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THE POSITIVE EFFECT OF RESVERATROL ON MOTILITY AND DNA FRAGMENTATION OF HUMAN SPERMATOZOA DURING CRYOPRESERVATION

PAVEL TSONEV[1], DIMITAR PARVANOV[1]*, RUMIANA GANEVA[1], ILIAN DZAGAROV[1], VLADIMIR STAIKOV[1], DIANA IVANOVA[1], GUEORGUI STAMENOV[1], TODOR CHAUSHEV[1]

[1] Nadezhda,Women's Health Hospital, 3 "Blaga vest" Street, Sofia, Bulgaria
**Corresponding author: e-mail: dimparvanov@abv.bg*

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Abstract: Freezing and thawing procedures of human spermatozoa trigger the excessive production of reaction oxygen species (ROS) that cause damage to spermatozoa. Resveratrol, a natural polyphenol, produced from several plants is being envisioned as a valuable antioxidant that prevents DNA damage.

The aim of this study was to analyze the effect of resveratrol supplementation into cryopreservation medium on the motility and DNA fragmentation of human spermatozoa after freezing-thawing procedure.

Semen was collected from 26 normozoospermic (N) patients. All samples were cryopreserved in nitrogen vapour after ten minutes incubation. After one week samples were thawed and sperm motion was measured. Using the Sperm Chromatin Structure Assay (SCSA), the number of DNA fragmented sperm, expressed as DNA fragmentation index (DFI) was evaluated in each sample.

The addition of 0.01 mM resveratrol into cryopreservation medium have lead to better motility in 81% of the studied patients. The number of progressive spermatozoa in the samples with resveratrol was significantly higher compared with the control semen samples measured after the thawing procedure (25.45 ± 12.65 vs. 15.75 ± 9.65, respectively). The impact of resveratrol on DNA fragmentation of investigated spermatozoa was relatively small and led to insignificant change in the DFI. However, in 63% of the patients the addition of resveratrol had a positive effect and resulted in lower DFI in comparison with the control semen samples.

In conclusion, the supplementation of 0.01 mM resveratrol significantly improves the post-thawed human spermatozoa progressive motility and decreases the level of DNA fragmentation.

INTRODUCTION

The improvement of cryopreservation methods for human spermatozoa and oocytes, and the application of new types of cryoprotectants are of major importance to ensure the success of frozen embryo transfer. During the process of cryopreservation, an uncontrolled increase in the production of reactive oxygen species (ROS) results in different types of sperm cell damages (Fraser and Strzezek, 2005; Aitken and Baker, 2006).

Human spermatozoa are highly susceptible to oxidative stress due to their high concentration of poly-unsaturated fatty acids (Storey, 1997). ROS has detrimental effect not only to the sperm membranes (Jones et al., 1979; Sikka, 2001) but also to DNA and proteins (Twigg et al., 1998; Stadtman and Levine, 2003; Moustafa et al., 2004). Seminal plasma and cell cytoplasm include specific complex of enzymes and antioxidants that could partly prevent human spermatozoa from undesired ROS induced damage. This complex includes glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase, vitamin E, vitamin C, urate and albumin (Smith et al., 1996). Based on this natural protection system one of the available treatment strategies is the supplementation of antioxidants into the cryopreservation medium in order to increase the scavenging capacity of the seminal plasma (Taylor et al., 2009; Collodel et al., 2011).

Resveratrol is a polyphenol with antioxidant activity that was found in the peanuts, grapes and red wine (Collodel et al., 2011). It was shown that its administration has a positive effect on sperm production and motility in animal models (Juan et al., 2005; Shin et al., 2008). Its protective effect against DNA fragmentation induced by a cryopreservation procedure of human spermatozoa has recently been observed (Garcez et al., 2010).

The purpose of this study was to assess the effect of resveratrol on motility and DNA fragmentation of sperm from normozoospermic men after freezing-thawing procedure.

