



The Effect of Magnesium Sulfate on Sperm Characteristics and Blood Hematology After Testicular Ischemia-Reperfusion Injury in Rats

Saadat Moshkelani¹ , Ahmad Asghari^{1*} , Gholamreza Abedi¹, Alireza Jahandideh¹, Pejman Mortazavi²

Abstract

Objectives: The aim of this study was to investigate the effect of magnesium sulfate ($MgSO_4$) administered single intraperitoneally on sperm characteristics after testicular ischemia-reperfusion (IR) injury in the male rats.

Materials and Methods: Thirty adult rats were randomly divided into 5 groups: sham operations (group 1), IR (group 2), IR + 125 mg/kg $MgSO_4$ (group 3), IR + 250 mg/kg $MgSO_4$ (group 4), and IR + 500 mg/kg $MgSO_4$ (group 5). Testicular ischemia was achieved by 720 degrees torsion of the left testis for 2 hours. Then, detorsion was performed and reperfusion was induced. One hour after ischemia, three different doses of $MgSO_4$ (125, 250, and 500 mg/kg) were administered intraperitoneally. The sperm count, motility, mobility, and blood hematology were evaluated.

Results: There was a significant increase in sperm count, motility, and mobility in groups treated with $MgSO_4$ compared to the sham group ($P < 0.05$). In addition, lymphocyte count in the sham group significantly increased compared to the IR group ($P < 0.05$).

Conclusions: Generally, this study demonstrated that $MgSO_4$ can improve sperm characteristics after testicular IR injury.

Keywords: Magnesium sulfate, Testis, Ischemia-reperfusion, Rat

Introduction

Infertility is regarded as one of the most common health concerns in couples and approximately 30% of the problems are related to males (1). Testicular torsion refers to the twisting of the spermatic cord structures and the subsequent loss of blood supply to the ipsilateral testicle (2). This is a urologic emergency that occurs frequently in neonates and adolescents (1); hence, early diagnosis and surgical intervention are vital to save the testicle and to preserve future fertility (2). The surgical correction of testicular torsion includes the detorsion of spermatic cord and the restoration of blood stream to the testicles. The reperfusion of ischemic tissue leads to a sequence of events that injure the tissue more severe than the injuries induced by ischemia, so-called ischemia-reperfusion (IR) injury. Reactive oxygen species (ROS) including hydrogen peroxide, hydroxyl radicals, and superoxide anions, along with the formation of nitric oxide (NO) and peroxynitrite are enhanced during the reperfusion of the ischemic tissue. These free radicals cause further cellular damage through the peroxidation of lipids in mitochondrial and cell membranes (3, 4). Moreover, the repeated succession of IR injury in testicular cells cause many biochemical and morphological changes which may lead to lipid peroxidation, protein denaturation, DNA damage, and apoptosis (5). During the past few years, many anti-

inflammatory, antioxidant, and free radical scavengers have been used for the treatment of testicular IR injury which have induced male infertility (6, 7). Magnesium sulfate ($MgSO_4$) possesses potent anti-oxidative and anti-inflammatory capacities that can prevent oxidative stress (8). On the other hand, Magnesium (Mg^{2+}), the second most abundant cation within the cell, plays an important role in numerous biological functions (9, 10). However, there exists scarce information about the role of Mg^{2+} in fertilization. It is believed that Mg^{2+} plays a prominent role in the human reproductive system and in semen, as well as fertilization (11). Furthermore, seminal plasma plays an important role in the protection of sperm and acts as a buffer for sperm motility. Moreover, semen is composed of lipids, ions such as citrate, calcium, Mg^{2+} , K^+ , Na^+ , zinc and chloride, proteins, and oxidative enzymes that protect the sperm from oxidative stress (12). Therefore, the aim of the present study was to evaluate the action of $MgSO_4$ injection on the sperm characteristics and blood hematology following experimental IR injury.

Materials and Methods

Animals

Thirty healthy adult male Wistar rats ($n=30$) weighing 250 ± 20 g were purchased from Razi Vaccine and Serum Research Institute, Iran. The rats were given ad libitum



access to standard commercial rodent chow pellets and filtered tap water. They were kept under constant conditions at 22-24°C with a 12-hour dark/light cycle for one week before the beginning of the study. This study was conducted according to the guidelines for the care and use of laboratory animals, Islamic Azad University, Faculty of Veterinary Medicine.

