

Selenium supplementation reduces induced stress, enhances semen quality and reproductive hormones in Saanen bucks

M.M. Mojapelo ^a, J.B.J. van Ryssen ^a, K.C. Lehloeny ^{a,b,*}

^a Department of Animal & Wildlife Sciences, Faculty of Natural and Agricultural Sciences, University of Pretoria, Private Bag X20, Hatfield, 0028, South Africa

^b Faculty of Science and Agriculture, Private Bag X1001, University of Zululand,

*Corresponding author. Email: LehloenyaK@unizulu.ac.za

Highlights

- Selenium supplementation reduces induced stress by lowering cortisol levels and increasing glutathione peroxidase activity.
- Selenium supplementation increases LH and testosterone concentrations regardless of induced stress.
- Selenium supplementation improves semen quality and quantity of Saanen goats.

Abstract

The study was aimed to evaluate the effect of selenium (Se) supplementation on semen quality of Saanen goats following induced stress. Forty Saanen bucks were divided into two main groups: selenium supplementation and control. The groups were further subdivided into two groups each comprising of induced stress (injected with adrenocorticotrophic hormone - ACTH) and not, leading to four groups (Se, Se + ACTH, ACTH and control). Semen was collected using electro-ejaculator. Selenium supplemented bucks received sodium selenite at two months interval while ACTH was administered at three weeks interval. Selenium supplementation led to higher ($P < 0.001$) ejaculate volume, sperm motility, concentration and viability regardless of induced stress. Se supplemented group produced creamy semen while Se + ACTH treatment led to thick milky semen with no significant difference ($P > 0.05$) between ACTH and control groups which led to milky semen. The semen pH from ACTH, SE + ACTH and control groups was significantly ($P < 0.001$) acidic compared to neutral (7.05 ± 0.05) pH from Se supplemented group. The percentage of primary sperm abnormalities were significantly ($P < 0.001$) lower in selenium supplemented group than the ACTH and control groups. Selenium supplementation significantly ($P < 0.001$) increased serum glutathione peroxidase activity, luteinising hormone and testosterone concentrations and reduced cortisol level. Therefore, oral supplementation with sodium selenite can be implemented in goats that are raised on selenium deficient soils to boost the oxidant status hence, lessen the stress effects by reducing cortisol levels and increasing glutathione peroxidase activity and eventually, improving semen quality.

Keywords: Electro-ejaculator; Selenium; Semen quality; Saanen goats; Cortisol; GSH-Px; Testosterone

1. Introduction

Reproductive performance of animals is largely affected by nutrition, environment, genetic make-up and management (Kumar et al., 2009; Ahsan et al., 2014a, b; Ibtisham et al., 2018). Amongst these factors plane nutrition and specific nutrients play a bigger role in regulating reproduction activities, particularly in male animals (Hafez and Hafez, 2000). Currently, some trace element nutrients have attracted more research as they seem to play important roles in regulating reproduction and therefore, emphasizing their need as being important similar to macro elements (Horky et al., 2017; Ibtisham et al., 2018). Selenium (Se) is one of the essential trace elements playing an important role in male fertility. The imbalance of this element is reported to impair reproduction and reduce immunity in animals (Kolodziej and Jacyno, 2005; Shi et al., 2018).

Se plays a key role in antioxidant defence system as it is a constituent of the enzyme glutathione peroxidase (GSH-Px). In male animals, Se enhances testicular growth and development of the seminiferous tubules, testosterone biosynthesis and spermatogenesis (Marin-Guzman et al., 2000; Shi et al., 2018). A deficiency of Se is reported to result in reduction of the antioxidant defence in farm animals, which in turn disrupts normal spermatogenesis (Behne et al., 1996; El-Mokadem et al., 2012). Additionally, higher percentages of abnormal sperm and lower fertilising ability of the oocytes by sperm were observed in animals that had lower Se levels (Marin-Guzman et al., 1997). Under extensive production systems, animals obtain Se from the pastures/grasses. As a result, soils deficient in Se can lead to pastures lower in Se, resulting in Se deficiency in animals. In South Africa, lower Se levels in soils has been observed in some areas of South Africa such as KwaZulu-Natal Midlands and Mpumalanga Bethal/ Hedrina (Van Ryssen, 2001). It is therefore expected that animals fed forages from Se deficient areas or raised under those environments will have a compromised fertility and lower semen quality (Ahsan et al., 2014a, b).

