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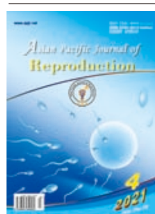

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Oral supplementation of selenium improves post−thaw sperm quality in Saanen bucks

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ABSTRACT

Objective: To determine the effect of oral selenium supplementation and semen collection methods on various post thaw semen quality parameters in Saanen bucks.

Methods: Sixteen healthy bucks were divided into two equal groups (*n*=8 each). The treatment group received selenium at 10-day intervals for three months. Sperm kinematic parameters, morphological parameters, mitochondrial membrane potential, plasma membrane functionality, and sperm DNA integrity were evaluated weekly pre and post-cryopreservation.

Results: The mean percentages of the morphological abnormalities were significantly lower in the selenium-supplemented samples when semen was collected by using artificial vagina method ($P<0.05$). Proximal droplet defects were significantly lower in the selenium supplementation group when semen was collected by electro-ejaculation ($P<0.05$). Post-thaw sperm parameters such as total motility and progressive motility were significantly higher when semen was obtained by artificial vagina in the selenium-supplemented bucks compared to the electro-ejaculation and the control groups ($P<0.05$). The sperm kinematic parameters such as curvilinear velocity, average path velocity, and amplitude of lateral head displacement were significantly higher when semen was collected by artificial vagina in the selenium-treated bucks ($P<0.05$). The percentages of sperm with intact and functional plasma membrane and functional mitochondria were significantly higher in the selenium-supplemented samples collected with artificial vagina compared to the electro-ejaculation method and the control groups ($P<0.05$). *In vitro* fertilizing potential was significantly higher in the selenium-supplemented samples collected with artificial vagina compared to the electro-ejaculation method and the control groups, respectively ($P<0.05$).

Conclusions: Oral supplementation of selenium and artificial vagina semen collection improve post thaw sperm quality parameters of Saanen buck.

KEYWORDS: Post thaw; Cryopreservation; Saanen buck; Selenium supplementation; Semen collection method; Sperm kinematic parameters; Sperm motility; Sperm quality

1. Introduction

Artificial insemination with cryopreserved semen is a tremendous technology to bring rapid genetic improvement in livestock and has a major breakthrough in cattle but the same results have not been translated in small ruminants. This may be attributed to the higher sensitivity of buck sperm to oxidative stress during cryopreservation and thus low post thaw motility[1,2]. However, there are several antioxidants in semen that are known to protect sperm against reactive oxygen species (ROS) such as vitamin E and C, as well as selenium (Se) and Zinc which are components of antioxidant systems[3]. But, these antioxidants may be insufficient in seminal plasma to protect spermatozoa against high levels of ROS produced during the freezing-thawing process.

Significance

Oral supplementation of selenium has been reported to improve sperm motility and morphology in goat. In addition, the quality of post-thawed sperm depends on the semen collection method used. However, there is limited information on the potential beneficial effects of selenium with regard to the post-thawed goat semen obtained either by artificial vagina or electro ejaculation methods. The present study revealed that supplementing bucks with selenium prior to semen collection using artificial vagina method can be an alternative option to enhance post thaw sperm quality.

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Trace elements have been reported to be associated with antioxidant profile in semen. However, modifications in their concentrations may decrease the ability of antioxidants to protect spermatozoa against oxygen species[4]. They may also lead to a reduction of cryo-resistance capability of the sperm cells[5]. Selenium is one of the indispensable trace elements for cellular defense systems against ROS[4]. Se, as component of glutathione peroxidase (GSH-Px) and selenoproteins[6], plays an important role in many physiological functions, including antioxidant defense, fertility, endocrine function and stability of cellular membranes[7,8]. Deficiency or excess may lead to infertility and abnormal development[9]. Selenium deficiency in male rats produces abnormal sperm shapes, structural abnormalities with a prominent increase in head, midpiece, tail, and irregular structure of mitochondria[10]. It has been associated with reduced reproductive performance and decreased sperm quality of mice, pigs, sheep, cattle, and goat[11].