The animals were randomly divided into 5 groups including: sham operations (group 1), IR (group 2), IR + 125 mg/kg MgSO₄ (group 3), IR + 250 mg/kg MgSO₄ (group 4), and IR + 500 mg/kg MgSO₄ (group 5).

Surgery Process

Anesthesia was induced by the combination of ketamine HCL (60 mg/kg) and xylazine HCL (10 mg/kg). The skin on the abdomen was shaved and prepared with 10% povidone-iodine solution. A midline longitudinal incision was made for access to both testes. Torsion was developed by twisting the left testis 720 degrees in counterclockwise direction and maintained by fixing the testis to scrotum with a 6/0 nylon suture passing through the tunica albuginea and dartos. After 2 hours of ischemia, the suture was removed and the left testis was detorted and replaced with scrotum for a 24-hour of reperfusion. After each surgical intervention, the incision was closed.

The sham group was subjected to all operative procedures except for vessels occlusion and IR group underwent 2 hours of ischemia and 24 hours of reperfusion. Groups 3, 4, and 5 received intraperitoneal (i.p.) injections of 125, 250, and 500 mg/kg MgSO₄ as medications one hour before the detorsion of the testis, respectively. In the end, orchietomy of the left testis was performed for all 5 groups after euthanizing the rats.

Sperm Characteristics

Sperms were obtained from the caudal pole of epididymis. They were placed in 1 mL of the medium containing Hams F10 + FBS in 9/1, and incubated at 37°C for 15 minutes. The sperm count, motility, and mobility were evaluated by

hemocytometer using Neubauer slide (13).

Blood Collections

Blood samples were collected from vena porta puncture of each rat at the termination of the trial. Cell count and RBC indices were evaluated in fresh anticoagulated blood with EDTA by means of an electronic cell counter. The percentage of hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and differential WBC counting were calculated by an electronic cell counter.

Statistical Analysis

The parametric data were analyzed by one-way analysis of variance (ANOVA) using SPSS software version 16.0 (SPSS, Inc., Chicago, IL, USA). Data were expressed as mean ± standard error of mean (SEM). A *P* value < 0.05 was considered statistically significant.

Results

The hematological analyses of the groups are summarized in Table 1. In none of the hemograms of the groups, significant changes were observed, except for lymphocyte count in the sham group compared to the IR group in which a significant increase from 62.18 ± 0.96 to 65.86 ± 0.57 was observed (*P* < 0.05).

The effects of MgSO₄ treatment followed by testicular IR injury on sperm count, motility, and mobility in the rats are summarized in Figures 1-3. Experimental unilateral testicular IR injury significantly diminished sperm count (*P* = 0.03), motility (*P* = 0.04), and mobility (*P* = 0.02) compared to the sham operation group (*P* < 0.05).

Based on the results of this study, intraperitoneal injections of different doses of MgSO₄ (125, 250, and 500 mg/kg) as medications caused a significant increase in sperm count (*P* = 0.03), sperm motility (*P* = 0.01), and sperm mobility (*P* = 0.02) after 24 hours compared to the IR group (*P* < 0.05).

Table 1. Hematological Results of the Study Groups (Mean±SE)