In farm animals, stress has been implicated as a contributory factor to infertility. Stress influences numerous mechanisms including the hypothalamic-pituitary-axis that in turn triggers the testicular endocrine function (Kumar et al., 2009). Excessive stress induces the increase in plasma concentration of the glucocorticoids which in turn suppresses luteinising hormone (LH) receptors and consequently reduces testosterone secretion of the animals (Brown et al., 1989). Animals exposed to stress were reported to have a reduced quality of semen and fertility as stress causes impairment of spermatogenesis (Hansen, 2009). Farm animals exposed to stress had reduced semen quality indicated by decline in sperm motility, concentration and increased morphologically abnormal spermatozoa in the ejaculate (Mieusset et al., 1992; Hansen, 2009). Therefore, we hypothesize that Se supplementation will reduce induced stress and improve semen quality collected with electro-ejaculator (EE) method. Thus, the aim of the present study was to determine the effect of Se supplementation on semen quality of Saanen goats treated with ACTH to induce stress.

2. Materials and methods

2.1. Study location

This study was conducted at University of Pretoria experimental farm, Hatfield, Pretoria (latitude 25°45' South, longitude 28°16' East). The experimental farm is situated in the Highveld region of South Africa, at an altitude of 1 327 m above mean sea level (Mengistu et al., 2016).

2.2. Experimental animals and management

The study was approved by the University of Pretoria Animal Ethics Committee under the project number EC 10613 and was conducted using 40 Saanen male goats. All the experimental animals were kept in an open pen for a period of 13 months and fed locally available milled Lucerne (*Medicago sativa*) obtained from the Vaalhartz Irrigation scheme and phosphate mineral lick *ad libitum*. The animals also had free access to water throughout the study. The animals had no access to fresh growing forages. Lucerne hay was milled and tested for Se concentration prior to supplementation and no Se traces were detected using the spectrophotometer (Perkin-Elmer 2380 Atomic Absorption Spectrophotometer; Varian, Australia). In South Africa, sheep and goats are raised under extensive production (graze on natural or cultivated pastures) and are supplemented with Lucerne (for protein) in winter when the pasture nutrients are considered low. For this study, the animals were given Lucerne hay produced from soils deficient in selenium (Van Ryssen, 2001) in order to have a control group. Furthermore, Lucerne hay is considered as good quality nutrition that can meet the nutritional requirements of the goats and therefore for sheep and goats. Besides providing high protein, Lucerne also contains the following minerals (Calcium, copper, iron, magnesium, Manganese, Phosphorus, Zinc and Silicon), vitamins (A, B, C, E, K), phytochemical substances (carotene, chlorophyll, coumarins, isoflames, alkaloids and saponins), secondary metabolites of plants phytates (L-canavanine, sponins) (Aganga and Tshwenyane, 2003; Gawel, 2012; Mielmann, 2013; Ponnampalam et al., 2020).

2.3. Experimental design and treatment

Forty Saanen male goats were used for conducting the trial. Data collection was carried out from winter to spring (July to October) for a period of four months. It should be noted that the animals used in this study were already under the same feeding strategy from the previous experiment (Mojapelo and Lehloenya, 2019) where they were fed Lucerne only. Meaning that the period they were on Lucerne and those on selenium was for 13 months although, the stress treatment and data collection for this study was for four months. For this study, the animals received 0.34 mg Se/kg orally (Van Ryssen et al., 1992; Mahmoud et al., 2013) at two months interval. The bucks aged 11–12 months at the beginning of the study, with average body weight of 27.01 kg. The animals were grouped according to body weight and randomly allocated into treatment groups. The study was a 2 × 2 factorial design with the main factors being Se and ACTH. Animals were first divided into the main groups of Se supplementation and control then each group was further subdivided into ACTH and not, leading to 4 groups [Se + ACTH (n = 10); Se (n = 10); ACTH (n = 10) and control (n = 10)].

