

OVOZOA

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Based on the decision of Asosiasi Departemen Reproduksi Veteriner Indonesia (ADERVI) or the Indonesian Association of Department of Veterinary Reproduction (IADVR) meeting on December 11th, 2019 in Surabaya, the publication of the OVOZOA e-journal starting in 2020 in collaboration with ADERVI. The Memorandum of Agreement between the Dean of the Faculty of Veterinary Medicine, Universitas Airlangga and the Chairperson of ADERVI, Number (FKH unair): 1445/UN3.1.6/KS/2020 and Number (ADERVI): 04/ADERVI/V/2020 signed on May 4th, 2020. Since March 27th, 2021 OVOZOA Journal of Animal Reproduction has also collaborated with the Indonesian Animal Reproduction Association (Asosiasi Reproduksi Hewan Indonesia, ARHI). Starting from Volume 9 – 2020, OVOZOA completes its name as the OVOZOA Journal of Animal Reproduction. It contains the publication of scientific work of lecturers, researchers, and practitioners of ADERVI members in the field of Veterinary Reproduction and Animal Reproduction Biotechnology. Ovozoa Journal of Animal Reproduction is published at <https://e-journal.unair.ac.id/OVZ/index> periodically three times a year: April, August, and December.

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Original article

Effect of taurine on histopathological features of spermatogenesis in seminiferous tubules of mice (*Mus musculus*) induced by paraquat

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ABSTRACT

This study aimed to determine the effect of taurine on the enhancement of the spermatogenic process in male mice (*Mus musculus*) induced by paraquat (PQ). Twenty-five male mice (*Mus musculus*) aged 2-3 months with a bodyweight of around 35 grams were divided randomly into five groups. The K + and the treatment group (P1, P2, and P3) mice were induced using PQ. PQ was given intraperitoneally (IP) twice a week for 21 consecutive days at a dose of 30 mg/kg BW. Two hours after the administration of PQ, P1, P2, and P3 groups were given taurine at a dose of 250, 500, and 1000 mg/kg BW/day for three weeks (Heidari *et al.*, 2019). K- group was given distilled water (IP) only. On day-29, mice were sacrificed for testicles histopathological preparations with hematoxylin-eosin staining. Results showed that the mice exposed to PQ only (the K+ group) had a reduced spermatogenesis score compared to those of the K- group ($p < 0.05$). Taurine treatment on PQ-exposed mice was followed by an increase spermatogenesis score. The optimal curative dose of taurine was 500 mg/kg (P2 group). However, a higher dose (1000 mg/kg BW) of taurine resulted in a decline in the spermatogenesis score than those of at the 500 mg/kg. It could be concluded that treatment with taurine could enhance the spermatogenic process of male mice (*Mus musculus*) induced by PQ.

Keywords: histopathology, paraquat, reproductive health, seminiferous tubules, spermatogenic cell, taurine

INTRODUCTION

Herbicides are pesticides with toxic chemical compounds that control unwanted weeds or plants (Gaines *et al.*, 2020). However, paraquat (PQ) also caused toxic effects on animals and humans' growth, development, and activities (Soni *et al.*, 2019). PQ was absorbed

through the bloodstream to almost all organs and tissues, not metabolized but reduced to unstable free radicals, which were then re-oxidized to form cations and produce superoxide anions (Wasiu and Abdulfatai, 2019). Free radicals could also attack the Leydig cells' microtubules and the Sertoli cells' mitochondrial membranes. If these two cells were damaged, it would

interfere with the maturation of germ cells in the seminiferous tubules, resulting in imperfect spermatozoa. In addition, damage to the mitochondrial membrane of sperm cells would reduce the amount of ATP needed for sperm movement, damaged the DNA structure, and led to sperm cell death. Decreased sperm motility could also be caused by sperm maturation in the epididymis (Anggraini *et al.*, 2019).

PQ was known to cause cell damage by ordering excessive amounts of Reactive Oxygen Species (ROS) due to oxidative stress (Chen *et al.*, 2012; See *et al.*, 2022). The toxicity mechanism of PQ elevated the increase in ROS in the form of hydrogen peroxide and superoxide anions. The two free radicals could cause severe damage to various vital organs and system disorders such as infertility (Elham *et al.*, 2015; Onur *et al.*, 2022). Oxidative stress occurred as a result of excess ROS production. ROS had a toxic effect on sperm quality due to damage to the plasma membrane, which contained large amounts of fatty acids, and caused sperm morphological defects by inducing lipid peroxidation (Mirzaee *et al.*, 2019). ROS could be captured by antioxidants (Ravi *et al.*, 2018). Taurine could act as an antioxidant, capacitation factor, membrane stabilizer, and spermatozoa motility factor (Yang *et al.*, 2010). As a membrane stabilizer taurine inhibited Na and K-ATPase activity to protect the sperm plasma membrane from free radicals and oxidation (Baliou *et al.*, 2021). It was reported that taurine could be synthesized by male organs. Taurine has been detected in the testes and identified as the main free amino acid in sperm cells and seminal fluid (Aaronson *et al.*, 2010). Taurine could also stimulate testosterone secretion in both in vivo and in vitro conditions (Yang *et al.*, 2010). Testosterone functioned to control the spermatogenesis process, increased the Sertoli cells, and played a role in determining the quality of spermatozoa (Anggraini *et al.*, 2019). Taurine, which was non-enzymatic, had a positive effect on spermatozoa during cryo conservation due to more minor damage to male gametes. Taurine inhibited lipid peroxidation and protects cells from the accumulation of ROS (Jong *et al.*, 2021). The antioxidant action of taurine was shown by protecting mitochondria against excessive superoxide radicals when taurine