| Parameters | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|---------------------------|--------------|-------------|--------------|--------------|-------------|
| RBC (10 ⁹ /μL) | 7.47± 0.94 | 7.68± 0.33 | 7.56± 0.81 | 7.02± 0.7 | 7.32± 0.66 |
| WBC (10 ³ /μL) | 6.22± 0.66 | 6.52± 0.46 | 6.08± 0.88 | 6.31± 0.76 | 6.14± 0.54 |
| Hb (g/dL) | 11.43± 0.25 | 12.67± 0.10 | 12.45± 0.10 | 11.12± 0.12 | 12.45± 0.52 |
| Hct (%) | 41.42± 0.60 | 40.44± 0.66 | 38.62± 0.58 | 41.23 ± 0.70 | 37.65± 0.70 |
| MCV (fl) | 50.11± 1.16 | 48.70±3.97 | 48.36±0.89 | 51.92± 1.32 | 52.50±0.16 |
| MCH (pg) | 18.71± 0.41 | 19.10±1.78 | 20.26± 0.24 | 18.28± 0.22 | 17.32± 0.24 |
| MCHC (g/dL) | 31.13± 0.42 | 29.80± 0.57 | 32.65± 0.49 | 30.57± 0.90 | 29.61± 0.86 |
| Neutrophils (%) | 26.00 ± 0.55 | 25.54± 0.41 | 24.10 ± 0.54 | 24.52± 0.55 | 29.62± 0.53 |
| Eosinophils (%) | 3.17± 1.10 | 2.49±0.18 | 1.81±0.20 | 1.83±0.30 | 1.79±0.22 |
| Basophils (%) | 0.50 ± 0.20 | 0.60± 0.18 | 0.79± 0.20 | 0.50 ± 0.20 | 0.48± 0.17 |
| Lymphocytes (%) | 62.18± 0.96 | 61.45± 0.57 | 67.31± 0.20 | 67.87± 0.70 | 67.43± 0.80 |
| Monocytes (%) | 2.65± 0.16 | 2.62± 0.26 | 2.22± 0.24 | 2.67± 0.30 | 2.34± 0.42 |

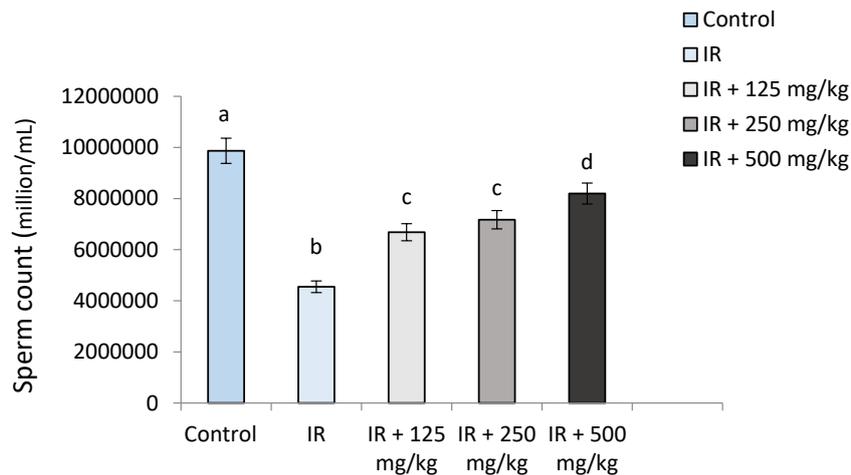


Figure 1. The Effect of Magnesium Sulfate on Sperm Count After Experimental Testicular IR Injury. Note. There are significant differences between groups with different superscripts in a column ($P<0.05$).

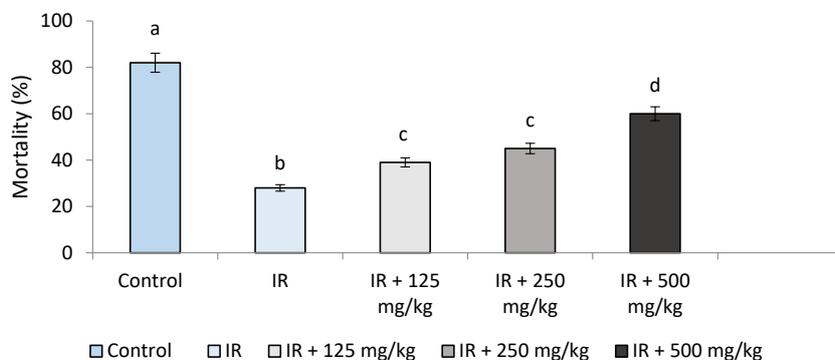


Figure 2. The Effect of Magnesium Sulfate on Sperm Motility After Experimental Testicular IR Injury. Note. There are significant differences between groups with different superscripts in a column ($P<0.05$).