For ACTH treatment, the animals were injected intramuscularly with 0.3 mg ACTH/kg (Porcine, ACTH fragment 1–39, Sigma Aldrich®, USA) every three weeks while those that were not receiving ACTH were injected with saline solution, intramuscularly (Van Lier et al., 2003).

2.4. Blood collection from Saanen goats over a period of 4 months

For determination of Se concentration, blood samples were collected using 4 mL BD vacutainer (lithium heparin) tubes (BD-Plymouth.PL6 7BP®, United Kingdom) from the jugular vein at the onset of the experiment and once a month throughout the experiment. The whole blood was stored at –20 °C until analysed for Se concentration.

For testosterone, LH and cortisol concentrations as well as glutathione peroxidase (GSH-Px) activity, the blood samples were collected every two weeks throughout the experiment using 4 mL BD sterile vacutainer tubes (BD-Plymouth.PL6 7BP®, United Kingdom). On the day of ACTH treatment, blood samples were also collected at 8 h intervals starting from ACTH administration and continued for 24 h. The blood samples were centrifugated at 3000 g for 10 min and serum was recovered and stored immediately at -20°C until assayed for glutathione peroxidase activity, LH, cortisol and testosterone concentration.

The LH, testosterone and cortisol concentrations as well as Glutathione peroxidase activity were determined using sheep ELISA kits (Elabscience Biotechnology Co, Ltd, E-ELS0783, Beijing for LH; Demeditec Diagnostics GmbH, D-24145 Kiel, Germany for testosterone; Demeditec, Germany for cortisol and ab102530, Abcam, England for Glutathione peroxidase activity) following the manufacturer's instructions. The concentrations were read using a spectrophotometer (Multiskan™ Go Micro-Plate, Thermo Scientific, United Kingdom) at absorbency rate of 340 nm. Blood samples for Se concentration were analysed using continuous hydride generation atomic absorption method (Horwitz, 2000). Samples were read using a Perkin-Elmer 2380 Atomic Absorption Spectrophotometer (Varian, Australia) at an absorbency of 196 nm and lamp energy of 16 mA. Bovine pancreas powder was used as a Standard Reference Material (SRM) and included in each batch of analyses to verify the accuracy of the Se assays.

2.5. Semen collection and evaluation

Semen was collected biweekly throughout the experiment. This experiment comprised of eight semen collections. Semen was collected using electro-ejaculator (EE) as previously described by Bopape et al. (2015). Immediately after semen collection, the samples were transported to the laboratory within 1 h for macroscopic (semen colour, volume and pH) and microscopic semen parameters (sperm motility, concentration, morphology and viability) evaluation which was done according to (Malejane et al., 2014).

Sperm mass motility was assessed with the aid of a microscope (Olympus, CX21FS1; Tokyo, Japan) under $\times 10$ magnification. A 5 μL of semen was placed on the pre-warmed microscope slide (Lasec®, South Africa) and evaluated using a score of 0–5 (scale for sperm waves) as it was indicated by Hafez and Hafez (2000).

Sperm progressive motility was determined by placing 5 μL of saline solution (Onderstepoort Pharmacy, South Africa) on the pre-warmed microscope slide placed followed by a drop (5 μL) of semen and covered with a cover slip (Lasec®, South Africa). Then progressive sperm motility was evaluated under $\times 40$ magnifications using a microscope according to Talebi et al. (2009).

The sperm viability was determined using eosin-nigrosine stain (Onderstepoort Pharmacy, South Africa). A mixture of 5 μL semen and 20 μL of eosin-nigrosine were prepared. Thereafter, a 5 μL of the mixture was placed on a clean warmed microscope slide and smeared using another slide. The slide was allowed to dry and thereafter, a drop of immersion oil (Onderstepoort Pharmacy, South Africa) was placed on top of the slide. A cover slip was then placed and examined at $\times 1000$ magnification using a microscope to determine the percentage live and dead sperm by counting a total of 100 sperm across the slide. The sperm that did not absorb stain (appeared as white or clear) were considered as live while those that absorbed stain and appeared pinkish were considered dead (Malejane et al., 2014).

