DYNAMIC OF THE SPERM DNA INTEGRITY IN FROZWN-THAWED SEMEN FROM ENDANGERED GREY STEPPE BREED BULLS

Sabina Andra Neculai-Văleanu[1], D.L. Dascălu[1], A.M. Ariton[1], M. Davidescu[1], Elena Ruginosu[1], G. Nacu[1,2], Şt. Creangă[1,2]*

[1]Research and Development Station for Cattle Dancu, Iasi, Romania
[2]Faculty of Animal Breeding, University of Agronomical Sciences and Veterinary Medicine of Iasi, Romania

Abstract

Aim of the study The purpose of this study was to evaluate the dynamic of the sperm DNA integrity of spermatozoa from endangered Grey Steppe cattle breed bulls in order to establish the potential use of sperm DNA fragmentation (SDF) assay for improving the routine fertility screening in the conservation program.

Material and methods Cryopreserved semen doses from two Grey Steppe bulls were thawed by placing the straws into a water bath at 38°C for 30 seconds. The dynamic of the sperm DNA integrity was analyzed by determining the sperm DNA fragmentation index using the Toluidine blue stain at T0 moment and subsequentely after 3, respectively 6 hours of incubation at 38°C, in a incubator. Briefly, wet smears were fixed in acetone:ethanol, washed, hydrolized in HCl and stained with Toluidine Blue.

Results Variability of percentages of spermatozoa with fragmented DNA was assessed in 2 Grey Steppe bulls. In the case of both bulls, the sperm DNA fragmentation index (DFI%) increased progresively during the 6 hours incubation, when the hightest DNA fragmentation index (DFI) was observed, 7.5% in bull 2, respectively, 4.5% in bull 1. At T0, the DFI% was greater for Bull 2, a value of 3.5% being registered as compared to bull 1, in which the DFI% was 2.5%.

Conclusions The semen from the two Grey Steppe bulls analyzed in this study presented moderate DNA fragmentation index and may be used for AI and IVF in conservation program of the Grey Steppe breed.

Key words: sperm chromatin, Grey Steppe, bull, spermatozoa

INTRODUCTION

The ex situ conservation became a valuble tool in the attempt to preserve endangered breeds. Assissted reproductive technologies (ART) and the development of genetic resource banks in which are stored cryopreserved male and female gametes, embryos and tissue samples, have contributed to the preservation of cattle breeds that face extinction and even the resuscitation of breeds that were thought to be extinct (Velazquez, 2008, Hiemstra et al., 2010).

One of the most frequently used methods of ART in conservation programs of rare breeds is the cryopreservation of semen. Unfortunately, spermatozoa which are cryopreserved and stored in genetic material banks are usually submitted to routine analysis such as motility, viability and morphology determination, their fertilising potential not being further tested. In the last years, various semen assays were developed to fulfill this purpose, since storing cryopreserved semen with unknown fertility is not a sustainable solution in conservation programs (Morrell, 2011).

The assessment of the sperm DNA integrity has become a mandatory assay in the evaluation of bull sperm quality, especially since DNA abnormalities are recognized as one of the main factors of infertility. Sperm DNA fragmentation negatively influences the rate of in vitro embryo development in both humans (Lopez et al., 2013) and cattle (Simoes et al., 2013).

*Corresponding author: creanga162@gmail.com
The manuscript was received: 20.09.2016
Accepted for publication: 06.11.2016

The sperm chromatin integrity may be altered during spermatogenesis due to factors such as genetic and breed traits (Bochnek and Smorag, 2010) health or envinronmental factors (extreme temperatures) or during processing of the semen, due to improper manipulation or oxidative damage (Agarwal and Allamaneni, 2005).

The purpose of this study was to evaluate the dynamic of the sperm DNA integrity of spermatozoa from endangered Grey Steppe cattle breed bulls in order to establish the potential use of sperm DNA fragmentation (SDF) assay for improving the routine fertility screening in the conservation program.

