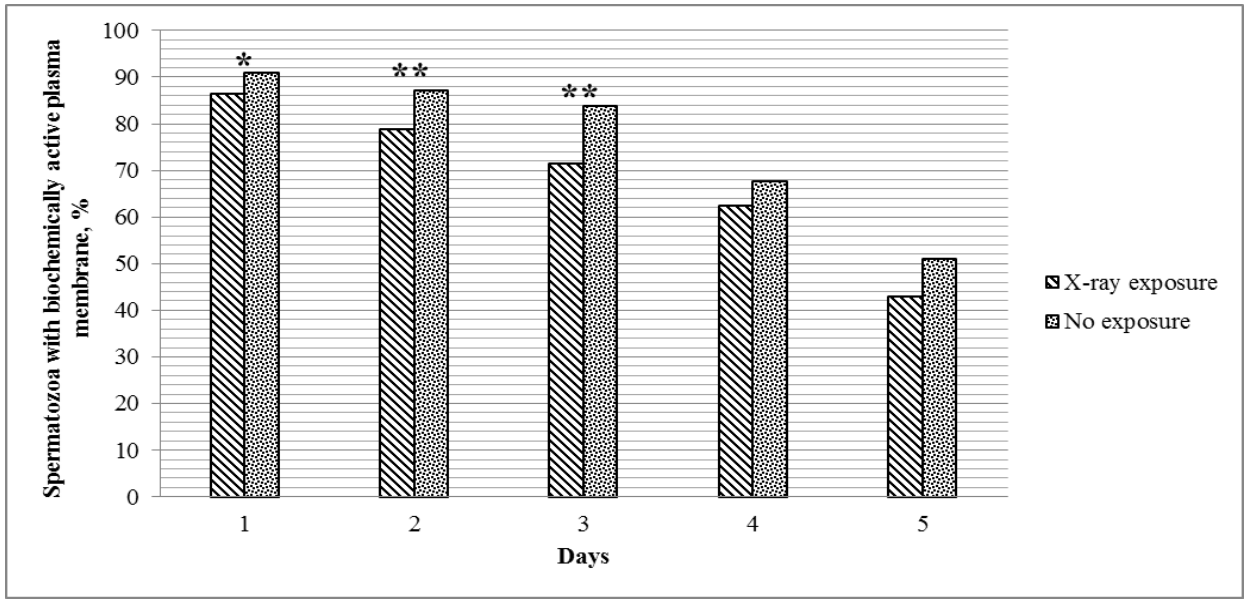

Fig. 2. **Difference of canine spermatozoa with a biochemically active plasma membrane between exposed and not exposed to X-ray over the storage period of 5 days**; **significant difference between groups **P ≤ 0.01, * P ≤ 0.05**

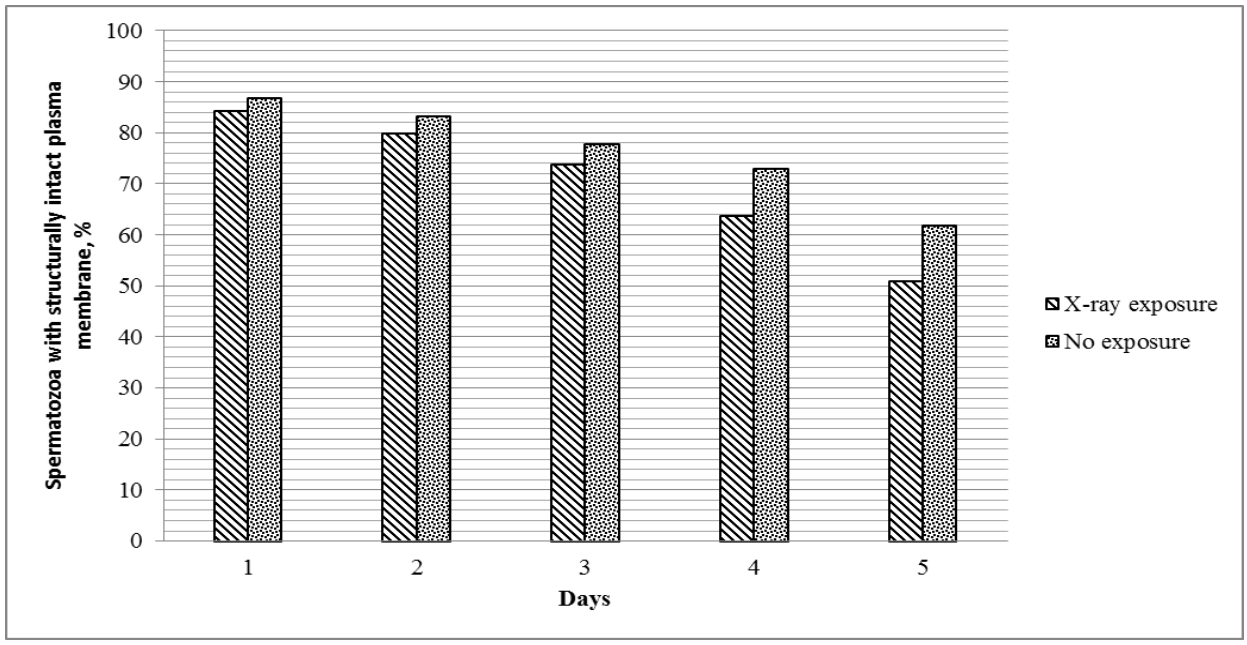

Fig. 3. **Difference of canine spermatozoa with a structurally intact plasma membrane between exposed and not exposed to X-ray over the storage period of 5 days**

Discussion and conclusions. This was the first investigation of the X-irradiation impact on spermatozoa motility and viability parameters of canine semen. X-ray produces damage to cells primarily by free radicals (Bonisoli-Alquati et al., 2011). The results of our study showed that airport X-ray screening had a significant effect on sperm plasma membrane integrity of chilled canine semen.