Dietary supplementation of Se has been demonstrated to improve reproductive performance of buck[12]. It has been reported to have a significant effect to stimulate GSH-Px activity in seminal plasma, spermatozoa, and testes[13,14]. It was also reported that Se supplementation for buck led to improved sperm motility and morphology[15].

In goat, the quality of post-thawed sperm invariably depends on the semen collection method used[16]. Ample evidence[12,16,17] indicate more optimal sperm quality characteristics with artificial vagina compared to electro ejaculation. In addition, remarkable variations in seminal plasma composition may exist between ejaculates obtained by artificial vagina and electro ejaculation[18].

Based on the available knowledge, there is limited information on the potential beneficial effects of Se with regard to the post-thawed goat semen obtained either by artificial vagina or electro ejaculation method. It is therefore hypothesized that sperm samples obtained by electro ejaculation could require Se supplementation to carry out a successful sperm cryopreservation. The objective of this study was to determine the effect of oral Se supplementation and semen collection methods on post-thawed sperm of Saanen buck spermatozoa.

2. Materials and methods

2.1. Study area

The present study was carried out at the University of Pretoria, Experimental Farm in Hatfield, South Africa, over a period of three months during summer and autumn (February–April, 2018). The mean average air temperatures during the experimental period ranged from 25.6 ℃ in February to 22.0 ℃ in April. The average annual rainfall is 674 mm.

2.2. Animals and experimental design

A total of 16 Saanen bucks aged between 18–19 months and weighing (57.73±3.47) kg were used in this study. Bucks were divided randomly into two groups: the Se treatment (n=8) and the control group (n=8). Each group was further subdivided into semen collection methods: artificial vagina (n=4) and electro ejaculation (n=4). The Saanen bucks were raised only with locally available milled Lucerne from weaning and they had no access to fresh growing forages or other feeds for four months before the start of the experiment. The Lucerne used was bought from Northern Cape, Jankempdorp. Fresh water was provided *ad libitum* during the experimental period. Lucerne hay was milled and tested for Se concentration prior to supplementation and no Se traces were detected by using the spectrophotometer (Perkin-Elmer 2380 Atomic Absorption Spectrophotometer; Varian, Australia). The treatment animals received Se (ACECHEM, South Africa) at the dose rate of 0.34 mg/kg body weight adjusted from injection doses reported by Mahmoud *et al*[19]. The Se was administered orally at 10-day intervals for three months.

2.3. Semen collection

A total of 144 semen samples (9 ejaculates from each buck)were collected by using artificial vagina and electro-ejaculation. Ejaculates were collected once weekly throughout the experiment. Semen collection procedure for both artificial vagina and electro ejaculation was carried out as previously described by our group[16]. Briefly, for artificial vagina bucks were trained prior to semen collection using a doe on heat. The artificial vagina was prepared by filling the space between the casing and liner with approximately 55 ℃ warm water[20]. Before collection, the prepuce of the buck was wiped clean with distilled water to reduce contamination. For electro ejaculation method, an electro-ejaculator (Ramsem, South Africa) with a rectal probe 28 cm long, 2 cm in diameter, standardized for small ruminants was used. The animal was physically restrained in a lateral position on the floor. The rectum was cleaned of faeces and the prepuce area was shaved and washed with distilled water and wiped with paper towel. An electro-stimulation was applied for 4–6 s intervals between stimuli for 4 to 5 times. The electrical current was gradually increased until a maximum of 5 voltages was reached[17].

2.4. Freezing procedure

The ejaculates were considered for freezing using standard criteria established by Hidalgo *et al*[21]. Each pooled semen sample was extended by using Tris-based extender (pH 6.8) containing Tris 2.422 g, citric acid monohydrate 1.360 g, glucose 1.000 g, gentamycin 1 000 μg/mL, kanamycin 1000 μg/mL, egg-yolk (v/v) 20%, glycerol (v/v) 16%, and distilled H_2O to final volume (mL).