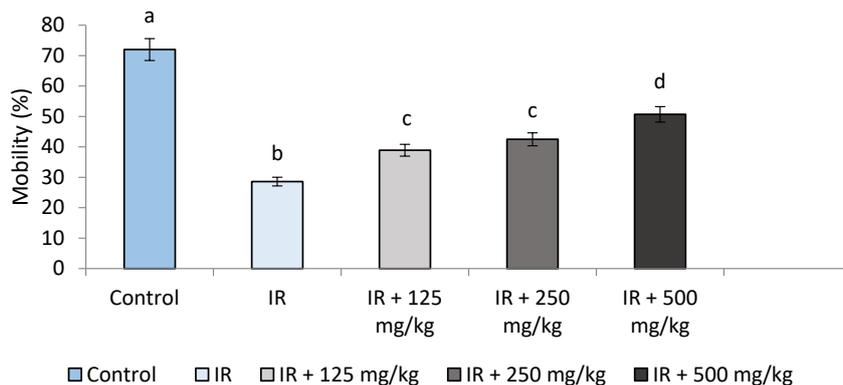


Figure 3. The Effect of Magnesium Sulfate on Sperm Mobility After Experimental Testicular IR Injury. Note. There are significant differences between groups with different superscripts in a column ($P<0.05$).

Discussion

Although the protective and antioxidant effects of $MgSO_4$ have been shown in different tissue injuries such as brain, spinal cord, neurons, and myocardia (14,15), limited studies have described the effect of $MgSO_4$ on sperm characteristics and blood hematology after testicular IR injury in the rats. As noted earlier, the single intraperitoneal

injection of $MgSO_4$ (dose dependently) one hour after IR injury caused the improvement of sperm count, motility, and mobility.

During IR injury, the increased production of ROS causes serious damage to ischemic tissues through oxidization of cell membrane lipids, proteins, and DNA (16). Oxidative stress resulting in an imbalance between

the production of free oxygen radicals and antioxidant capacity (17), causes cell damage (18). The ROS severely produced from abnormal spermatozoa decrease the level of antioxidant defenses in the seminal plasma (19). Renewed interest has developed in using Mg^{2+} as a neuroprotective and antivasospastic agent. Studies have confirmed the usefulness of $MgSO_4$ treatment in diminishing traumatic brain edema and blood brain barrier injury (20). Latest research has shown that the stabilizing effect of antenatal $MgSO_4$ treatment could relieve the risk of cerebral vascular catastrophes in the vulnerable areas of the brain in premature infants with respiratory distress syndrome (21). Moreover, most ATP-dependent cellular processes require Mg^{2+} as a cofactor (22). Eshraghi et al investigated the impacts of $MgSO_4$ on cholestasis-induced hepatic injury after bile duct ligation in male rats. The results suggested $MgSO_4$ treatment may be beneficial for cholestasis-induced hepatotoxicity (23).

A previous study revealed that the administration of different doses of $MgSO_4$ (25, 50, and 100 mg/kg) for 6 weeks can improve sperm characteristics against varicocele in rats (24). It has also been mentioned in the other study that Lomodex and $MgSO_4$ as adjunct pharmacotherapy in cases of testicular torsion can affect testicular salvage with a possible subsequent improvement in semen quality, fertility, and a reduction in long-term morbidity (25). The results of the current study were in line with those of previous reports.

In another study, the effects of sildenafil citrate on torsion/detorsion-induced changes in red blood cells and plasma lipid peroxidation, antioxidants, and blood hematology of male rats were evaluated. Results indicated that the administration of low-dose sildenafil citrate led to a significant increase in the levels of erythrocyte glutathione, plasma paraoxonase, NO, and blood lymphocyte count (26). Furthermore, the results of Lysiak et al suggested that strategies that block neutrophil recruitment may be useful for reducing IR injury in certain affected tissues. These beliefs verify the potential of medical therapies in improving the rescue of the testis, especially, after surgical treatment of torsion (27). In addition, Taati et al investigated the protective effects of ghrelin against IR damage. They found that malondialdehyde values were significantly reduced in the treated group, and ghrelin significantly enhanced sperm movement, motility, and concentration. This protective effect could be attributed to the anti-apoptotic and anti-inflammatory effects of ghrelin (28). Recent studies have indicated that the simultaneous administration of melatonin and vitamin E to homocysteine-treated animals could impede the decrease of antioxidant enzyme activities in plasma, testosterone level, the epididymal sperm concentration, and motility (29). In a research that evaluated the effect of pomegranate juice on sperm concentration in a rat model after testicular torsion-detorsion, the results indicated that pomegranate juice significantly improved

the concentration of spermatids, spermatocytes, and spermatogonia compared to those in the IR group (30).