MATERIAL AND METHOD

The dynamic of the sperm DNA integrity was analyzed at T0 moment and subsequentely after 3, respectively 6 hours of incubation at 38°C, in a incubator. Cryopreserved semen doses from two Grey Steppe bulls were thawed by placing the straws into a water bath at 38°C for 30 seconds. Wet smears were prepared according to the „feather" technique on pre-cleaned, degresed slides and air dried for 30 min. Fixation of the dried slides was done in a mixture of freshly made 96% ethanol:acetone (1:1), at 4°C for 30 min.

Subsequentely, slides were set to dry for 2 hours and then submitted to hydrolyzation in HCl 0.1 N, at 4°C for 5 min, followed by 3 washes in distilled water, for 2 minutes each. Finally, the smears were subjected to Toluidine Blue staining (0.05% in 50% McIlvain's citrate phosphate buffer at pH 3.5,) for 5 min, dried and analyzed using oil immersion with ×1,000 magnification according to the protocol described by Agarwal and Said, 2004. A total number of 100 spermatozoa were counted and divided in two distinct categories based on their color : dark blue or violet, sperm cells with various degrees of fragmented DNA and light blue or violet, cells with DNA integrity (Figure 1). Immature sperm and somatic cells were discharged from the analysis. As the Grey Steppe breed is an endagered one, currently, there is cryopreserved semen available from only 2 tested bulls, therefore statistical analysis could not be performed.

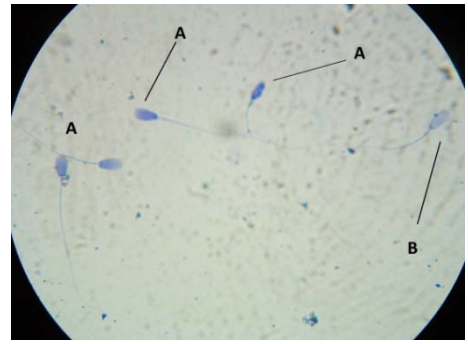

Figure 1 Sperm DNA integrity assessed by Toluidine Blue stain. A- sperm cell with abnormal chromatine; B- sperm cell with normal chromatin

RESULTS

Variability of percentages of spermatozoa with fragmented DNA was assessed in 2 Grey Steppe bulls. In the case of both bulls, the sperm DNA fragmentation index (DFI%) increased progresively during the 6 hours incubation, when the hightest DNA fragmentation index (DFI) was observed, 7.5% in bull 2, respectively, 5.5% in bull 1. At T0, the DFI% was greater for Bull 2, a value of 3.5% being registered as compared to bull 1, in which the DFI% was 2.5%.

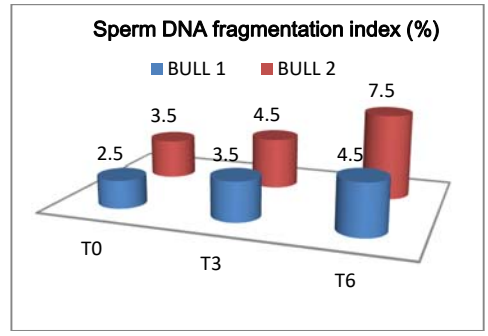

Figure 2 Sperm DNA fragmentation index (%) in bulls from Grey Steppe Cattle Breed

DISCUSSION

Artificial insemination and in vitro fertilization using frozen-thawed bull semen are two well establish biotechnologies used in conservation programs with the purpose of obtaining offspring. Unfortunately, the outcome of such procedures are influenced by many factors, among which sperm DNA

integrity have attracted increasingly more interest in recent years (Sharma et al., 2004; Fernández et al. 2005; Zini and Libman 2006; Boe-Hansen et al., 2005; Simões et al., 2013). Besides, routine semen usually focuses on the evaluation of parameters such as concentration, viability, motility and morphology although studies have showed that these parameters are poor indicators of fertility and pregnancy outcome.