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The semen samples from all treatments were diluted by using a 2-step dilution method. In the first step, extender portion A (without glycerol) was added to semen samples at 33 ℃, to obtain a sperm concentration of (150×10^6) sperm/mL. Diluted semen was cooled for 2 h (slow cooling) in a refrigerator at 4 ℃. Following this, an equal volume of extender portion B (containing 16% glycerol) was added at 4 ℃ to obtain a final sperm concentration of (75×10^6) sperm/mL and equilibrated for 2 h. After equilibration time, the semen samples were aspirated into 0.25 mL French mini straws, sealed with polyvinyl alcohol powder, and suspended in liquid nitrogen vapour inside a styrofoam box container at height 4 cm above liquid nitrogen for 10 min. They were subsequently submerged into liquid nitrogen at –196 ℃, where they were stored at for 24 h before analysis[16].

2.5. Semen evaluation

All collected ejaculates were grossly evaluated for colour, pH and volume, mass motility and progressive motility. Only those semen samples with at least 80% sperm progressive motility were selected for further analysis. The sperm morphological abnormalities were assessed by using nigrosin/eosin-stain smears. The sperm motility and kinematic parameters such as curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), linearity coefficient, straightness coefficient, Wobble coefficient, amplitude of lateral head displacement (ALH) and beat/cross-frequency (BCF) were evaluated by computer assisted sperm analyser (CASA) as per the method described by Lukusa and Kabuba[16].

2.5.1. Sperm mitochondrial membrane potential

At post-thaw, mitochondrial membrane potential assay of spermatozoa was evaluated with the probe JC-1(5,5’,6,6’-tetrachloro-1,1’,3,3’-tetraethylbenzimidazolylcarbocyanine iodide), JC-1 (Molecular Probes, USA), according to Silva *et al*[22]. About 20 µL JC-1 (0.15 mM indimethylsulfoxide) was added in 100 µL of each sample (sperm concentration; 5×10^6 cells) and the mixture was incubated at 37 ℃ for 10 min. Two hundred spermatozoa with orange mid-piece (high mitochondrial membrane potential) and with green mid-piece (low mitochondrial membrane potential) were evaluated under epifluorescence microscope (magnification, ×400; Nikon, Optiphot, Marunouchi, Chiyoda-ku, Tokyo, Japan) with excitation and emission filters around 490 and 550 nm.

2.5.2. Plasma membrane functionality

The functionality of sperm plasma membrane was evaluated by using the hypo-osmotic swelling test[24]. A volume of 10 µL of semen was added to 1 mL of the hypo-osmotic solution (100.0 mOsm/L, 57.6 mM fructose and 19.2 mM sodium citrate) and incubated at 37 ℃ for 30 min[23]. After incubation, one drop of semen was placed on a glass slide, covered with a coverslip and evaluated under a phase contrast microscope (OLYMPUS, CX21FS1; Tokyo, Japan) at 40× magnification[12]. At least 200 sperm were counted, and the proportion of sperm with swollen or coiled tail was counted as hypo-osmotic swelling test positive or reacted spermatozoa.

