

A Comparison of the Oxidant-antioxidant Status of Serum and Seminal Plasma from Infertile Male Camels after Zinc, Selenium, and Vitamin E Treatment

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Article History: 23-206

Received: 13-May-23

Revised: 29-Jun-23

Accepted: 13-Jul-23

ABSTRACT

The aim of this study was to investigate how Zn, Se, and Vit E treatment affected the oxidant-antioxidant status of serum and seminal plasma from infertile male dromedary camels. Catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), total antioxidant capacity (TAC), and malondialdehyde (MDA) levels in serum and seminal plasma were all measured before and after treatment. The treatment increased CAT and TAC expression while decreasing MDA activity in serum and seminal plasma. In conclusion, Zn, Se, and Vit E treatments improve the antioxidant status of infertile camel serum and seminal plasma, implying that trace element supplementation may be effective in treating camel infertility. This program may benefit male dromedaries suffering from idiopathic infertility.

Key words: Antioxidant; Male camels; Infertility; Trace elements; Vitamin E.

INTRODUCTION

A constant and balanced ratio of oxidants and antioxidants is one of the most widely used treatments for male infertility in the world today (Pintus and Ros-Santaella 2021; El-Hamid 2021; Ritchie et al. 2021). Oxidative stress and a decrease in seminal antioxidant capacity cause approximately 30-80% of male infertility (Agarwal et al. 2018; Darbandi et al. 2019; Alkadi 2020). As a result of a variety of stresses, cells generate free radicals (reactive oxygen species, ROS). Cells are protected from ROS by antioxidants (Smits et al. 2018). Antioxidants can be exogenous, such as vitamin E (Vit E) and selenium (Se), or endogenous, such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) (Yang et al. 2018).

Post-coital infertility refers to the male's inability to conceive the female, which can be permanent or temporary. It is the most common cause of infertility in dromedaries in Saudi Arabia (Ali et al. 2021a). More research is needed in this area because male camel infertility has increased over the last decade (Ali et al. 2014; 2019; 2021a).

Several studies have indicated that adequate amounts of trace elements, minerals, and vitamins are beneficial to sperm health and male fertility (Contri et al. 2011;

Domosławska et al. 2018; Ali et al. 2021b; Beygi et al. 2021). There is little known about how these elements affect dromedary camels' serum and seminal plasma oxidant-antioxidant capacity. In this study, Zn, Se, and Vit E were administered to post-coital infertile male dromedary camels to determine the oxidant-antioxidant status of serum and seminal plasma.

MATERIALS AND METHODS

This study was approved by the Deanship of Deanship of Higher Studies, Qassim University, Kingdom of Saudi Arabia.

Animals

Thirty-three male dromedary camels (age: 7.17±0.29 years; body condition score [BCS]: 3±0.11 on scale of 1-5 (Sghiri and Driancourt 1999) and duration of infertility: 2.76±0.26 rutting seasons) were examined for post-coital infertility during the rutting season, at Qassim University's Veterinary Teaching Hospital. In addition to sexual desire and mating ability, previous fertility, diseases or injuries, and prescriptions were also documented. Most of the animals were released into open desert areas and fed alfalfa hay and barley concentrate.

Cite This Article as: Ali A, Derar DR, Alhassun TM, and Zeitoun MM, 2023. A comparison of the oxidant-antioxidant status of serum and seminal plasma from infertile male camels after zinc, selenium, and vitamin E treatment. International Journal of Veterinary Science x(x): xxx. <https://doi.org/10.47278/journal.ijvs/2023.082>

Breeding Sound Examination

Male camels were placed in a sternal position and underwent a general physical examination. The testes were examined by palpation, caliper and ultrasound (Aloka SSD-500, Aloka Co., Ltd, Tokyo, Japan). The prostate body and bulbourethral gland dimensions were also estimated using ultrasound.

Treatment Regime

Infertile camels were given intramuscular injections of vitamin E (-tocopherol acetate, 1mg/kg bw) and selenium (sodium selenite, 0.088mg/kg bw) (B0 SE, Heritage Animal Health, IA, USA) once a week for three weeks and an oral administration of 360mg zinc gluconate (Vita-Vigor, FL, USA) for five weeks (Fig. 1). Oxidant-antioxidant status was assessed in serum and seminal plasma before and after treatment.