The same slide used for analysis of sperm viability was also used to determine sperm morphology. The live spermatozoa were classified into normal and abnormal sperm. Sperm abnormalities were evaluated in two ways. The first method was for the evaluation of abnormalities that occurred at a specific location by observing the head, mid piece and tail of the sperm cell (Bearden et al., 2004). Then, the sperm abnormalities were further categorised into primary, secondary and tertiary abnormalities. The primary sperm abnormalities included abnormal acrosomes and elongated mid-piece of the sperm cells. The secondary sperm abnormalities observed were detached and loose heads as well as cytoplasmic droplets (Chandler et al., 1988). While the tertiary sperm abnormalities included bent and broken tails (Chenoweth and Lorton, 2014).

The sperm concentration was determined by counting sperm cells using an improved Neubauer haemocytometer (Lasec®, South Africa). Semen was diluted thoroughly and gently with distilled water (5 mL of water and 25 µL of semen) to kill the sperm cells. Then, the diluted sample (10 µL) was loaded into the two sides of the chamber using a micro pipette (Merck, South Africa®) in the V-shape groove of the haemocytometer. The haemocytometer was covered with a coverslip then allowed the contents to settle for 5 min. A phase contrast microscope under ($\times 40$) magnification was used to count the sperm cells. The sperm cells counted were multiplied with the dilution factor (201 for the current study) Then the following equation was used to calculate the sperm concentration: $\text{Concentration/mL} = (\text{Dilution Factor}) (\text{Count in 5 squares}) (0.05 \times 10^6)$ to get the concentration of sperm cells in millions per mL semen (Matthews et al., 2003).

2.6. Statistical analysis

All data collected for semen parameters and the blood components were analysed using general linear model (GLM) of statistical analysis software (SAS). Data of sperm characteristics were compared using ANOVA for repeated measures. When ANOVA revealed a significant effect, values were separated using the Duncan's multiple range tests. The results were expressed as mean \pm SEM.

3. Results

Se supplemented group produced creamy semen while Se + ACTH treatments lead to thick milky semen with no significant difference between ACTH and control groups which produced milky semen (Table 1). The mean ejaculate volume was significantly ($P < 0.05$) higher from the Se, Se + ACTH and ACTH groups than the control. The semen pH was significantly ($P < 0.001$) acidic from other groups compared to neutral pH (7.05 ± 0.05) from Se supplemented group. Higher ($P < 0.001$) progressive sperm motility and concentration were recorded from Se supplemented group and the lowest from the ACTH group. The highest ($P < 0.001$) percentage of live sperm was recorded in Se and Se + ACTH than the other groups. However, there were no significant ($P > 0.05$) differences concerning the percentage of live and dead sperm between the ACTH treated and control groups. The percentage of abnormal sperm was lower ($P < 0.001$) from Se and Se + ACTH groups compared to the other groups. The percentage of primary, secondary and tertiary sperm abnormalities was lower ($P < 0.05$) in the Se supplemented and Se + ACTH than from ACTH and control groups (Table 2).

Table 1. Effect of selenium supplementation and induced stress semen parameters of Saanen bucks collected with electro- ejaculator (mean \pm SE).

Treatments	SAP (0–5 scale)	Semen volume	pH	PM (%)	SC ($\times 10^6$ /mL)	LS (%)	AS (%)
Se \times ACTH	3.90 \pm 0.09 ^B	1.15 \pm 0.08 ^{ba}	6.74 \pm 0.05 ^b	81.20 \pm 1.67 ^B	2617.64 \pm 73.60 ^B	93.14 \pm 0.83 ^A	6.42 \pm 0.62 ^C
Se	4.34 \pm 0.09 ^A	1.30 \pm 0.08 ^a	7.05 \pm 0.05 ^a	88.40 \pm 1.67 ^A	3143.51 \pm 73.60 ^A	91.22 \pm 0.83 ^A	9.12 \pm 0.62 ^B
ACTH	2.60 \pm 0.13 ^C	1.10 \pm 0.12 ^{ba}	6.83 \pm 0.07 ^b	50.40 \pm 1.67 ^D	1683.64 \pm 104.0 ^D	65.12 \pm 1.18 ^C	27.44 \pm 0.88 ^A
Control	2.60 \pm 0.13 ^C	0.92 \pm 0.08 ^c	6.76 \pm 0.05 ^b	64.80 \pm 2.37 ^C	1971.02 \pm 73.60 ^C	71.36 \pm 0.83 ^B	27.06 \pm 0.62 ^A
P-Value	<.0001	0.0224	0.0004	<.0001	<.0001	<.0001	<.0001