Conclusions

In summary, the results of this study, along with those of the above-mentioned studies indicated that the administration of $MgSO_4$ can improve sperm count, motility, and mobility. It can also increase the fertility in adult males after spermatic cord torsion/detorsion. Additionally, the current results showed an increased lymphocyte count after IR injury.

Conflict of Interests

Authors declare that there is no conflict of interests.

Ethical Issues

All protocol of the study was approved by the Ethics Committee of Islamic Azad University, Science and Research Branch, Tehran, Iran (Code of Ethics: 25876).

Financial Support

None.

References

1. Barkhordari A, Hekmatimoghaddam S, Jebali A, Khalili MA, Talebi A, Noorani M. Effect of zinc oxide nanoparticles on viability of human spermatozoa. *Iran J Reprod Med.* 2013;11(9):767-71.
2. Asghari A, Akbari G, Beigi A, Mortazavi P. Tramadol reduces testicular damage of ischemia-reperfusion rats. *Anim Reprod.* 2016;13(4):811-9. doi: 10.21451/1984-3143-AR823
3. Makgür F, Kilinç K, Tanyel EC, Büyükpamukcu N. Ipsilateral and contralateral testicular biochemical acute changes after unilateral testicular torsion and detorsion. *Urology.* 1994;44(3):413-8.
4. Tuglu D, Yuvanc E, Yilmaz E, et al. The antioxidant effect of dexmedetomidine on testicular ischemia-reperfusion injury. *Acta Cir Bras.* 2015;30(6):414-21. doi: 10.1590/S0102-865020150060000007.
5. Kanter M. Protective effects of melatonin on testicular torsion/detorsion-induced ischemia-reperfusion injury in rats. *Experimental and molecular pathology.* 2010;89(3):314-20. doi: 10.1016/j.yexmp.2010.07.006.
6. Takhtfooladi MA, Jahanshahi A, Sotoudeh A, Khansari M, Takhtfooladi HA. The antioxidant role of N-acetylcysteine on the testicular remote injury after skeletal muscle ischemia and reperfusion in rats. *Pol J Pathol.* 2013;64(3):204-9.
7. Asghari A, Akbari G, Meghdadi A, Mortazavi P. Effects of melatonin and metformin co-administration on testicular ischemia/reperfusion injury in rats. *J Pediatr Urol.* 2016;12(6):410.e1-e7. doi: 10.1016/j.jpuro.2016.06.017
8. Kao M-C, Jan W-C, Tsai P-S, Wang T-Y, Huang C-J. Magnesium sulfate mitigates lung injury induced by bilateral lower limb ischemia-reperfusion in rats. *J Surg Res.* 2011;171(1):e97-e106. doi: 10.1016/j.jss.2011.03.028.
9. Flatman PW. Magnesium transport across cell membranes. *J Membr Biol.* 1984;80(1):1-14.
10. Romani A, Scarpa A. Regulation of cell magnesium. *Arch Biochem Biophys.* 1992;298(1):1-12. doi: 10.1016/0003-