2.5.3. Sperm DNA integrity

At post-thaw, Tunel assay was performed with the *In Situ* Cell Death Detection, Fluorescein Kit (In, Roche, Indianapolis, IN, USA), according to manufacturer’s instructions. Frozen-thawed sperm were fixed with 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS) for 30 min at room temperature. After fixation, the samples were centrifuged at 300×*g* for 10 min and resuspended in a permeability-enhancing solution containing 0.1% Triton X-100 in 0.1% sodium citrate for 5 min. Then the cells were washed twice in PBS–polyvinylpyrrolidone and incubated in Tunel reaction mixture for 1 h at 37 ℃ in a dark and humidified atmosphere. For a positive control from the kit, slides were treated with RNase-free DNase I at room temperature for 10 min before incubation with the Tunel reagent. For a negative control, slides were incubated with all the components of the labelling solution, except for the terminal deoxynucleotidyl transferase enzyme. A minimum of 100 spermatozoa were examined in each sample by using a fluorescent microscope (magnification, ×400; Nikon, Optiphot, Marunouchi, Chiyoda-ku, Tokyo, Japan), under ultraviolet light, with excitation DAPI (460 nm for blue fluorescence) and FITC (520 nm for green fluorescence) filters. Digital images were acquired by using NIS-Elements-F software and a high-resolution color digital camera (Digital Sight DS-Fi 1C; Nikon, Japan), and the numbers of total (blue) and Tunel -positive (Tunel+, green) nuclei were registered[24].

2.5.4. Fertilizing potential

The heterologous *in vitro* fertilization test was performed based on a previously described method[25]. Briefly, bovine ovaries were collected from a local slaughterhouse and transported within 2 h in a thermic container to the laboratory at room temperature. The cumulus–oocyte complexes with homogeneous ooplasm were collected from ovarian follicles and washed three times in modified M199 supplemented with 0.5% HEPES (w/v). The cumulus–oocyte complexes were cultured in TCM-199 supplemented with 50 µg/mL gentamycin, 5.5 mM Ca lactate, 2.3 mM Na pyruvate, 36 mM $NaHCO_3$, 5 mM HEPES, 0.01 UI/mL rhFSH (Gonal F-75, Serono, UK). Maturation was performed in four-well plates (Nunc, Roskilde, Denmark) in groups of 50 cumulus–oocyte complexes in 400 µL of maturation media for 24 h at 38.5 ℃ under 5% CO_2 in air with maximum humidity. Matured cumulus–oocyte complexes were transferred to a new four-well plate containing 400 µL of fertilization medium (synthetic oviducial fluid) supplemented with 10% of oestrous sheep serum and 40 µg/mL gentamycin. Thawed sperm were capacitated in the fertilization medium for 10 min.

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In vitro capacitation status was assessed by the chlortetracycline staining assay as described by Anand *et al*[26]. Sperm was co-incubated with oocytes at a final concentration of 1×10^6 mL for 10 h at 38.5 ℃ in 5% CO_2. The oocytes were fixed and stained by washing in PBS labelled with 1 mg/mL Hoechst 33342 Stain solution for 30 min at room temperature, and they were then washed again in PBS. Fertilization rate was assessed 40 h later with an inverted microscope by the presence of cleaved oocytes (two to eight cells) or the presence of two or more nuclei.

2.6. Statistical analysis

Statistical analysis was performed by the General Linear Model procedures using statistical software SPSS (2015) (IBM SPSS Statistics for Windows, Version 23.0. NY, USA). Normality distribution of data was verified prior to its analysis using the Shapiro–Wilk test as a parametric test assumption. All sperm parameters passed the normality test. For the analysis, a completely randomized block design in a 2 × 2 factorial arrangement for each Se treatment (Se supplemented and control) and collection methods (artificial vagina and electro ejaculation) was used. The fixed effects were the Se treatment, collection method and their interaction. The response variables were CASA motility, kinematics, plasma membrane integrity, sperm viability, acrosome integrity mitochondria membrane potential, and DNA integrity. Data were expressed as mean±standard deviation (mean±SD). The analysis of variance was used to test differences between the treatments for each variable. Mean±SD were separated by using Duncan's multiple range tests. A probability of $P<0.05$ was considered to be statistically significant.

2.7. Ethics statement

All animal care and procedures used were performed in accordance with Animal Ethics Committee of the University of Pretoria, South Africa (Project No: EC079-14).