Estimation of Oxidant-antioxidant Biomarkers in Serum and Seminal Plasma

Blood (obtained from the jugular vein) and semen (by electroejaculation, Ali et al. 2014) were collected from all animals before and after treatment. Food and water were withheld for 24 hours prior to the procedure in order to collect sperm. Camels were sedated in lateral recumbency with xylazine HCl Bomazine (BOMAC Laboratories Ltd., New Zealand). The ElectroJac 6 electroejaculator probe (Neogen, Lexington, KY, USA) was inserted into the rectum and set to automatic mode. The ejaculate was collected in a sterile collecting tube that was mounted on a collection funnel that was held close to the preputial orifice. Serum and seminal plasma were harvested by centrifugation (1200 x g for 10min) and kept at -20°C until analysis. Serum and seminal plasma were examined spectrophotometrically for CAT, TAC, MDA, GPx, and SOD using commercial kits (Biodiagnostic, Giza, Egypt, Cat. No: MD 25 29, TA 25 13, SD 25 21, GR 25 24, CA 25 17).

Statistical Analysis

For the analysis, the IPM-SPSS program, version 25.0 (Chicago, IL, USA, 2017), was used. To compare CAT, TAC, GPx, and SOD levels before and after treatment, a paired samples t-test was used. The data were presented as means±SE. P<0.05 was chosen as the level of significance.

RESULTS AND DISCUSSION

The sizes of the testes and accessory glands (Table 1) were within the normal range based on Arabian camel reference values (Derar et al. 2012). Furthermore, ultrasound revealed no abnormalities in the testes or accessory sex glands. Similarly, neither visual, testicular parenchyma nor computerized pixel analysis of testicular ultrasonic echo texture predicted bull semen quality consistently (Pinho et al. 2013). The treatment had no effect on the testes or accessory glands size or echotexture. As a result, the current treatment regimen may be advised for infertile male dromedaries with no visible genital pathology.

Zn, Se, and Vit E treatment increased CAT and TAC expression while decreasing MDA activity in serum and seminal plasma (Table 2 and 3). Similarly, taking Zn, Se, and Vit E supplements in stallions resulted in higher antioxidant levels in seminal plasma 30 days later (Contri et al. 2011).

Table 1: The effect of Zn, Se, and Vit E treatment on testicular and accessory gland sizes in infertile male camels (n=33)

Organ		Before treatment	After treatment	
Testes (cm)	Right	length	6.84±0.4	6.83±0.3
		Width	3.42±0.3	3.44±0.2
		Depth	3.67±0.2	3.69±0.4
	left	length	7.66±0.5	7.68±0.6
		Width	3.53±0.2	3.56±0.2
		Depth	3.81±0.3	3.83±0.4
Prostate gland (cm)	Length	3.55±0.2	3.57±0.2	
	Width	2.43±0.1	2.42±0.1	
Bulbo-urethral glands (cm)	Right Diameter	1.47±0.05	1.48±0.06	
	Left Diameter	1.49±0.07	1.49±0.08	

Values (mean±SE) with different letters in the same row differ significantly (P<0.05).

Table 2: The effect of Zn, Se, and Vit E treatment on the oxidant-antioxidant capacity of infertile camel serum (n=33)

	Before treatment	After treatment
Catalase (U/L)	236.36±25.6 ^a	299.36±27.4 ^b
Total antioxidant (Mm/100 mL)	2.45±0.3 ^a	3.06±0.2 ^b
Malondialdehyde (nmol/mL)	8.73±2.2 ^a	2.29±0.7 ^b
Glutathione peroxidase (U/mL)	0.089±0.01	0.087±0.01
Superoxide dismutase (U/mL)	270.16±10.6	258.96±14.1

Values (mean±SE) with different letters in the same row differ significantly (P<0.05).