MATERIALS AND METHODS

Study design

A prospective study was performed at the Nadezhda Women's Health Hospital, Sofia, Bulgaria during the period May 2017 – June 2017. A native sperm from 26 normozoospermic patients were used for analysis of sperm concentration, morphology, motion and DNA fragmentation. Samples were divided into three aliquots prior to cryopreservation. The first aliquot was analyzed before the cryopreservation. The second (control) aliquot was mixed with SpermFreeze solution (Vitrolife) and the last aliquot was mixed with the same solution and resveratrol (0.01 mMol). These two aliquots were cryopreserved in nitrogen vapors after ten minutes incubation with or without resveratrol. After one week samples were thawed and sperm parameters were measured again.

Semen analysis

In all patients, after 3-5 days of sexual abstinence, semen samples were collected. All samples for evaluation were allowed to liquefy for at least 20 minutes at 37°C and then the first aliquot was evaluated for sperm concentration, motility, and morphology according to the guidelines of the World Health Organization (WHO, 2010). Sperm concentration, motility and viability were assessed using a Sperm Class Analyzer CASA System (Microoptic S.L, Barcelona, Spain), Mackler's camera and Olympus microscope. The SCSA was done following the established protocol of Evenson and Jost (1994). More than 10000 sperm were evaluated for each semen sample and the results were expressed as percent DNA fragmentation index (%DFI).

Statistical analysis

Statistical data analysis was performed using SPSS v.21. Data were reported as mean ± SD. Paired-samples t-test was performed to find out whether any significant mean difference exist between the studied groups. The P value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Seminal analysis performed before and after cryopreservation showed that the studied population of normozoospermic men had significant decrease in sperm motility after the performed freezing-thawing procedure (Table 1). On the other hand, the cryopreservation did not change significantly sperm concentration or morphology.

Table 1. Semen characteristics of normozoospermic (N) patients before and after cryoopreservation (mean ± SD).

Variable		Patients (n=26)		
		Prefreezing	Post-thawing without resveratrol	Post-thawing with 0.01 mM resveratrol
	Age (years)		38 ± 4	
Semen variables	Sperm concentration (cells x 10^6)	79.11 ± 34.45	68.24 ± 36.11	72.38 ± 19.52
	Sperm motility (A %)	22.47 ± 12.25	5.05 ± 3.63	8.31 ± 6.93
	Sperm motility (B %)	31.20 ± 14.51	10.7 ± 8.41	17.14 ± 10.11
	Sperm progressive motility (A+B %)	53.67 ± 18.97	15.75 ± 9.65*	25.45 ± 12.65*
	Sperm morphology (Kruger) (%)	5.25 ± 3.11	5.12 ± 2.69	5.06 ± 3.27
	DNA fragmentation index (DFI) (%)		17.52 ± 10.19	16.60 ± 10.72

* Significant difference between studied patient groups (P<.05).

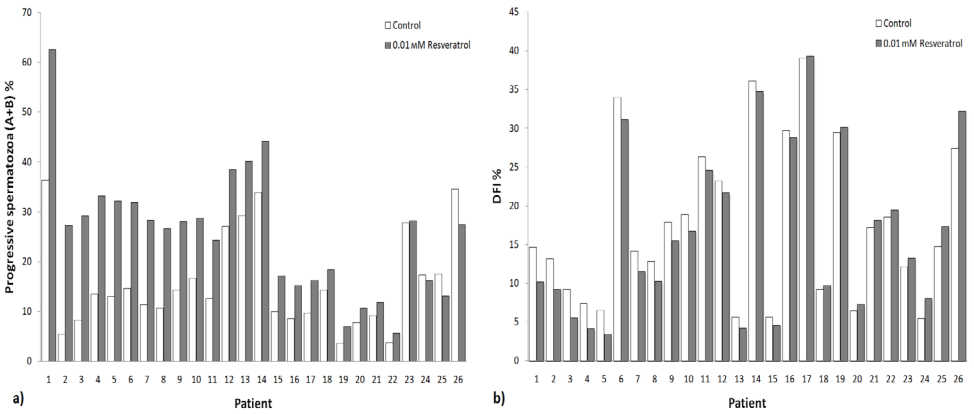

Fig. 1 Sperm motility (a) and DNA fragmentation index (DFI) (b) in normozoospermic men (n = 26) analyzed after cryopreservation with and without resveratrol.