3. Results

3.1. Sperm morphological abnormalities

The mean percentages of abnormalities such as detached head, distal mid-piece reflex, dag-like defects, and bent tail were significantly lower in Se-supplemented samples when semen was collected by using artificial vagina method ($P<0.05$). Proximal droplet defects were significantly lower in control group when semen was collected by electro ejaculation ($P<0.05$). Coiled tail defects were significantly higher in the Se-supplemented group when semen was collected by artificial vagina ($P<0.05$). No significant difference was observed regarding distal droplets and defects in size and shape in both artificial vagina and electro ejaculation group in Se supplemented bucks ($P<0.05$) (Table 1).

3.2. Sperm motility and kinematic parameters

Sperm parameters such as total motility and progressive motility were significantly higher when semen was obtained by artificial vagina in Se-supplemented bucks compared to electro ejaculation ($P<0.05$). However, rapid and medium spermatozoa were similar in both collection methods in Se-supplemented bucks although decreased percentages were observed in the control groups (Table 2).

The mean values of kinematic parameters in post-thaw sperm of the Se-supplemented and control groups collected by artificial vagina or electro ejaculation are shown in Table 3. The sperm kinematic parameters such as VCL, VAP, and ALH were significantly higher when semen was collected by artificial vagina from Se-treated bucks compared to electro ejaculation ($P<0.05$), while sperm VSL and BCF were similar for semen collected with both artificial vagina and electro ejaculation of Se-supplemented bucks. The percentages of Wobble coefficient were significantly higher ($P<0.05$) when semen was collected by electro ejaculation in Se-supplemented bucks compared to the artificial vagina method group and the control group.

Table 1. Effects of semen collection methods and selenium supplementation on the fresh sperm morphological abnormalities of Saanen bucks.

Sperm defects (%)	Artificial vagina		Electro-ejaculation	
	Se supplemented	Control	Se supplemented	Control
Head defects				
Detached head	2.92±0.15[a]	3.81±0.13[b]	3.82±0.07[b]	5.51±0.11[c]
Defects in size and shape	0.53±0.05[a]	0.73±0.05[b]	0.53±0.05[a]	0.92±0.06[c]
Mid–piece defects				
Distal mid-piece reflex	2.81±0.13[a]	3.33±0.14[b]	3.61±0.11[b]	4.22±0.12[c]
Proximal droplet	3.52±0.15[a]	4.42±0.15[b]	2.72±0.11[c]	1.23±0.07[d]
Tail defects				
Distal droplet	1.23±0.11[a]	1.72±0.13[b]	1.23±0.06[a]	1.22±0.07[a]
Dag-like defects	0.51±0.06[a]	0.71±0.07[b]	0.82±0.06[c]	0.64±0.05[d]
Bent tail	1.11±0.08[a]	1.53±0.09[b]	1.71±0.08[c]	0.91±0.08[d]
Coiled tail	1.82±0.14[a]	1.22±0.11[b]	1.33±0.07[b]	0.92±0.08[c]
Total abnormalities	15.52±0.87[a]	16.41±0.87[b]	15.64±0.61[a]	17.53±0.71[c]

Data are expressed as mean±SD, which are separated by using Duncan's multiple range tests. Means with different superscripts (a, b, c, d) in a row differ significantly at $P<0.05$. Se: selenium.

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Table 2. Interaction effect between selenium treatment and collection methods in terms of overall motility parameters in post-cryopreserved sperm (%).

Parameters	Artificial vagina		Electro-ejaculation	
	Se supplemented	Control	Se supplemented	Control
Total motility	66.12±0.13[a]	61.33±0.32[b]	63.82±1.02[c]	60.44±0.23[b]
Rapid speed	28.12±0.01[a]	23.34±1.11[b]	26.85±0.33[a]	23.33±1.23[b]
Medium speed	36.82±1.13[a]	33.01±1.45[b]	37.13±1.03[a]	30.72±1.23[c]
Progressive motility	41.01±0.61[a]	37.11±1.63[b]	38.44±1.04[b]	37.13±1.41[b]
Non-progressive motility	25.12±1.01[a]	24.23±0.15[a]	25.45±2.65[a]	23.03±1.21[b]

Data are expressed as mean±SD, which are separated by using Duncan's multiple range tests. Means with different superscripts (a, b, c, d) in a row differ significantly at $P<0.05$. Se: selenium.