Table 3: The effect of Zn, Se, and Vit E treatment on the oxidant-antioxidant capacity of infertile camel seminal plasma (n=33)

	Before supplementation	After supplementation
Catalase (U/L)	220.67±40.5 ^a	281.05±43.84 ^b
Total antioxidant (Mm/100 mL)	2.33±0.01 ^a	2.73±0.02 ^b
Malondialdehyde (nmol/mL)	5.7±1.8 ^a	1.64±0.4 ^b
Glutathione peroxidase (U/mL)	0.08±0.01	0.09±0.01
Superoxide dismutase (U/mL)	219.61±19.8	252.3±15.2

Values (mean±SE) with different letters in the same row differ significantly (P<0.05).

Zn is a potent antioxidant that scavenges ROS (Rahman et al. 2014). Zn supplementation improved seminal plasma antioxidant capacity and sperm characteristics in Beetal rams (Rahman et al. 2014), decreased MDA content in Kashmir goat spermatozoa (Liu et al. 2020), and improved sperm quality in Osmanabadi bucks (Mayasula et al. 2020) and male camels (Ali et al. 2019; 2021b). Zn supplementation can be used for idiopathic infertility in male dromedaries.

Se protects the mitochondrial membrane from damage caused by oxygen and various fatty acid-derived peroxides, controls the metabolism of sperm, and participates in the composition of PHGPx, which is important in ensuring proper sperm assembly, particularly in the mid-piece (Domosławska et al. 2018). Adding se to the diet increased the antioxidant content in the seminal plasma of stallions (Contri et al. 2011). Due to the prevalence of Se deficiency in the Arabian Peninsula (Ali et al. 2019), dietary supplementation may be required.

Vitamin E is one of the membrane defendants against ROS (Domosławska et al. 2018). Supplementation with Vit E has been shown to enhance the quality of camel sperm (Ali et al. 2021b), stallions (Contri et al. 2011), buck (Zhu et al. 2010) and ram (Yue et al. 2010). Se and Vit E have a synergistic effect and should be taken together (Domosławska et al. 2018).