The addition of 0.01 mM resveratrol into cryopreservation medium lead to better motility in 81% of the studied patients (Fig. 1, a). In addition, the number of progressive spermatozoa (A+B) in the samples with resveratrol was significantly higher compared with the control semen samples measured after the thawing procedure (25.45 ± 12.65 vs. 15.75 ± 9.65, respectively). The impact of resveratrol on DNA fragmentation of investigated spermatozoa led to insignificant change in the DFI (Fig. 1, b). However, in 63% of the patients the addition of resveratrol had a positive effect and resulted in lower DFI in comparison with the control semen samples.

The production of high quantities of reactive oxygen species (ROS) and the absence of sufficient antioxidant systems and natural scavengers is tightly bound with DNA fragmentation of human spermatozoa and the presence of specific types of morphological defects (Aziz et al., 2004; Parvanov et al., 2017). As a consequence this could have an effect on IVF success and the frequency of spontaneous abortions (Georgieva et al., 2017).

Resveratrol has a similar molecular structure to estradiol and it was found to be produced in several plants in response to different types of stress (Gehm et al., 1997). It has already proven anti-inflammatory, antioxidant, cardioprotective, analgesic, anti-apoptosis and anti-aging functions (Juan et al., 2005; Sharma et al., 2014; Liu et al., 2015; Orihuela Campos et al., 2015). The application of antioxidants such as resveratrol in order to neutralize the effect of ROS and other types of free radicals during the process of cryopreservation is still being debated (Tremellen, 2008). It was discovered that the supplementation of another antioxidant - vitamin E into the cryopreservation medium lead to better human sperm motility after thawing procedure but it did not have a significant effect on vitality and DNA damage (Taylor et al., 2009).

Our study showed that the cryopreservation process did not change sperm concentration and morphology but result in significant decrease in motility

parameters. The supplementation of 0.01 mM resveratrol diminished the negative effect of cryopreservation on human spermatozoa motility but it has an insignificant impact on DNA fragmentation levels. Similar results were obtained in an animal model by another research group who treated mouse spermatozoa with 0.015 mM resveratrol. These spermatozoa exhibited increased motility, viability, mitochondrial transmembrane potential and decreased ROS production (Mojica-Villegas et al., 2014). Other authors have shown that the supplementation of RVT in higher concentrations (0.1 to 10 mM) decreases oxidative damages induced by the cryopreservation of human spermatozoa, but it was not able to restore the decrease in sperm motility (Garcez et al., 2010; Branco et al., 2010).

As it is already known, the addition of resveratrol before cryopreservation of sperm cells leads to diminished levels of damages caused by oxidative stress (Garcez et al., 2010). The mechanism of action of resveratrol is not completely clear. Its activity is mostly attributed to ROS scavenging function (Garcez et al., 2010), but also it can induce an increase in superoxide dismutase activity (Soleas et al., 1997). In addition, it inhibits the activity of complex III by competing with coenzyme Q (Gambini et al., 2015). And in such way it determines its additional antioxidant activity by inhibiting the respiration complex that generates ROS (Zini et al., 1999). Furthermore, this chemical compound could easily be incorporated into the membrane's lipid bilayer, inhibiting the formation of lipid radicals and keeping the membrane integrity and the ionic equilibrium of the cell (Halliwell and Gutteridge, 1999). Resveratrol resemble cholesterol action on biological membranes and contributes to the regulation of cell membrane structure and fluidity, which may influence the activity of transmembrane proteins and hence control the cell signaling pathways (Neves et al., 2016). However, high concentrations of resveratrol could even lead to the production of cytotoxic ROS and quinones as a result of an autooxidation process (Klaus et al., 2010). The obtained oxidized form, in turn, can generate complexes with copper that can fragment DNA (Hadi et al., 2010).

CONCLUSION

In conclusion, the supplementation of 0.01 mM resveratrol during cryopreservation significantly improves progressive motility of human spermatozoa and decreases their DNA fragmentation index. Although further studies are needed, the present work showed that resveratrol could be considered as a suitable antioxidant ingredient in cryopreservation media.