Studies developed on both animals (Bochenek et al., 2001) and human semen (Evenson, 1999; Larson et al., 2000; Zini et al., 2001) revealed a strong relationship between fertility and sperm DNA abnormalities. Unlike humans, in whom a threshold of 30% sperm DNA damage was set as high risk of infertility, in domestic animals, such thresholds are presently not set. However, according to a study conducted by Karoui et al. (2012), the dna fragmentation index may be used as a marker for field bull fertility, values around 7% to 10% indicating a possible low AI success.

Furthermore, according to studies conducted by Palma & Sinowatz (2004), Alomar et al. (2008) the integrity of sperm chromatin might be responsible for the high variability registered in the outcome of bovine embryo production (IVP).

In our study, the degree of sperm chromatin damage in the two examined bulls was moderate, higher values being registered in bull 2, during the 6 hours incubation. According to a study conducted by Dogan et al. (2015), sperm chromatin condensation abnormalities may negatively influence both DNA integrity and sperm chromatin protamination, thus reducing the vivo fertility.

According to Saacke, 2008, the semen quality traits may be divided in the following two categories: compensable, which are characteristics that can be compensated by increasing the number of sperm cells per insemination dose (e.g. motility) and uncompensable, those which enable spermatozoa to participate in the fertilization process and development of the embryos. The sperm DNA abnormalities cannot be compensated by increasing the total number per dose.

In both natural conception and assisted reproduction, the integrity of the sperm DNA directly influences the outcome of the pregnancy. According to Wyrobek et al. (2006) the oocyte or embryo may repair minor abnormalities of the sperm chromatin, thus the development of the pregnancy may be successful. On the contrary, a high degree of sperm DNA fragmentation may determine embryo death in the first stages of the pregnancy or miscarriage in the last stage (Tesarik et al. 2004).

Although when predicting fertility many factors should be taken into consideration, including the reproductive health of the female, we may conclude that the semen from the two Grey Steppe bulls analyzed in this study presented moderate DNA fragmentation index and may be used for AI and IVF in conservation program of the Grey Steppe breed.

CONCLUSIONS

The semen from the two Grey Steppe bulls analyzed in this study presented moderate DNA fragmentation index and may be used for AI and IVF in the conservation program of the Grey Steppe breed.

We may conclude that in future, an alternative for improving the pregnancy outcome in this endangered cattle breed might be the cryopreservation of bull spermatozoa previously selected trough swim up, gradient centrifugation or microfluidic technique. The total number of sperm cell per insemination dose will be reduced in this case, but the sperm population obtained after selection will have specific characteristics such as high motility, chromatin integrity, viability and morphology.

ACKNOWLEDGMENTS

The study was carried out as part of the project ADER 5.3.2./2015 Conservation and preservation of endangered breed Grey Steppe Cattle for ensuring the biodiversity of genetical resources of animal populations, financed by the Ministery of Agriculture and Rural Development.

REFERENCES

[1] Velazquez M. A., 2008: Assisted Reproductive Technologies in Cattle: Applications in Livestock Production, Biomedical Research and Conservation Biology, ARBS Annual Review of Biomedical Sciences, DOI: 10.5016/1806-8774.2008.v10p36.

[2] Hiemstra S.J, Haas Y, Mäki-Tanila A., Gandini G., 2010: Local cattle breeds in Europe Development of policies and strategies for self-sustaining breeds

[3] Morrell J.M., 2011: Artificial Insemination: Current and Future Trends, "Artificial Insemination in Farm Animals", ISBN 978-953-307-312-5.

[4] López G., Lafuente R., Checa M.A., Carreras R., Brassesco M., 2013: Diagnostic value of sperm DNA fragmentation and sperm high-magnification for predicting outcome of assisted reproduction treatment. Asian J Androl., 15(6): p790–794.