Although sperm motility was lower in the samples exposed to X-ray than in the control group, there was no significant difference detected. To the contrary, in one study, motility of frozen felid semen significantly decreased ($P < 0.05$) even after one-time exposure to X-ray (Gloor et al., 2006). These diverse results can be due to a difference in morphology of investigated felid and canine semen. In our study, there were 75.47–90.37% of morphologically normal spermatozoa, while in a study with frozen felid semen 47–61% only.

There was a lack of scientific information about the influence of the X-ray effect on the spermatozoa plasma membrane. Our study showed that while membrane integrity assessed by SYBR 14/PI was lower in the semen samples affected by an airport security screening machine, there was no significant difference between the

groups. However, membrane integrity of the spermatozoa assessed by the HOST was significantly lower on the first 3 days of the study in the samples exposed to X-irradiation compared with the samples of the control group. Our results showed that X-ray had impact on the plasma membrane function but not on the structure of spermatozoa.

In conclusion, canine semen exposure to X-ray at airport checkpoints has an effect on the membrane of spermatozoa, which in turns may have a negative effect on fertility. However, further studies are required to confirm the potential detrimental effects of X-irradiation at airport security checkpoints on other canine semen parameters, such as DNA integrity.

References

1. Amorim E. A. M., Torres C. A. A., Graham J. K., Amorim L. S., Santos L. V. L. The hypoosmotic swelling test in fresh rabbit spermatozoa. Anim. Reprod. Sci. 2009. 111. P. 338–343.

2. Bonisoli-Alquati A., Møller A. P., Rudolfsen G., Saino N., Caprioli M., Ostermiller S., Mousseau T. A. The effects of radiation on sperm swimming behavior depend on plasma oxidative status in the barn swallow (*Hirundo rustica*). Comp. Biochem. Physiol. A. 2011. 159. P. 105–112.

3. Cabrita E., Alvarez R., Anel E., Herráez M. P. The hypoosmotic swelling test perfoomed wiith Counter: a method to assay functional integrity of sperm membrane in rainbow trout. Anim. Reprod. Sci. 1999. 55. P. 279–287.

4. Donoghue A. M., Garner D. L., Donoghue D. J., Johnson L. A. Viability assessment of turkey sperm using fluorescent staining and flow cytometry. Poult. Sci. 1995. 74. P. 1191–1200.

5. Gloor K. T., Winget D., Swanson W. L. Conservation science in a terrorist age: the impact of airport security screening on the viability and DNA integrity of frozen felid sperm. J. Zoo. Wildlife Med. 2006. 237. P. 327–235.

6. Goericke–Pesch S., Failing K. Retrospective analysis of canine semen evaluations with special emphasis on the use of the hypoosmotic swelling (HOS) test and acrosomal evaluation using Spermac®. Reprod. Dom. Anim. 2013. 48.P. 213–217.

7. Hendricks K. E. M., Penfold L. M., Evenson D. P., Kaproth M. T., Hansen P. J. Effects of airport screening X–irradiation on bovine sperm chromatin integrity and embryo development. Theriogenology. 2010. 73. P. 267–272.

8. Januškauskas A. Žemės ūkio gyvūnų reprodukcija ir sėklinimas. Kaunas: Vetinfo leidybinis centras. 2010. 34. P. 19–26.

9. Jeyendran R. S., Van der Ven H. H., Perez–Pelaez M., Crabo B. G., Zaneveld L. J. D. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J. Reprod. Fert. 1984. 70. P. 219–228.

10. Kargei S., Arlt S., Haimerl P., Heuwieser W. A Systematic Review of Studies Performing the Hypo-Osmotic Swelling Test to Evaluate the Quality of Canine Spermatozoa. Reprod. Dom. Anim. 2014. 49. P. 1–6.

11. Kmenta I., Strohmayer C., Müller–Schlösser F., Schäfer–Somi S. Effects of a lecithin and catalase containing semen extender and a second dilution with different enhancing buffers on the quality of cold–stored canine spermatozoa. Theriogenology. 2011. 75. P. 1095–1103.

12. Mocé E., Graham J. K. *In vitro* evaluation of sperm quality. Anim. Reprod. Sci. 2008. 105. P. 104–118.

13. Santos E. A. A., Sousa P. C., Dias C. E. V., Castelo T. S., Peixoto G. C. X., Lima G. L., Ricarte A. R., Simão B. R., Freitas C. I., Silva A. R. Assessment of sperm survival and functional membrane integrity of the six–banded armadillo *(Euphractus sexcinctus)*. Theriogenology. 2011. 75. P. 623–629.

Received 29 June 2016
Accepted 01 September 2016