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REFERENCES

1. Aitken R.J., Baker M.A. 2006. Oxidative stress, sperm survival and fertility control. *Mol Cell Endocrinol,* 250:66–9.
2. Aziz N., Saleh R.A., Sharma R.K., Lewis-Jones I., Esfandiari N., Thomas A.J. Jr, et al. 2004. Novel association between sperm reactive oxygen species production, sperm

morphological defects, and the sperm deformity index. *Fertil Steril,* 81:349–54
3. Branco C., Garcez M.E., Pasqualotto F.F., Erdtman B., Salvador M. 2010. Resveratrol and ascorbic acid prevent DNA damage induced by cryopreservation in human semen. *Cryobiology*, 60: 235–237
4. Collodel G., Federico M.G., Geminiani M., Martini S., Bonechi C., Rossi C., Figura N., Moretti E. 2011. Effect of trans-resveratrol on induced oxidative stress in human sperm and in rat germinal cells. *Reproductive Toxicology,* 31: 239–246
5. Evenson, D., Jost, L. 1994. Sperm chromatin structure assay: DNA denaturability. In: Darzynkiewicz Z, Robinson JP, Crissman HA, *eds. Methods in Cell Biology. Vol 42, 2nd ed. Orlando, Fla: Academic Press,* pp.159–176.
6. Fraser L., Strzezek J. 2005. Effects of freezing-thawing on DNAintegrity of boar spermatozoa assessed by neutral comet assay. *Reprod Domest Anim,* 40:530–6.
7. Gambini J., Inglés M., Olaso G., Lopez-Grueso R., Bonet-Costa V., Gimeno-Mallench L., Mas-Bargues C., Abdelaziz K.M., Gomez-Cabrera M.C., Vina J., and Borras C. 2015. Properties of Resveratrol: In Vitro and In Vivo Studies about Metabolism, Bioavailability, and Biological Effects in Animal Models and Humans. *Oxid Med Cell Longev.* ID: 837042, pp.1-13.
8. Garcez M.E., Branco C.D., Lara L.V., Pasqualotto F.F., Salvador M. 2010. Effects of resveratrol supplementation on cryopreservation medium of human semen. *Fertil Steril,* Vol. 94, No. 6, 2118-2121.
9. Gehm B.D., McAndrews J.M., Chien P.-Y., Jameson J.L. 1997. Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor. P*roceedings of the National Academy of Sciences of the United States of America,* 94(25):14138–14143.
10. Georgieva V., Parvanov D., Taskudi E., Stamenov G., Chaushev T. 2017. Relation between morphological defects in human spermatozoa and spontaneous abortions in patients undergoing ICSI. *Annuaire de l'Université de Sofia "St. Kliment Ohridski" Faculte de Biologie,* volume 102, livre 4, pp. 186-193
11. Hadi S.M., Ullah M.F., Azmi A.S., et al. 2010. Resveratrol mobilizes endogenous copper in human peripheral lymphocytes leading to oxidative DNA breakage: a putative mechanism for chemoprevention of cancer. *Pharmaceutical Research*, 27(6):979–988.
12. Halliwell B., Gutteridge JMC. 1999. Free radicals in biology and medicine. 3d ed. Oxford, U.K.: *Oxford Science Publications,* 543 pp.
13. Jones R., Mann T., Sherins R.J. 1979. Peroxidative breakdown of phospholipids in human spermatozoa: spermicidal effects of fatty acid peroxides and protective action of seminal plasma. *Fertil Steril,* 31:531–7.
14. Juan M.E., González Pons E., Munuera T., Ballester J., Rodríguez Gil J.E. and Planas J.M. 2005. trans Resveratrol, a natural antioxidant from grapes, increases sperm output in healthy rats. *J Nutr,* 135: 757 760.
15. Juan M.E., González-Pons E., Munuera T., Ballester J., Rodríguez-Gil J.E., Planas J.M. 2005. Trans-resveratrol, a natural antioxidant from grapes, increases sperm output in healthy rats. *J Nutr,* 135:757–60.
16. Klaus V., Hartmann T., Gambini J., et al. 2010. 1,4-Naphthoquinones as inducers of oxidative damage and stress signaling in HaCaT human keratinocytes. *Archives of Biochemistry and Biophysics,* 496(2):93–100.
17. Liu J., Yi L., Xiang Z., Zhong J., Zhang H. and Sun T. 2015. Resveratrol attenuates spinal cord injury induced inflammatory damage in rat lungs. *Int J Clin Exp Pathol*, 8: 1237 1246.