helped mitochondrial proteins (Bai *et al.*, 2021). Therefore, this study aimed to determine the effect of taurine on enhancing the spermatogenesis of mice (*Mus musculus*) exposed to PQ.

MATERIALS AND METHODS

Twenty-five male mice (*Mus musculus*) aged 2-3 months with a bodyweight of around 35 grams were divided randomly into five groups. The K+ and the treatment group (P1, P2, and P3) mice were induced using PQ. PQ was given intraperitoneally (IP) twice a week for 21 consecutive days at a dose of 30 mg/kg BW (El-Aarag *et al.*, 2019). Two hours after the administration of PQ, P1, P2, and P3 groups were given taurine at a dose of 250, 500, and 1000 mg/kg BW/day for three weeks (Heidari *et al.*, 2019). K- group was given distilled water (IP) only. On day-29, mice were sacrificed for testicles histopathologic preparations with hematoxylin-eosin staining. The procedures conducted in this study was approved by the Animal Care and Use Committee (ACUC) Faculty of Veterinary Medicine, Universitas Airlangga No. 1.KE.108.11.2020.

Assessment of testicular histopathology

The histopathological slides of the seminiferous tubules were examined by light microscopy (Nikon Eclipse E200 LED, Tokyo, Japan) and Optilab Plus software (PT MICONOS, Jakarta, Indonesia) using a 400x magnification of five different fields of view for each slide. Spermatogenesis was quantified according to the profile of the cells encountered along the seminiferous tubules using the Johnsen scoring system (Teixeira *et al.*, 2019). A Johnsen score of 10 indicates maximum spermatogenesis activity, whereas a score of 1 indicates complete absence of germ cells (Table 1).

Data analysis

The spermatogenesis score was evaluated using the Kruskal-Wallis test followed by the Mann - Whitney test. Statistical analysis used SPSS V21 (International Business Machines/ IBM Corporation, Armonk, New York, USA) with a 95% confidence level.

Table 1 The Johnsen score criteria for spermatogenesis quantification

score	histological criteria
10	Complete spermatogenesis and complete tubules
9	The number of spermatozoa is large, but the spermatogenesis is irregular
8	The number of spermatozoa is low
7	There are no spermatozoa, but there are many spermatids
6	There are very few spermatids
5	There are no spermatozoa or spermatids but many spermatocytes
4	There are only a few spermatocytes
3	There are only spermatogonia
2	No germ cells
1	No germ cells or Sertoli cells

(modified from Teixeira et al., 2019)

RESULTS

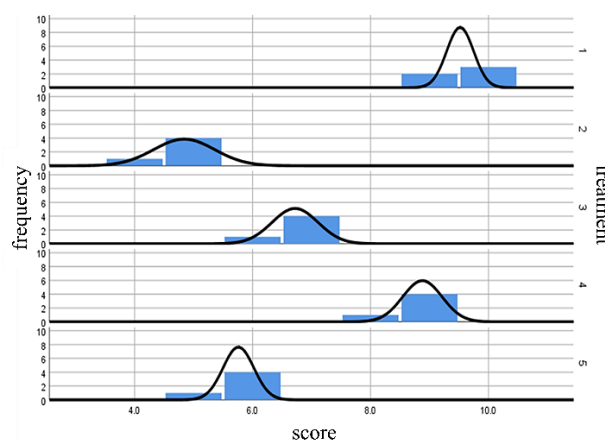
Table 2 Quantification of spermatogenesis process (scored, means \pm SD) in mice (*Mus musculus*) given taurine after being induced with paraquat.

	means \pm SD
K-	9.52 \pm 0.23 ^a
K+	4.84 \pm 0.52 ^e
P1	6.72 \pm 0.39 ^c
P2	8.80 \pm 0.33 ^b
P3	5.76 \pm 0.26 ^d

Different superscripts in the same column indicate significant differences ($p < 0.05$); K-: negative control, mice were given distilled water intraperitoneally; K+: positive control, mice were given paraquat; P1, P2, P3: mice were given paraquat and taurine at a dose of 250, 500, and 1000 mg/kg BW respectively; PQ was given intraperitoneally (IP) twice a week for 21 consecutive days at a dose of 30 mg/kg BW.