Table 3. Interaction effect between selenium treatment and collection methods in terms of overall kinematic parameters in post-cryopreserved sperm.

Parameters	Artificial vagina		Electro ejaculation	
	Se supplemented	Control	Se supplemented	Control
VCL, μm/s	69.42±0.51[a]	66.13±0.12[b]	65.35±0.24[b]	62.66±0.02[c]
VSL, μm/s	58.12±0.42[a]	53.24±1.23[b]	57.95±2.06[a]	48.83±1.22[b]
VAP, μm/s	63.21±1.52[a]	57.52±0.53[b]	59.24±1.65[b]	55.83±0.58[c]
Linearity coefficient, %	66.63±0.34[a]	65.92±1.15[a]	65.71±7.11[a]	64.94±1.44[a]
Straightness coefficient, %	86.44±0.11[a]	82.13±1.97[b]	87.51±0.40[a]	86.85±2.11[a]
Wobble coefficient, %	76.22±0.42[a]	75.13±0.09[a]	79.85±1.37[b]	72.44±0.42[c]
ALH, μm	3.13±0.74[a]	2.45±1.16[b]	2.01±0.72[b]	1.32±1.02[c]
BCF, Hz	18.22±1.12[a]	15.14±0.82[b]	17.25±0.57[a]	13.63±0.60[c]

Data are expressed as mean±SD, which are separated by using Duncan's multiple range tests. Means with different superscripts (a, b, c, d) in a row differ significantly at $P<0.05$. VCL: curvilinear velocity; VSL: straight-line velocity; VAP: average path velocity; ALH: amplitude of lateral head displacement; BCF: beat/cross-frequency; Se: selenium.

Table 4. Effects of selenium treatment and collection methods on post-thawed sperm parameters (%).

Selenium treatment and collection methods	Hypo-osmotic swelling test positive sperm	High MMP sperm or Low MMP sperm	DNA integrity	Fertilization rate
Selenium treatment				
Selenium supplemented	40.06±3.04[a]	33.83±2.26[a]	43.21±1.04[a]	43.48±2.43[a]
Control (No selenium)	30.58±2.11[b]	27.14±2.33[b]	38.34±2.23[b]	35.28±2.5[8b]
Semen collection methods				
Artificial vagina	38.52±2.57[a]	32.73±2.25[a]	41.45±3.65[a]	41.93±2.78[a]
Electro ejaculation	32.12±2.58[b]	28.24±3.32[b]	39.23±2.34[a]	36.68±2.34[b]
Interaction between selenium treatment and collection methods				
Artificial vagina				
Selenium supplemented	42.01±3.02[a]	36.22±2.34[a]	42.35±2.51[a]	45.63±2.45[a]
Control	35.04±2.12[b]	29.24±1.04[b]	40.55±1.75[a]	38.23±3.46[b]
Electro ejaculation				
Selenium supplemented	38.12±3.12[ab]	31.45±3.43[b]	43.32±1.53[a]	41.34±2.42[ab]
Control	26.12±2.07[c]	25.04±3.23[c]	35.14±1.23[b]	32.34±2.31[c]

Data are expressed as mean±SD, which are separated by using Duncan's multiple range tests. For each factor, different superscripts (a, b, c, d) within a column differ significantly ($P<0.05$). MMP: mitochondrial membrane potential.