Values with different superscripts in the same column differ significantly (^{A, B, C, D} $P < 0.001$; ^{a, ab, b, ba, c}, $P < 0.05$). SAP = Semen Semen samples were evaluated over a period of four months. Appearance, PM = Progressive motility, SC = sperm concentration, LS = Live sperm, AS = Abnormal sperm.

Table 2. Effect of selenium supplementation and induced stress on morphological sperm abnormalities of Saanen bucks collected with electro- ejaculator (mean \pm SE).

Treatments	PA (%)	SA (%)	TA (%)
Se \times ACTH	3.78 \pm 0.30 ^C	3.24 \pm 0.25 ^C	4.02 \pm 0.32 ^B
Se	3.46 \pm 0.30 ^C	2.50 \pm 0.25 ^C	1.94 \pm 0.32 ^C
ACTH	12.16 \pm 0.43 ^A	13.08 \pm 0.35 ^A	11.48 \pm 0.46 ^A
Control	8.62 \pm 0.30 ^B	12.28 \pm 0.25 ^B	10.76 \pm 0.32 ^A
P-Value	<.0001	<.0001	<.0001

Values with different superscripts in the same column differ significantly (^{A, B, C} $P < 0.001$). PA = Primary abnormalities, SA = Secondary abnormalities, TA = Tertiary abnormalities.

Whole blood Se concentrations were higher ($P < 0.001$) in Se and Se + ACTH groups than in ACTH and control groups (Table 3). Similarly, the mean serum testosterone and LH concentrations were highest ($P < 0.001$) in the Se and Se + ACTH compared to the ACTH and control groups. The GSH-Px activity was higher ($P < 0.001$) in Se and Se + ACTH groups than in ACTH and control groups. The lowest ($P < 0.001$) cortisol level was observed in bucks that received Se and Se + ACTH compared to ACTH and control groups, throughout the study (Table 3).

Table 3. Effect of selenium supplementation and induced stress on the concentration of selenium, reproductive hormones, glutathione peroxidase and cortisol of Saanen bucks (mean \pm SE).

Treatments	Se (ng/g)	Te (ng/mL)	LH (ng/mL)	GSH-Px (U/mL)	Cortisol (ng/mL)
Se \times ACTH	86.09 \pm 4.98 ^A	2.35 \pm 0.05 ^B	1.40 \pm 0.04 ^B	5.78 \pm 0.14 ^A	1.30 \pm 0.12 ^C
Se	94.05 \pm 4.98 ^A	3.47 \pm 0.05 ^A	2.34 \pm 0.04 ^A	5.96 \pm 0.14 ^A	1.04 \pm 0.24 ^C
ACTH	16.64 \pm 4.98 ^B	0.94 \pm 0.05 ^C	0.61 \pm 0.04 ^C	2.27 \pm 0.14 ^B	5.52 \pm 0.13 ^A
Control	11.41 \pm 4.98 ^B	0.96 \pm 0.05 ^C	0.56 \pm 0.04 ^C	2.43 \pm 0.16 ^B	4.97 \pm 0.12 ^B
P-Value	<.0001	<.0001	<.0001	<.0001	<.0001

Blood samples were collected over a period of four months. Values with different superscripts in the same column differ significantly (^{A, B, C} $P < 0.001$). Se = Selenium, Te = Testosterone, LH = Luteinising hormone, GSH-Px = Glutathione peroxidase.