[5] Simões R., Feitosa W., Perez Siqueira A.F., Nichi M., Paula-Lopes F.F, Marques M.G., Peres M.A., Barnabe V.H., Visintin J.A., Ortiz Assumpção M.E., 2013: Influence of bovine sperm DNA fragmentation and oxidative stress on early embryo in vitro development outcome, Reproduction, doi: 10.1530/REP-13-0123.

[6] Bochnek M. and Smorag Z, 2010: The level of sperm DNA fragmentation in bulls of different breeds. Annual Animal Sciences, 10, (4): p379–384.

[7] Agarwal A. & Allamaneni SS., 2011: Free radicals and male reproduction. Journal of the Indian Medical Association no. 109 :p 184–187.

[8] Agarwal A. and Said TM., 2004: Carnitines and male infertility. Reprod Biomed Online. 2004;8:376–384.

[9] Sharma RK, Said T, Agarwal A., 2004: Sperm DNA damage and its clinical relevance in assessing reproductive outcome, Asian J Androl 6: p139–148

[10] Zini A, Libman J., 2006: Sperm DNA damage: clinical significance in the era of assisted reproduction. CMAJ;175(5): p495-500.

[11] Boe-Hansen G.B., Morris I.D., Ersboll A.K., Greve T., Christensen P., 2005: DNA integrity in sexed bull sperm assessed by neutral comet assay and sperm chromatin structure assay, Theriogenology, 63: p1789–1802.

[12] Bochenek M., Smorag Z., Pilch J., 2001: Sperm chromatin structure assay of bulls qualified for artificial insemination.
Theriogenology 56: p557–567

[13] Evenson DP, Jost LK, Marshall D, Zinaman MJ, Clegg E, Purvis K, de Angelis P., Claussen OP, 1999: Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Human Reproduction, 14: p1039–1049. (doi:10.1093/humrep/14.4.1039)

[14] Larson KL, DeJonge CJ, Barnes AM, Jost LK, Evenson DP., 2000: Sperm chromatin structure assay parameters as predictors of failed pregnancy following assisted reproductive techniques, Hum Reprod.; 15(8): p 1717-722.

[15] Zini A., Bielecki R., Phang D., Zenzes M.T., 2001: Correlations between two markers of sperm DNA integrity, DNA denaturation and DNA fragmentation, in fertile and infertile men. Fertil Steril.; 75(4): p674-677.

[16] Karoui S, Díaz C, González-Marín C, Amenabar ME, Serrano M, Ugarte E, Gosálvez J, Roy R, López-Fernández C, Carabaño MJ, 2012: Is sperm DNA fragmentation a good marker for field AI bull fertility?, Journal of Animal Science, 90(8), p2437-2449, doi: 10.2527/jas.2011-4492.

[17] Palma G.A. and Sinowatz F., 2004: Male and female effects on the in vitro production of bovine embryos. Anat Histol Embryol.; 33(5): p 257-262.

[18] Alomar M, Tasiaux H, Remacle S, George F, Paul D, Donnay I., 2008. Kinetics of fertilization and development, and sex ratio of bovine embryos produced using the semen of different bulls. Animal Reproduction Science, 107: p 48–61.

[19] Dogan S, Vargovic P, Oliveira R, Belser LE, Kaya A, Moura A, Sutovsky P, Parrish J, Topper E, Memili E., 2015: Sperm protamine-status correlates to the fertility of breeding bulls. Biol Reprod, 92: p1-9.

[20] Wyrobek A.J, Eskenazi B., Young S., Arnheim N., Tiemann-Boege I., Jabs E.W., Glaser R.L., Pearson F.S., Evenson D., 2006. Advancing age has differential effects on DNA damage, chromatin integrity, gene mutations, and aneuploidies in sperm. Proc Natl Acad Sci USA.; 103:9601-9606. doi:10.1073/pnas.0506468103.

[21] Tesarik J., Greco E., Mendoza C., 2004: Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. Human Reproduction 19,p:611–615. (doi:10.1093/humrep/deh127).