3.3. Post–thawed sperm parameters

Table 4 presents the effects of Se supplementation, semen collection technique and their interaction on post-thaw sperm quality of goat semen. Semen quality was significantly affected by Se supplementation and method of semen collection ($P<0.05$). Regardless of the method of semen collection, Se supplementation had a significant effect on sperm characteristics ($P<0.05$). The percentages of all post-thaw sperm parameters were significantly higher in Se-supplemented group compared to the control group ($P<0.05$). On the other hand, hypo-osmotic swelling test positive spermatozoa, sperm with high mitochondrial membrane potential or sperm with low mitochondrial potential, fertilization rate were significantly higher in semen collected with artificial vagina than electro ejaculation ($P<0.05$). The sperm DNA integrity did not differ between the two semen collection methods. The interaction between treatment and semen collection methods were significant ($P<0.05$). The percentages of sperm with hypo-osmotic swelling test positive spermatozoa, sperm with high mitochondrial membrane potential and the *in vitro* fertilization rate were significantly higher in Se-supplemented samples when semen was collected with artificial vagina compared to electro ejaculation method and control groups ($P<0.05$). The percentages of sperm DNA integrity were not different between semen collection methods in Se-supplemented bucks.

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4. Discussion

In the present study, it was observed that sperm defects affecting sperm head and mid-piece were reduced when semen was obtained from Se-supplemented bucks by artificial vagina method. These reduced sperm abnormalities may be attributed to Se supplementation and not the artificial vagina method because these abnormalities are usually related to disruptions during spermatogenesis and sperm maturation in the epididymis[27] and not during ejaculation. The higher percentages of head and mid-piece abnormalities in semen obtained by electro ejaculation in Se supplemented bucks might be due to the stress induced during semen collection[28], therefore leading to lower GSH-Px activity. The lower GSH-Px has been reported to be associated with damage of the chromatin structure of the sperm in the epididymis. thereby leading to increased number of abnormal sperm morphology[29].

To the best of our knowledge, this is the first report on the interaction between Se supplementation and method of semen collection for sperm motility and kinematic parameters. Here we report better total sperm and progressive motility as well as values of sperm kinematic parameters such as VCL, VAP, and ALH of semen collected by artificial vagina from Se-supplemented bucks. This corroborates well with the studies of Lukusa and Lehloenya[12] and Mojapelo and Lehloenya[15] who also reported increased percentages of sperm progressive motility and mass motility in Se-supplemented bucks. However, Jiménez-Rabadán *et al*[17] reported increased post-thaw total sperm motility and progressive motility with artificial vagina method, but no differences were found between artificial vagina and electro ejaculation in terms of post-thaw kinematic parameters in bucks. It is clear thatthe combination of artificial vagina and Se supplementation could be one of the reasons why sperm kinematic parameters are better improved in our study, indicating that artificial vagina is better than electro ejaculation for collection of semen from Se-supplemented bucks in order to get acceptable post-thaw motility and kinematic parameters for better results after artificial insemination.

The increased values of post-thaw sperm parameters such as hypo-osmotic swelling test positive spermatozoa, sperm with high mitochondrial membrane potential and DNA integrity reported in Se-supplemented group are in agreement with earlier published findings[12,30]. Protective effect of Se supplementation on post-thaw sperm membrane integrity, functional mitochondria, and DNA integrity may be explained by the increased concentrations of antioxidant enzymes GSH-Px in the blood plasma. The enzyme has been implicated in the protection of cellular membranes and lipid containing organelles from peroxidative damage[30]. Numerous reports have shown that Se is localized in the keratinous outer membrane of sperm mitochondria suggesting that Se might have played a major role in maintaining properly its structural composition[31]. Furthermore, it has been reported that biological function associated with Se which is present in various enzymatic selenoproteins including GSH-Px is to protect membranes and DNA integrity from oxidative damage due to free radicals[6].