18. Mojica-Villegas M.A., Izquierdo-Vega J.A., Chamorro-Cevallos G., Sánchez-Gutiérrez M. 2014. Protective effect of resveratrol on biomarkers of oxidative stress induced by iron/ascorbate in mouse spermatozoa. *Nutrients*, 6, 489-503
19. Moustafa M.H., Sharma R.K., Thornton J., Mascha E., Abdel-Hafez M.A., Thomas Jr A.J., et al. 2004. Relationship between ROS production, apoptosis and DNA denaturation in spermatozoa from patients examined for infertility. *Hum Reprod,1*9:129–38.
20. Neves A.R., Nunes C., Amenitsch H., Reis S. 2016. Effects of resveratrol on the structure and fluidity of lipid bilayers: A membrane biophysical study. S*oft Matter,1*2(7):2118-26.
21. Orihuela Campos R.C., Tamaki N., Mukai R., Fukui M., Miki K., Terao J. and Ito H.O. 2015. Biological impacts of resveratrol, quercetin, and N acetylcysteine on oxidative stress in human gingival fibro¬blasts. *J Clin Biochem Nutr,* 56: 220 227.
22. Parvanov D., Georgieva V., Staykov V., Stamenov G., Chaushev T. 2017. Higher frequency of tail defects in human spermatozoa correspond to higher levels of DNA fragmentation. *Annuaire de l'Université de Sofia "St. Kliment Ohridski" Faculte de Biologie,* volume 102, livre 4, pp. 97-104
23. Sharma P., Huq A.U. and Singh R. 2014. Cypermethrin induced repro¬ductive toxicity in the rat is prevented by resveratrol. *J Hum Reprod Sci,* 7: 99 106.
24. Shin S., Jeon J.H., Park D., Jang M.J., Choi J.H., Choi B.H., et al. 2008. Trans-resveratrol relaxes the corpus cavernosum ex vivo and enhances testosterone levels and sperm quality in vivo. *Arch Pharm Res,* 31:83–7.
25. Sikka S.C. 2001. Relative impact of oxidative stress on male reproductive function. *Curr Med Chem,* 8:851–62.
26. Smith R., Vantman D., Ponce J., Escobar J., Lissi E. 1996. Total antioxidant capacity of human seminal plasma. *Hum Reprod*, 11:1655–60.
27. Soleas G.J., Diamandis E.P., Goldberg D.M. 1997. Resveratrol: a molecule whose time has come? And gone. *Clin Biochem,* 30:91–113.
28. Stadtman E.R., Levine R.L. 2003. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids,* 25:207–18.
29. Storey B.T. 1997. Biochemistry of the induction and prevention of lipoperoxidative damage in human spermatozoa. *Mol Hum Reprod*, 3:203–13.
30. Taylor K., Roberts P., Sanders K., Burton P. 2009. Effect of antioxidant supplementation of cryopreservation medium on post-thaw integrity of human spermatozoa. *Reprod Biomed Online,*18:184–9.
31. Tremellen K. 2008. Oxidative stress and male infertility a clinical perspective. *Hum Reprod Update,* 14:243–58.
32. Twigg J., Irvine D., Aitken R. 1998. Oxidative damage to DNA in human spermatozoa does not preclude pronucleus formation at intracytoplasmic sperm injection. *Hum Reprod,* 13:1864–71.
33. WHO. 2010. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva: *WHO Press*; pp. 271
34. Zini R., Morin C., Bertelli A., Bertelli A. A. E., Tillement J.-P. 1999. Effects of resveratrol on the rat brain respiratory chain. *Drugs under Experimental and Clinical Research,* 25(2-3):87–97.