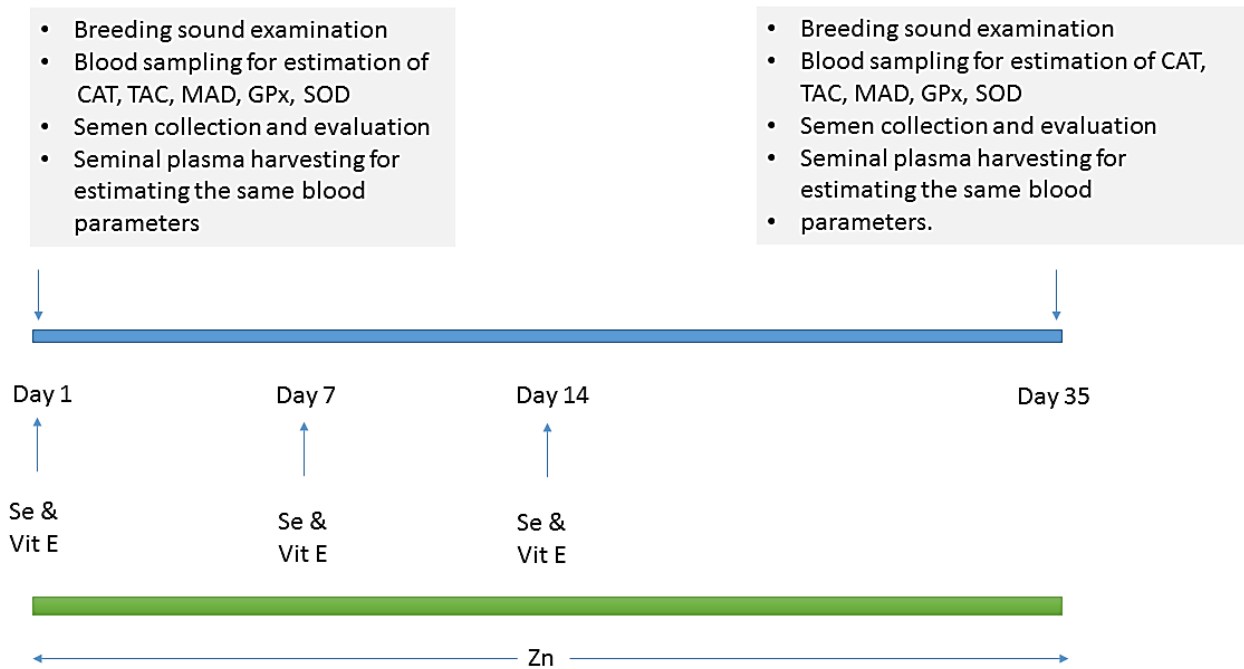


Fig. 1: Illustration of the treatment regime and timing of sampling for infertile camels (n=33). Zn: daily oral administration of 360 mg zinc gluconate (Vita-Vigor, Florida, USA) for 5 consecutive weeks. Vitamin E (α -tocopherol acetate, 1mg/kg body weight) and Selenium (sodium selenite, 0.088mg/kg body weight): once a week for three consecutive weeks (B0 SE, Heritage Animal Health, Iwoa, USA).

CAT acts as a scavenger to remove excess hydrogen peroxide from the cell, protecting cells from oxidative damage (Albarrak 2023). The addition of CAT semen extenders improved semen quality in dogs (Prete et al. 2019) and camels (Malo et al. 2019).

MDA is one of the byproducts of free radical-induced polyunsaturated fatty acid peroxidation in cells (Kumar et al. 2017). Lipid peroxidation contributes to sperm plasma membrane damage. MDA content in seminal plasma was lower in highly fertile bulls than in subfertile bulls (Kumar et al. 2017). MDA accumulation correlated negatively with sperm motility and morphology in infertile patients, indicating oxidative damage to lipids that impairs sperm quality (Benedetti et al. 2012).

TAC denotes the number of free radicals scavenged by a solution under investigation; as a result, it has been used to assess the antioxidant capacity of biological samples (Ghiselli et al. 2000). Infertile patients had lower TAC in semen than fertile men (Benedetti et al. 2012; Gupta et al. 2020). In stallions, a positive correlation was found between progressive motility and seminal plasma TAC levels in both treated and control groups (Contri et al. 2011). Furthermore, TAC and Vit E concentrations in the seminal plasma of infertile men were lower than in fertile men (Benedetti et al. 2012).

Conclusion

Zn, Se, and Vit E treatments enhance the antioxidant status of infertile camel serum and seminal plasma. This suggests that trace element supplementation may help with camel infertility, particularly idiopathic infertility.

Compliance with Ethical Standards

This study was approved by the Animal Care and Welfare Committee, Deanship of Scientific Research, Qassim University, Kingdom of Saudi Arabia.

Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

- Agarwal A, Rana M, Qiu E, AlBunni H, Bui AD and Henkel R, 2018. Role of oxidative stress, infection and inflammation in male infertility. *Andrologia* 50: e13126. <https://doi.org/10.1111/and.13126>
- Albarrak SM, 2023. Evaluation of somatic cells count, antioxidants and antimicrobial proteins in milk samples obtained from different breeds of the dromedary camel. *International Journal of Veterinary Science* 12(3): 462-469. <https://doi.org/10.47278/journal.ijvs/2022.221>
- Ali A, Derar DR and Almundarij TI, 2021a. Aetiological analysis and diagnosis of reproductive disorders in male dromedary camels. *Reproduction in Domestic Animals* 56: 1267–1273. <https://doi.org/10.1111/rda.13988>
- Ali A, Derar DR, Abdel-Elmoniem EM and Almundarij TI, 2019. Impotentia generandi in male dromedary camels: heavy metal and trace element profiles and their relations to clinical findings and semen quality. *Tropical Animal Health and Production* 51: 1167–1172. <https://doi.org/10.1007/s11250-019-01803-7>
- Ali A, Derar DR, Alhassun TM and Almundarij TI, 2021b. Effect of Zinc, Selenium and Vitamin E Administration on Semen Quality and Fertility of Male Dromedary Camels with Impotentia Generandi. *Biological Trace Elements and Research* 199: 1370-1376. <https://doi.org/10.1007/s12011-020-02276-8>
- Ali A, Derar R, Al-Sobayil F, Mehana S and Al-Hawas A, 2014. Impotentia generandi in male dromedary camels: clinical findings, semen characteristics, and testicular histopathology. *Theriogenology* 82: 890–896. <https://doi.org/10.1016/j.theriogenology.2014.07.001>
- Alkadi H, 2020. A Review on Free Radicals and Antioxidants. *Infectious Disorders-Drug Targets* 20: 16-26. <https://doi.org/10.2174/1871526518666180628124323>