The mice exposed to PQ only (the K+ group) showed a reduced spermatogenesis score compared to those of the K- group ($p < 0.05$). The taurine treatment on the PQ-exposed mice

was followed by an increase of spermatogenesis score. The optimal curative dose of taurine was 500 mg/kg (the P2 group). However, a higher dose (the P3 group, 1000 mg/kg BW) of taurine resulted in decreasing in the spermatogenesis score than those of the P2 group ($p < 0.05$) (Table 2, Figure 1, Figure 2).

**Figure 1.** The mean histogram of spermatogenesis scores in mice using the Johnson criteria.

DISCUSSION

The group which was not induced by PQ (only injected with distilled water) had the highest spermatogenesis score compared to other treatment groups. This group did not experience intoxication due to PQ, so they had a normal testicle histological picture. PQ exposure to the mice caused a reduction in spermatogenesis score compared to normal mice. The group that received PQ induction without taurine administration had the lowest spermatogenesis score and the worst histopathological picture of spermatogenesis compared to other groups. The seminiferous tubules of mice given PQ resulted in an abnormal spermatogenesis process due to interference with endogenous antioxidant mechanisms and an imbalance in the number of free radicals and antioxidants in the body. ROS, which has an unpaired electron final layer, would stabilize itself by confiscating electrons from other biomolecules so that they are unstable. Therefore, homeostatic instability can lead to cell death (Aguilar et al., 2016). PQ caused cells oxidative stress due to excessive amounts of

ROS (See *et al.*, 2022). ROS decreased sperm count and motility and inhibited sperm-oocyte fusion. PQ was not metabolized but was reduced to unstable free radicals, then re-oxidized to form cations and produced superoxide anions (Wasiu and Abdulfatai, 2019). Oxidative damage to the membrane caused changes in the degree of membrane fluidity which could compromise the integrity of the membrane, and inactivation of membrane-bound receptors and enzymes, which in turn could damage normal cell function and increase tissue permeability (Aprioku, 2013).

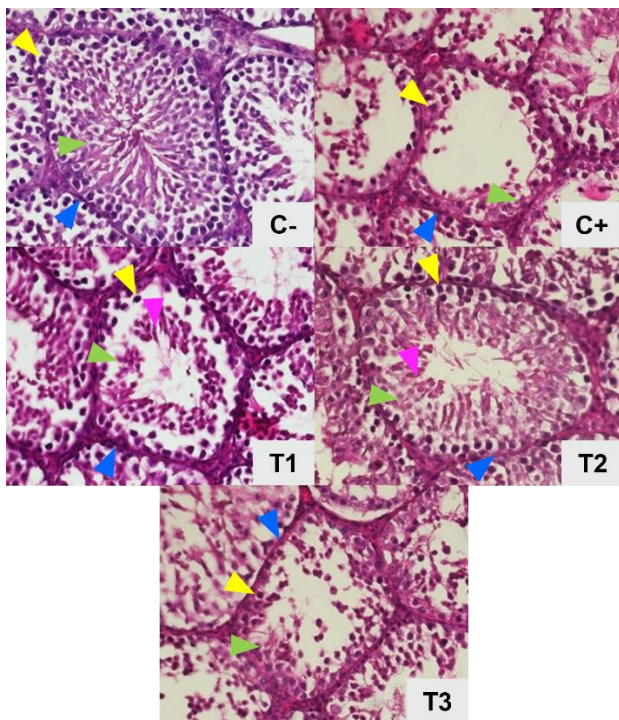


Figure 2 Representative histological image of the seminiferous tubules of mice (*Mus musculus*) exposed to paraquat and treated with taurine (Nikon Eclipse E200, 400x magnification, HE staining); blue arrowheads: seminiferous tubular membrane; yellow arrowheads: spermatogonia cells; green arrowheads: Sertoli cells; pink arrowheads: spermatid; K-: negative control, mice were given distilled water intraperitoneally; K+: positive control, mice were given paraquat; P1, P2, P3: mice were given paraquat and taurine at a dose of 250, 500, and 1000 mg/kg BW respectively; PQ was given intraperitoneally (IP) twice a week for 21 consecutive days at a dose of 30 mg/kg BW.