The higher post-thaw values of sperm functional plasma membrane and functional mitochondria in Se fed bucks for artificial vagina method support the findings of Jiménez-Rabadán *et al*[17] and Jiménez-Rabadán *et al*[32], which suggested that spermatozoa obtained by artificial vagina were more resistant to cryo-injury than those obtained by electro ejaculation. This can be explained by the fact that the electro ejaculation method might have changed the secretory function of accessory glands that could influence the amount of fluid produced and the chemical composition of the seminal plasma. This event could probably modify the cryo-survival ability of sperm samples obtained by this method[17].

Similar to our study, Jiménez-Rabadán *et al*[17] reported greater percentages of intact sperm plasma membrane and active mitochondria when semen was obtained by artificial vagina method. Contrary to our results, Ledesma *et al*[25] reported that sperm cells obtained by electro ejaculation were more resistant to cryo-damage than sperm collected by artificial vagina in ram. Marco-Jiménez *et al*[33] also observed a higher number of acrosome intact in post-thawed sperm cells collected by electro ejaculation than by artificial vagina in ram. However, this can be attributed to different species used and lack of Se supplementation to provide protection against oxidative damage. Lukusa and Lehloenya[12] reported higher percentages of viability, sperm acrosome integrity, and morphologically normal sperm collected by artificial vagina in Se-supplemented group compared to semen collected with both artificial vagina and electro ejaculation in the control group. This may be attributed to the combined role of Se and artificial vagina method in the protection of sperm membrane integrity and lowering enzyme leakages during cooling process[34]. In addition, Se has been implicated to play a pivotal role in the maintenance of mitochondrial structural integrity, leading to the increase in adenosine triphosphate of spermatozoon, therefore causing an increase in sperm motility[35]. The present results confirm that Se supplementation prior to semen collection using artificial vagina method is required to reduce damage to Saanen buck sperm cells during the freezing process.

Our experiment shows that Se treatment and artificial vagina collection method can improve *in vitro* fertilization outcomes. It has been well documented that Se deficiency results in significant reduction of fertilized eggs in mice[36]. Further, Lukusa and Lehleonya[12] reported that Se supplementation improved reproductive performance of Saanen goat. Moreover, a prospective randomized trial has shown that multi-nutrient supplementation containing Se in women results in better embryo quality[37].

In the present study, we compared the effects of Se on different sperm parameters between artificial vagina and electro ejaculation

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methods samples. Our results indicated that Se is more effective on different parameters of artificial vagina samples. It can be inferred that supplementing animals with Se prior to semen collection using artificial vagina method can eliminate ROS and improve the effectiveness of antioxidants.

Measurement of sperm motility and kinematic parameters as well as other parameters such as DNA fragmentation index, mitochondrial membrane potential, and heterologous *in–vitro* fertility tests will help to predict the buck fertility of post-thaw sperm using the combination of oral Se supplementation and collection methods. However, measurement of these parameters alone is not sufficient. Measurement of pregnancy rates and the complex changes that occur in the female reproductive tract before fertilization could be assessed to understand the level of effectiveness of Se supplementation and collection methods on post-thaw sperm quality.

In conclusion, Se supplementation prior to semen collection using artificial vagina improves post-thaw sperm motility and kinematic parameters, as well as increases the percentages of sperm with intact and functional plasma membrane, functional mitochondria and the *in vitro* fertilizing potential, and thus could be considered as a promising strategy to obtain high quality semen samples from Saanen bucks for better cryosurvivability and their use in artificial insemination with optimal pregnancy rate.

Conflict of interest statement

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. None of the authors of this paper has a financial or personal relationship with other persons or organizations that might inappropriately influence or bias its content.

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Authors' contributions

Dr. Kambulu Lukusa is a corresponding author of this manuscript; he has contributed substantially to conception, sample collection and design, revising it critically for important intellectual content; and final approval of the version to be published. Dr. John Kabuba is the co-author of this manuscript; he contributed substantially in data analysis and interpretation as well as drafting of the manuscript.

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