- Benedetti S, Tagliamonte MC, Catalani S, Primiterra M, Canestrari F, De Stefani S, Palini S and Bulletti C, 2012. Differences in blood and semen oxidative status in fertile and infertile men and their relationship with sperm quality. *Reproductive BioMedicine Online* 25: 300-306. <https://doi.org/10.1016/j.rbmo.2012.05.011>
- Beygi Z, Forouhari S, Mahmoudi E, Hayat SMG and Nourimand F, 2021. Role of Oxidative Stress and Antioxidant Supplementation in Male Fertility. *Current Molecular Medicine*, 21: 265-282. <https://doi.org/10.2174/1566524020999200831123553>
- Contri A, De Amicis I, Molinari A, Faustini M, Gramenzi A, Robbe D and Carluccio A, 2011. Effect of dietary antioxidant supplementation on fresh semen quality in stallion. *Theriogenology* 75: 1319–1326. <https://doi.org/10.1016/j.theriogenology.2010.12.003>
- Darbandi M, Darbandi S, Agarwal A, Baskaran S, Dutta S, Sengupta P, Khorram Khorshid HR, Esteves S, Gilany K, Hedayati M, Nobakht F, Akhondi MM, Lakpour N and Sadeghi MR, 2019. Reactive oxygen species-induced alterations in H19-Igf2 methylation patterns, seminal plasma metabolites, and semen quality. *Journal of Assisted Reproduction and Genetics* 36: 241-253. <https://doi.org/10.1007/s10815-018-1350-y>
- Derar DR, Hussein HA and Ali A, 2012. Reference values for the genitalia of male dromedary before and after puberty using caliper and ultrasonography in subtropics. *Theriogenology* 77: 459-465. <https://doi.org/10.1016/j.theriogenology.2011.08.023>
- Domosławska A, Zdunczyk S, Franczyk M, Kankofer M and Janowski T, 2018. Selenium and vitamin E supplementation enhances the antioxidant status of spermatozoa and improves semen quality in male dogs with lowered fertility. *Andrologia* 50: e13023. <https://doi.org/10.1111/and.13023>
- El-Hamid IS, 2021. Blood constituents, antioxidant activities and hormonal profile in she-camels (*Camelus dromedarius*) during different physiological statuses in the Northwestern Coast of Egypt. *International Journal of Veterinary Science* 10(4): 247-258. <https://doi.org/10.47278/journal.ijvs.2021.055>
- Ghiselli A, Serafini M, Natella F and Scaccini C, 2000. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. *Free Radical Biology and Medicine* 29:1106–1114. [https://doi.org/10.1016/s0891-5849\(00\)00394-4](https://doi.org/10.1016/s0891-5849(00)00394-4)
- Gupta S, Finelli R, Agarwal A and Henkel R, 2020. Total antioxidant Capacity-Relevance, Methods and clinical implications. *Andrologia* 12: e13624. <https://doi.org/10.1111/and.13624>
- Kumar P, Saini M, Kumar D, Bharadwaj A and Yadav PS, 2017. Estimation of endogenous levels of osteopontin, total antioxidant capacity and malondialdehyde in seminal plasma: Application for fertility assessment in buffalo (*Bubalus bubalis*) bulls. *Reproduction in Domestic Animals*, 52: 221-226. <https://doi.org/10.1111/rda.12882>
- Liu H, Sun Y, Zhao J, Dong W and Yang G, 2020. Effect of Zinc Supplementation on Semen Quality, Sperm Antioxidant Ability, and Seminal and Blood Plasma Mineral Profiles in Cashmere Goats. *Biological Trace Elements and Research* 196: 438-445. <https://doi.org/10.1007/s12011-019-01933-x>