4. Discussion

Induced stress treatment with injection of ACTH decreased the semen appearance quality and led to a milky colour in the present study. This observation suggests that elevated stress induces free radicals and attacks the germ cells within the seminiferous tubules, which in turn, causes extensive apoptosis and the disruption of spermatogenesis (Aitken and Baker, 2013). A disruption in spermatogenesis causes a decline in concentration of sperm which then alters the semen appearance (Hafez and Hafez, 2000). Thus, a decline in the number of sperm produced was reflected by milky semen appearance in the ACTH induced stress group while the semen appearance was creamy in Se supplemented group, even when bucks were under induced stress. This observation indicated that Se supplementation can minimise stressful effects in semen.

When looking at the semen volume results obtained, there were many confounding effects that makes it very difficult to explain the results of the present study. Firstly, the semen was collected using the electro ejaculator that is known to increase the semen volume (Bopape et al., 2015) and secondly, the animals were supplemented with selenium also known to increase semen volume (Lukusa and Lehloenya, 2017). Therefore, higher semen volume under induced stress and when Se was supplemented could be due to a common procedure for these groups that is electro ejaculation, causing the stimulation of the accessory gland to produce more seminal plasma, eventually to a higher semen volume (Lukusa and Lehloenya, 2017).

The semen pH was slightly acidic from the control and Se supplemented bucks with induced stress and was neutral only from Se supplemented group. The acidic semen pH is considered unacceptable as it may have a negative impact on the fertility (Latif et al., 2005). From the present study, decreased sperm motility has been observed in semen obtained from the control and stress induced groups, signifying that the acidic semen pH has a negative impact on sperm characteristics. It was previously reported that the acidic semen pH leads to reduced sperm motility as a consequence of the change in the metabolic activity and a disturbance in the cellular respiration of the sperm (Latif et al., 2005; Bonato et al., 2012). The semen pH obtained from the Se supplemented bucks was considered to be optimum (7.05 ± 0.05) (Hafez and Hafez, 2000). Thus, this confirms the importance of Se supplementation as the control group that was fed Se deficient Lucerne had slightly acidic pH but also that stress related acidic pH cannot be corrected by Se supplementation.

The sperm motility was decreased under induced stress compared to the control group. From the induced stress group, the GSHP-x declined in this study, which is an indication of a decline in the antioxidant status under stress conditions. Therefore, the decline in sperm motility can be associated with a decline in the GSHP-x levels in the sperm (Foresta et al., 2002). However, Se supplementation reduced the harmful effects of induced stress, as reflected by high sperm motility from Se supplemented group. This can also be associated with the protection provided by GSHP-x to the developing sperm from oxidative stress-induced (Qazi et al., 2019). The positive effect of selenium supplementation on the increased sperm motility from the Se supplemented group has been previously confirmed in rams and goats (Xu et al., 2003; Mojapelo and Lehloenya, 2019).

The sperm concentration of the bucks decreased under induced stress compared to the control group. This observation could ascribed to Se deficiency in this animals and it has been reported that animals se deficient animals are more likely to have a lower mGPx4 expression

and hence lower GSHP-x activity that is associated with the reduced sperm concentration (Foresta et al., 2002).

Similar to the control group in this study, the induced stress group had higher percentages of abnormal sperm. The increase in the percentage of abnormal sperm when stress was induced and also in the control group might be attributed to the lower GSH-Px activity that was observed from these groups. Previous report showed that lower GSH-Px activity can be associated with damage of the chromatin structure of the sperm in the epididymis thereby leading to increased number of abnormal sperm morphology (Hansen, 2009). Clearly reflected in this study, when Se was supplemented, the percentage of the abnormal sperm morphology was reduced. Se is a component of the selenoproteins and selenoenzymes which are involved in spermatocytogenesis by providing protection to the spermatozoa (Ahsan et al., 2014a, b).