Antioxidants were essential in preventing oxidative damage to sperm DNA (Gualtieri *et al.*, 2021). Antioxidants were widely used to detoxify excess ROS that caused oxidative stress (Agarwal *et al.*, 2021). Antioxidant nutrition supplementation proved to prevent mortality in healthy humans and patients with various diseases (Bjelakovic *et al.*, 2014). The antioxidant content was found in taurine, which affected male reproduction. Taurine has been detected in vascular endothelial cells, Leydig cells, and several other interstitial cells of the testes, efferent duct epithelial cells, and could be bio-synthesized by male reproductive organs. Additionally, taurine has been identified as the main free amino acid from sperm cells and seminal fluid. Taurine could act as an antioxidant for sperm membrane and sperm motility (Yang *et al.*, 2010).

The taurine-treated group of mice at a dose of 250 mg/kg BW improved the spermatogenesis score. However, that was lesser than the dose of 500 mg/kg BW. This could be caused by the number of ROS produced by PQ was more than the amount of taurine as an antioxidant. The amount of ROS must be balanced with the need for antioxidants so that it could improve the spermatogenesis process (Wagner *et al.*, 2018). This study's optimal curative dose of taurine was 500 mg/kg BW. This result followed the previous report that the optimal dose of taurine 500 mg/kg BW could improve pulmonary fibrosis and be nephroprotective (Heidari *et al.*, 2019). Certain antioxidant nutrients obtained through the diet could be beneficial for disease risk, whereas high doses of the same nutritional supplement could cause damage to others (Smits *et al.*, 2019). Taurine was a compound required for spermatozoa capacitation, fertilization, and embryo development. Taurine was essential in maintaining and stimulating spermatozoa motility and stimulating capacitation and acrosome reactions in vivo and in vitro. Taurine could also inhibit lipid peroxidation in spermatozoa and prevent loss of motility (Slanina *et al.*, 2018).

The higher dose (1000 mg/kg BW) of taurine decreased the spermatogenesis score than those of 500 mg/kg BW. Antioxidant overuse was associated with adverse effects on the large doses of dietary antioxidants. The excessive use

of antioxidant in therapy might cause male infertility (Henkel et al., 2019). Free radicals have multiple biological functions, dangerous and beneficial. In moderate concentrations, free radicals were an essential mediator of reactions in which unwanted cells were eliminated from the body (Bjelakovic et al., 2012). However, the lower free radicals could interfere with essential defense mechanisms such as apoptosis, phagocytosis, and detoxification. It could disrupt the delicate balance between oxidative stress and antioxidants in body cells (Bisht et al., 2017).

CONCLUSION

The administration of taurine improved spermatogenesis in the seminiferous tubules of mice (*Mus musculus*) induced by paraquat with the optimal dose of taurine of 500 mg/kg BW for 21 days of treatment. Future study is needed to assess semen quality in mice exposed to paraquat and treated with taurine.

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Author Guidelines

OVOZOA Journal of Animal Reproduction is a journal that contains scientific articles on animal reproduction and animal reproductive biotechnology published by the Department of Veterinary Reproduction of Airlangga University together with the Association of the Indonesian Veterinary Reproduction Department (ADERVI) 3 times per year on April, August and December. OVOZOA received manuscripts in the form of original research articles, review articles and case reports in Indonesian and English. Manuscripts received must be original, current and have never been published or are being planned to be published in other scientific journals.

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The first page contains titles in Indonesian and English, followed by full names of all authors without titles and initials (bold, centered), followed by the name and complete address of the respective institution (marked with numeric superscripts) and e-mail of corresponding authors (marked with *superscript).

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Title should be concise but informative, as far as possible in no more than 12 words.

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Keywords maximum of 6 (six) words or phrases, written after the abstract in each languages, alphabetically ordered. As far as possible avoid using keywords from the title.

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Acknowledgements are delivered to the research funders (state the name, number and recipient name of the grant, if applicable), and to those who have helped carry out the research.

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Journals

Nakamura K, Kusama K, Ideta A, Imakawa K, Hori M. 2020. IFNT-independent Effects of Intrauterine Extracellular Vesicles (EVs) in Cattle. *Reproduction* 159: 503-11.

Windeyer MC, Gamsjager L. 2019. Vaccinating Calves in the Face of Maternal Antibodies:

Challenges and Opportunities. *Review Vet Clin North Am Food Anim Pract.* 35: 557-73.

Books

Gordon I. 2003. *Laboratory Production of Cattle Embryos*. 2nd Ed. CABI Publishing. UK

Chapter in books

Parkinson TJ, Vermunt Jos J, Noakes DE. 2019. Maternal Dystocia: Causes and Treatment. In: Noakes DE, Parkinson TJ, England GCW (Ed). *Veterinary Reproduction and Obstetrics*. 10th Ed. Elsevier, UK. 236-49.

Thesis/Dissertation

Utama S. 2012. *Monitoring and Assessment of Nuclear Transfer Pregnancies using Maternal*

Pregnancy Recognition Proteins. PhD. Thesis. Monash University. Melbourne, Australia.

Internet

Akhir N. 2020. Title of article from the internet. <http://www.link>. access date.

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