- Malo C, Grundin J, Morrell JM and Skidmore JA, 2019. Individual male dependent improvement in post-thaw dromedary camel sperm quality after addition of catalase. *Animal Reproduction Science* 209: 106168. <https://doi.org/10.1016/j.anireprosci.2019>
- Mayasula VK, Arunachalam A, Sellappan S, Guvvala PR, Naidu SJ, Dintaran P and Bhatta R, 2020. Organic Zn and Cu supplementation imprints on seminal plasma mineral, biochemical/antioxidant activities and its relationship to spermatozoal characteristics in bucks. *Reproductive Biology* 20: 220-228. <https://doi.org/10.1016/j.repbio.2020.02.007>
- Pintus E and Ros-Santaella JL, 2021. Impact of Oxidative Stress on Male Reproduction in Domestic and Wild Animals. *Antioxidants (Basel)* 10:1154. <https://doi.org/10.3390/antiox10071154>
- Pinho RO, Deiler Sampaio Costa DS, Siqueira JB, Martins LF, Teixeira LA and Guimaraes SEF, 2013. Lack of relationship between testicular echotexture and breeding soundness evaluation in adult Nelore bulls. *Livestock Science* 154: 246–249. <https://doi.org/10.1016/j.livsci.2013.03.001>
- Prete CD, Ciani F, Tafuri S, Pasolini MP, Valle GD, Palumbo V, Abbondante L, Calamo A, Barbato V, Gualtieri R, Talevi R and Cocchia N, 2018. Effect of superoxide dismutase, catalase, and glutathione peroxidase supplementation in the extender on chilled semen of fertile and hypofertile dogs. *Journal of Veterinary Science* 19: 667-675. <https://doi.org/10.4142/jvs.2018.19.5.667>
- Ritchie C and Ko EY, 2021. Oxidative stress in the pathophysiology of male infertility. *Andrologia* 53:e13581. <https://doi.org/10.1111/and.13581>
- Rahman HU, Qureshi MS and Khan RU, 2014. Influence of dietary zinc on semen traits and seminal plasma antioxidant enzymes and trace minerals of beetal bucks. *Reproduction in Domestic Animals* 49: 1004–1007. <https://doi.org/10.1111/rda.12422>
- Sghiri A and Driancourt MA, 1999. Seasonal effects on fertility and ovarian follicular growth and maturation in camels (*Camelus dromedarius*). *Animal Reproduction Science* 55: 223–237. [https://doi.org/10.1016/s0378-4320\(99\)00017-2](https://doi.org/10.1016/s0378-4320(99)00017-2)
- Smits RM, Mackenzie-Proctor R, Fleischer K and Showell MG, 2018. Antioxidants in fertility: impact on male and female reproductive outcomes. *Fertility and Sterility* 110: 578-580. <https://doi.org/10.1016/j.fertnstert.2018.05.028>
- Yang CS, Ho CT, Zhang J, Wan X, Zhang K and Lim J, 2018. Antioxidants: Differing Meanings in Food Science and Health Science. *Journal of Agricultural and Food Chemistry*, 66: 3063-3068. <https://doi.org/10.1021/acs.jafc.7b05830>
- Yue D, Yan L, Luo H, Xu X and Jin X, 2010. Effect of Vitamin E supplementation on semen quality and the testicular cell membranial and mitochondrial antioxidant abilities in Aohan fine-wool sheep. *Animal Reproduction Science* 118: 217–222. <https://doi.org/10.1016/j.anireprosci.2009.08.004>
- Zhu H, Luo H, Meng H, Zhang G, Yan L and Yue D, 2010. Effect of vitamin E supplement in dieton antioxidant ability of testis in Boer goat. *Animal Reproduction Science* 117: 90–94. <https://doi.org/10.1016/j.anireprosci.2009.03.016>