- 9861(92)90086-C
11. Valsa J, Padmanabhan Skandhan K, Sahab Khan P, Sumangala B, Gondalia M. Split ejaculation study: semen parameters and calcium and magnesium in seminal plasma. *Cent European J Urol.* 2012;65(4). doi: 10.5173/cej.2012.04.art7.
 12. Agarwal A, Durairajanayagam D, Halabi J, Peng J, Vazquez-Levin M. Proteomics, oxidative stress and male infertility. *Reprod Biomed Online.* 2014;29(1):32-58. doi: 10.1016/j.rbmo.2014.02.013.
 13. Seed J, Chapin RE, Clegg ED, et al. Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: a consensus report. *Reprod Toxicol.* 1996;10(3):237-44.
 14. Esen F, Erdem T, Aktan D, et al. Effect of magnesium sulfate administration on blood-brain barrier in a rat model of intraperitoneal sepsis: a randomized controlled experimental study. *Crit Care.* 2004;9(1):R18. doi: 10.1186/cc3004
 15. Saeki H, Matsumoto M, Kaneko S, et al. Is intrathecal magnesium sulfate safe and protective against ischemic spinal cord injury in rabbits? *Anesth Analg.* 2004;99(6):1805-12. doi:10.1213/01.ANE.0000138039.04548.3D
 16. Wei S-M, Yan Z-Z, Zhou J. Curcumin attenuates ischemia-reperfusion injury in rat testis. *Fertil Steril.* 2009;91(1):271-7. doi: 10.1016/j.fertnstert.2007.10.082.
 17. Asghari A, Akbari G, Meghdadi A, Mortazavi P. Protective effect of metformin on testicular ischemia/reperfusion injury in rats. *Acta Cirurgica Brasileira.* 2016;31(6):411-6. doi: 10.1590/S0102-865020160060000008.
 18. Zhang X-Y, Liu Z-M, Wen S-H, et al. Dexmedetomidine administration before, but not after, ischemia attenuates intestinal injury induced by intestinal ischemia-reperfusion in rats. *Anesthesiology.* 2012;116(5):1035-46. doi: 10.1097/ALN.0b013e3182503964.
 19. Agarwal A, Virk G, Ong C, du Plessis SS. Effect of oxidative stress on male reproduction. *World J Mens Health.* 2014;32(1):1-17. doi: 10.5534/wjmh.2014.32.1.1
 20. Feng D, Zhu Z, Lu Y. Effects of magnesium sulfate on traumatic brain edema in rats. *Chin J Traumatol.* 2004;7(3):148-52.
 21. Rantonen T, Grönlund J, Jalonen J, et al. Comparison of the effects of antenatal magnesium sulphate and ritodrine exposure on circulatory adaptation in preterm infants. *Clin Physiol Funct Imaging.* 2002;22(1):13-7. doi/10.1046/j.1475-097X.2002.00387.
 22. Abeş M, Sarihan H, Değer O, Uydu H. The effect of ATP-MgCl₂ on prevention of reperfusion injury after unilateral testicular torsion. *Eur J Pediatr Surg.* 2001;11(04):255-8.
 23. Eshraghi T, Eidi A, Mortazavi P, Asghari A, Tavangar SM. Magnesium protects against bile duct ligation-induced liver injury in male Wistar rats. *Magnes Res.* 2015;28(1):32-45. doi: 10.1684/mrh.2015.0380.
 24. Asghari A, Akbari G, Galustanian G. Magnesium Sulfate Improves Sperm Characteristics Against Varicocele in Rat. *Crescent Journal of Medical and Biological Sciences.* 2016;3(2):55-59.
 25. Adivarekar PK, Bhagwat SS, Raghavan V, Bandivdekar A. Effect of Lomodex-MgSO₄ in the prevention of reperfusion injury following unilateral testicular torsion: an experimental study in rats. *Pediatr Surg Int.* 2005;21(3):184-90.
 26. Yildiz H, Durmuş AS, Şimşek H, Yaman I. Effects of sildenafil citrate on torsion/detorsion-induced changes in red blood cell and plasma lipid peroxidation, antioxidants, and blood hematology of male rats. *Eur J Obstet Gynecol Reprod Biol.* 2011;159(2):359-63. doi: 10.1016/j.ejogrb.2011.07.023.
 27. Lysiak JJ, Turner SD, Nguyen QAT, Singbartl K, Ley K, Turner TT. Essential role of neutrophils in germ cell-specific apoptosis following ischemia/reperfusion injury of the mouse testis. *Biol Reprod.* 2001;65(3):718-25.
 28. Taati M, Moghadasi M, Dezfoulian O, Asadian P. Effects of ghrelin on testicular ischemia/reperfusion-induced injury. *Acta Med Iran.* 2016;54(1):32-8.
 29. Sönmez M, Yüce A, Türk G. The protective effects of melatonin and vitamin E on antioxidant enzyme activities and epididymal sperm characteristics of homocysteine treated male rats. *Reprod Toxicol.* 2007;23(2):226-31. doi:10.1016/j.reprotox.2006.11.003
 30. Atilgan D, Parlaktas B, Uluocak N, et al. Pomegranate (*Punica granatum*) juice reduces oxidative injury and improves sperm concentration in a rat model of testicular torsion-detorsion. *Exp Ther Med.* 2014;8(2):478-82. doi: 10.3892/etm.2014.1782

Copyright © 2020 The Author(s); This is an open-access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.