Se concentration in the induced stress and control groups was low and beyond recommendations (Puls, 1994) as it was expected. Thus, the lower Se concentration in induced stress group gives a better explanation for observation of poor semen quality and quantity, reduced testosterone level and GSH-Px activity obtained in the present study (Falana and Oyeyipo, 2012). On the other hand, the higher Se concentration in Se supplemented and also when Se was supplemented in the induced stress group were associated with the improved semen quality and quantity of bucks (Shi et al., 2010; Surai and Fisinin, 2015). Thus, it can be suggested that the blood Se concentration must be high in the testis and epididymides of goats in order to induce its physiological activities in sperm and semen quality (Surai and Fisinin, 2015).

The level of blood serum LH of induced stress group was lower. This suggests that stress has a regulatory effect on the hypothalamo-pituitary-axis, leading to destruction in GnRH pulses hence lowering the LH production (Clarke et al., 1999). However, in Se supplemented even under induced stress groups, the LH concentration increased. Similar results have been reported previously (Lukusa and Lehloenya, 2017). Se is known to accumulate in the anterior pituitary (Thorlacius-Ussing and Danscher, 1998). Therefore, it is possible that increase in serum Se concentration may have activated GnRH receptors in the anterior pituitary gonadotrophes, leading to increased LH production in Se supplemented group (Ottinger et al., 2004).

Induced stress decreased testosterone concentration compared to the control group. Similar results were reported in rams that, testosterone declines under stress (Van Lier et al., 2003). It has been reported that, as cortisol level increases, the testosterone concentration decreases (Fenske, 1997) thus, similar results were observed in the present study that cortisol level increased and testosterone declined in the stress induced bucks. However, it was noted that the testosterone level increased in goats which were supplemented with Se and under induced stress suggesting that, the addition of Se supplementation support testosterone production. The increase in testosterone concentration might be attributed to the increased GSH-Px activity, as it was observed in this study (Murakoshi et al., 1983; Qazi et al., 2019). It has been reported that in the Leydig cells, the GSH-Px is required for metabolic pathway of testosterone biosynthesis as this enzyme is essential for protection against unfavourable effects of ROS attacks (El-Sheshtawy et al., 2014).

As expected induced stress increased cortisol (a stress hormone) levels in blood serum of goats. This emphasizes that induced stress interferes with the hypothalamo-pituitary-adrenal

which regulates cortisol release (Collodel et al., 2008), as it has been previously demonstrated in pigs (Fenske, 1997). However, the levels of blood serum cortisol were lower in the Se supplemented and also in the goats which were exposed to stress but also supplemented with Se. This observation shows that supplementation with Se can reduce the detrimental effects of induced stress in goats.

Glutathione peroxidation (GSH-Px) activity in blood serum of goats was lower in both induced stress and control groups. A decrease in GSH-Px activity shows high usage of GSH-Px under stress and lack of Se concentration in both groups as it was expected. Since Se is an integral component of GSH-Px (Arthur et al., 2003), it can be suggested that a decrease in GSH-Px activity is an indication of the lower antioxidant status of the goats in both groups. Which in turn explains the poor semen quality obtained from the bucks in both induced stress and control groups from the present study. The low GSH-Px in the control group also indicates that the goats were under stress that might be related to numerous factors including environment and handling (for husbandry practices, semen collection *etc.*).

However, GSH-Px activity in blood serum of goats was increased in Se supplemented group and even in goats treated with Se and under induced stress. In agreement to the present study, it has been reported that Se supplementation increases GSH-Px (El-sharaky et al., 2007). This observation validates selenium supplementation as GSH-Px was high in Se supplemented groups even under induced stress.

5. Conclusion

Supplementing male goats with selenium had beneficial effects expressed as improved semen quality (high sperm motility, increased sperm concentration, high percentage of live sperm and decline in percentage of abnormal sperm). Selenium supplementation also increased the concentrations of selenium, testosterone, luteinising hormone, glutathione peroxidase activity and led to a decline in cortisol level. Therefore, oral supplementation with sodium selenite can be implemented in goats that are raised on selenium deficient soils, in order to boost the oxidant status and hence lessen the stress effects by reducing cortisol levels and increasing glutathione peroxidase activity leading to improved semen quality.

Declaration of Competing Interest

There is no conflict of interest.

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